INFLUENCE OF THERMAL CHALLENGE ON MEAT QUALITY OF TURKEY

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ABSTRACT

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Consumer demand for lean, inexpensive meat products has resulted in unprecedented growth of the poultry industry. Turkey breeders responded with intensive genetic selection to increase body weight, breast muscle yield, and feed conversion efficiency. However, this success has come at the cost of increased susceptibility to heat stress in the fast-growing birds, which led to increased frequency of pale, soft, exudative (PSE) meat. Previous studies have led to a general understanding of the etiology of PSE meat; however, the precise molecular mechanisms, which could inform development of a mitigation strategy for this defect, remain unclear.

This work comprises two studies. The first study was based on an observation that pyruvate dehydrogenase kinase 4 (PDK4) is significantly less abundant in PSE samples compared to normal turkey meat. We hypothesized that reduced PDK4 expression would result in reduced phosphorylation of pyruvate dehydrogenase which would alter the course of postmortem metabolism. Turkeys from two lines were exposed to a pre-market thermal challenge. Following slaughter and exsanguination, pectoralis major samples were removed, and the birds were then processed according to standard commercial practice. Meat quality data were collected at 24 hours postmortem and used to categorize meat samples as PSE or normal. The phosphorylation state of pyruvate dehydrogenase (PDH) in normal and PSE turkey meat and metabolite abundances were analyzed using the western blot technique. Interestingly, the results showed that PSE samples had a significantly lower mean total PDH. However, when normalized to the PDH level in each sample, there was no significant difference in the phosphorylation state of PDH between normal and PSE

samples. Analysis of these samples for various metabolites associated with aerobic and anaerobic metabolism showed no significant differences in the levels of glycogen, lactate, glycolytic potential, or ATP when compared to control samples.

The second study aimed to elucidate the effects of embryonic thermal manipulation on post-hatch thermotolerance and its consequent effects on meat quality of turkeys. Eggs from two turkey lines were exposed to pre-hatch thermal challenge; birds were hatched, raised, then exposed to three days of pre-market thermal challenge at 16 weeks. Following slaughter, breast muscle samples were collected, and birds were processed according to standard industry practice. Histological analysis was conducted for perimysial space and fiber diameter, metabolite levels were quantified, and samples were assessed for meat quality parameters including pH, color, drip loss, cook loss, and marinade uptake. The results showed that the pre-hatch treatment did not improve the thermotolerance of a premarket heat stress, and there were no significant differences in the fiber diameter, perimysial space, and meat quality characteristics. Moreover, embryonic thermal manipulation did not affect the levels of metabolites from samples of 24 h postmortem, nor of the levels of glucose, G6P, lactate, glycolytic potential, and ADP at 15 min postmortem. However, the glycogen and ATP levels at 15 min postmortem were significantly higher while the levels of IMP and AMP were significantly lower in the samples exposed to embryonic thermal manipulation compared to control when both were exposed to the pre-market thermal challenge.

The results of this research do not support a mechanistic role for reduced PDK4 abundance in altering postmortem muscle metabolism which would possibly lead to reduced meat quality. In addition, the embryonic thermal manipulation was not successful in improving thermotolerance of turkeys. Future studies are needed to define the molecular mechanisms associated with development of PSE meat and to develop a successful mitigation strategy.

This dissertation is dedicated to my family, friends, and country.

أهدي رسالة الدكتوراة للعائلة والأصدقاء ووطني المعطاء.

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INTRODUCTION

Poultry meat is a noteworthy component of the human diet, serving as an important source proteins, vitamins, minerals, and energy. Moreover, it is a primary contributor to the economy throughout the world including the US. In the US, there has been a continuous rise in the consumption of poultry since the 1950s compared with red meat (Daniel et al., 2011). Annual turkey production increased from approximately 131.9 million birds yielding 2.4 billion pounds live weight with a value of $0.68 billion in 1974, to 242 million birds yielding 7.5 billion pounds live weight with a value of approximately $4.8 billion in 2017 (USDA-NASS, 2018). These data not only show an increase in turkey consumption, but describe a significant increase in the body weight of the individual bird.

Poultry, like most meat animals, have been subjected to intense genetic selection for large size and rapid growth (Solomon et al., 1998; Owens and Apple, 2010). The economic driver is usually selection traits to produce higher body weight and more muscle yield per animal (Solomon et al., 1998; Updike et al., 2005). However, intense genetic selection has also resulted in adverse effects on the muscle structure, causing muscle abnormalities such as loss of connective tissue integrity and fiber necrosis (Sosnicki and Wilson, 1991; Solomon et al., 1998; Owens and Apple, 2010). Moreover, genetic selection has been linked with decreased protein functionality in the meat compromising the quality of the processed meat products (Updike et al., 2005; Owens and Apple, 2010).

Genetic selection has succeeded economically by increasing the body weight and the muscle yield, but the outcome of producing fast-growing birds has been accompanied by an increase in the heat stress susceptibility (Owens and Apple, 2010). Studies on broilers subjected to chronic thermal challenge showed a reduction in weight gain, and an increase in abdominal,

subcutaneous, and intermuscular fat deposition (Ain Baziz et al., 1996). Moreover, thermal challenge affects fast-growing birds to a greater extent than slow-growing birds. When slow-growing and fast-growing lines of turkeys were exposed to thermal challenge, fast-growing turkeys exhibited higher mean body temperature and reduced thermoregulation efficiency compared with the slow-growing line (Mills et al., 1999). Similarly, fast-growing broilers showed reduced weight gain and the meat had a lighter color (pale) and greater drip loss when exposed to chronic thermal challenge (Lu et al., 2007). The thermal challenge of turkeys prior to slaughter has a significant impact in lowering the meat quality; breast muscle from challenged birds showed a rapid decline in pH, as well as more cook loss, drip loss, and paler color (L*) in comparison with unstressed birds (McKee and Sams, 1997).

One of the challenges facing the meat (including turkey) industry regarding meat quality is the pale soft exudative (PSE) syndrome, which is mainly caused by antemortem heat stress, and may or may not be coupled with a genetic predisposition. This syndrome, first described in pork, is characterized by protein denaturation leading to poor protein solubility, poor binding in cooked products, paler color, softer texture, and reduced water-holding capacity. Thus, the use of PSE meat for the production of processed meat usually results in inferior quality products and reduced processing yields (Owens et al., 2000b). The incidence of turkeys producing PSE meat ranges from 5% to 30% due to the differences in turkey line and the season of the year (McCurdy et al., 1996; Owens et al., 2009). These incidences increases up to 40% with the onset of hot weather (McKee and Sams, 1997; Owens et al., 2000a). This causes a severe financial loss to the turkey industry which is estimated to exceed $200 million annually (Owens et al., 2009).

The molecular basis underlying the development of PSE is the unusually high rate of postmortem glycolysis. This will rapidly deplete the remaining oxygen and shift the metabolism

from aerobic to anaerobic for energy production, leading to lactic acid accumulation and pH drop. The combination of high temperature and reduced pH in the early postmortem conversion of muscle to meat is believed to result in protein denaturation and consequent production of PSE meat (Barbut et al., 2008). In an effort to more specifically define the molecular mechanism of PSE development, Malila et al. (2013, 2014) conducted transcriptome analysis to determine whether differences in expression of key genes could contribute to differences in postmortem metabolic rates in muscles of PSE compared with normal turkeys. This comparison found that both the transcriptional and translational expression of pyruvate dehydrogenase kinase 4 (PDK4) is reduced in PSE muscles (Malila et al., 2014). PDK4 is a key metabolism regulator; it regulates glucose metabolism by reducing the conversion of pyruvate to acetyl-CoA. This regulation is achieved by phosphorylating the pyruvate dehydrogenase complex (PDC). Phosphorylation by PDK4 inactivates pyruvate dehydrogenase (PDH), part of PDC, which subsequently decreases glucose oxidation and the production of ATP (Baumgard and Rhoads, 2013). Thus, PDK4 is a potentially significant candidate gene associated with the development of PSE meat. What is not fully understood is whether there is a mechanistic basis for the differential expression of PDK4 in PSE development in turkey meat. This knowledge gap represents an opportunity for the turkey meat industry to better understand of the development mechanism of PSE meat and reduce its incidence through management practices that will reduce economic losses for processors.

There is evidence that exposure of poultry to thermal challenge early in growth and development may positively influence the ability of birds to withstand pre-market heat stress and thereby improve overall meat quality. In studies using chickens, birds were exposed to thermal challenges during pre-hatch (embryonic), post-hatch, or pre-market periods to determine whether these thermal challenges may enable birds to adapt to temperature variation and thus, improve

thermotolerance. These strategies resulted in a reduced mortality rate, increased muscle yield, food conversion rate, or improved muscle growth. Correspondingly, they all concluded that these strategies improved the thermal tolerance compared with the control birds (Yahav and Hurwitz, 1996; Halevy et al., 2001; Piestun et al., 2008, 2011, 2013). Although turkeys have not yet been studied for implementation of such strategies, it is hypothesized that turkeys will respond similarly to chickens.

The long-term goal of Dr. Strasburg's research is to define molecular mechanisms associated with the development of superior meat quality, so that effective breeding, nutritional, and management strategies can be developed to promote the production of consistent, high quality muscle food products. The central hypothesis of this study is that the thermal challenge will induce differential gene expression in turkey skeletal muscle and thus early heat exposure can be used as an adaptation technique to produce turkeys with thermotolerance.

This project's central hypothesis will be tested in experiments that address the following objectives:

1. The first objective is to ***define the effects of pre-market thermal challenge in turkey skeletal muscle in normal and PSE meats.*** This aim is supported by the following sub-aims:
    1.1. To investigate the phosphorylation state of PDH in PSE and normal muscle samples. The working hypothesis is that down-regulation of PDK4 in PSE turkey meat will reduce the degree of phosphorylation of PDH, thereby maximizing the conversion of pyruvate to acetyl-CoA and the production of ATP.
    1.2 To determine changes in the metabolite levels of PSE meat samples compared with those meats classified as normal. The working hypothesis is that down-regulation of PDK4 in PSE samples will shift the metabolism to produce less lactate, more ATP, and faster

glycogen consumption.

2. The second objective is to ***identify the effects of an early thermal challenge on the turkey meat quality.*** This aim is supported by the following sub-aims:

   2.1. To determine the effects of the combination of pre-hatch and pre-market thermal challenges on the muscle structural characteristics of turkey. The working hypothesis is that the early pre-hatch thermal challenge will improve the muscle structural properties in response thermal challenge at market age.

   2.2 To determine changes in the metabolite levels of post-rigor meat samples of turkeys that were exposed to a combination of pre-hatch and pre-market thermal challenges. The working hypothesis is that the early pre-hatch thermal challenge will improve the thermotolerance and will shift the metabolism to produce less lactate, more ATP, and faster glycogen consumption.

   2.3 To compare the changes in the quality characteristics of turkey meat after exposing turkeys to different pre-hatch and pre-market thermal challenges. The working hypothesis is that early thermal challenge will improve the thermotolerance and produce improved protein functionality and improved meat quality, even under the pre-market thermal challenge.

By the end of this project, this research will provide a description of the role of PDK4 expression in the etiology of the development of PSE turkey. Moreover, it will establish a strategy to improve the thermotolerance in turkeys to overcome the negative effect of the thermal challenge on the meat quality of turkey. These outcomes are crucial to find a solution to overcome, or at least to reduce, the negative effect of the thermal challenge on the meat quality of turkey and eventually reducing the economic loss that the turkey industry suffers. Furthermore, these findings will

provide turkey breeders with a management strategy to raise turkeys that are more thermotolerant and subsequently producing superior quality meat, providing consumers with better products, and decreasing the economic losses in the turkey industry. Finally, it is expected that the outcomes and knowledge from this project will serve as the basis for further investigation in other poultry species.

CHAPTER 1: LITERATURE REVIEW

1.1 MUSCLE STRUCTURAL CHARACTERISTICS

In vertebrates, there are three types of muscle tissue: skeletal muscle, smooth muscle, and cardiac muscle. Smooth muscle is involuntary muscle commonly found within the walls of organs and structures such as the intestines. Skeletal muscle, which serves as the primary muscle source of meat, is voluntary muscle and it is usually attached to the bone by tendons. Skeletal muscle fibers are primarily classified into red, slow-twitch (Type I) or fast-twitch (Type II) based on histochemical staining for myosin ATPase activity (Maclntosh et al., 2006). Moreover, within Type II there are three subcategories, IIA, IIB, IIC (IIX) classified based on the ATPase staining after acidic pre-incubation of pH 3.9-4.9 (Brooke and Kaiser, 1970; Schiaffino et al., 1989).

Most mammalian muscles consist of both Type I and Type II fibers, with most having a higher percentage of Type II fibers than of Type I. On the other hand, poultry meat can have either type dominating depending on the muscle. Turkey breast muscle (Pectoralis major) comprises almost exclusively white, Type II fibers, whereas the Biceps femoris muscle (found in the leg) is darker in color than breast because it consists of both red and white fiber types. Type I muscle fiber is also called slow-oxidative (slow-twitch) and its dark red color is due to the greater infiltration of blood vessels, and high abundance of mitochondria and myoglobin which support oxidative metabolism (Brooke and Kaiser, 1970; Peter et al., 1972; Schiaffino and Reggiani, 1996; Larzul et al., 1997; Karlsson et al., 1999). Type II muscle fibers are also called fast-glycolytic (or fast-twitch) and are lighter in color due to the presence of fewer blood vessels, and reduced mitochondria and myoglobin compared to Type I fibers. Type II muscle fibers also have higher levels of glycogen compared to slow-twitch muscles (Brooke and Kaiser, 1970; Peter et al., 1972;

Schiaffino and Reggiani, 1996; Larzul et al., 1997; Karlsson et al., 1999). However, the subcategories of Type II muscle fibers have different predominant type of metabolism; fast oxido-glycolytic (Type IIA), fast-glycolytic (Type IIB) muscle fiber (Furuichi et al., 2014). Type IIX is often grouped with type IIA, but Type IIX is more glycolytic (Kerth, 2013).

The size of the muscle is determined by the size and number of muscle fibers. The number of muscle fibers is established during embryonic development, whereas after birth, muscle growth takes place by fiber hypertrophy (Picard et al., 2006; Rehfeldt et al., 2008). One of the characteristics of genetically selected birds is the larger size of breast muscles compared to muscles of unselected birds. The increased muscle size is generally thought to result from an increase in Type IIB fiber length and diameter (growth by hypertrophy), but not number of muscle fibers (growth by hyperplasia) (Aberle and Stewart, 1983; Remignon et al., 1994, 1995; Guernec et al., 2003; Ryu and Kim, 2005). However, one study on chicken breast muscle showed that the fast-growing line not only had a larger muscle fiber size but also showed an approximately 20% increase in the number of muscle fibers compared to samples from the slow-growing line (Remignon et al., 1995).

An important consequence of genetic selection and muscle fiber hypertrophy in poultry is that it has resulted in a limitation of available space in both the perimysium and endomysium, the connective tissue systems that encase fiber bundles and individual fibers, respectively (Wilson et al., 1990; Velleman et al., 2003). This limitation results in constricted capillaries that reduce their capacity to supply oxygen and nutrients to muscle, and to remove metabolic end products such as lactate from muscle (Sosnicki and Wilson, 1991; Velleman and Clark, 2015). Moreover, the fast-growing selected birds are associated with the production of lighter color breast meat with a

decrease in meat quality characteristics when birds undergo stress (Le Bihan-Duval et al., 1999; Karlsson et al., 1999; Velleman et al., 2003).

The muscle composition, including muscle fibers, connective tissues, and adipose tissues, can determine the amounts of glycogen, lipids, blood vessels, mitochondria and myoglobin as well as the extent of the tricarboxylic acid cycle and oxidative metabolism (Foegeding et al., 1996). Thus, muscle composition is one of the major contributors to the meat quality affecting the pH, color, water-holding capacity, tenderness, and flavor (Listrat et al., 2016).

1.2 POSTMORTEM MUSCLE METABOLISM

Delivery of nutrients and oxygen to cells stops after slaughter with the cessation of blood circulation, and organs such as muscle mobilize biochemical reserves in an effort to maintain homeostasis by sustaining ATP levels (England et al., 2015). In muscle, creatine phosphate is a source of high energy phosphate which regenerates ATP; creatine phosphate donates its phosphate group to ADP in a reaction catalyzed by creatine kinase (Bangsbo et al., 1991). Also, glycogen becomes the primary substrate for anaerobic metabolism, where the glycogen, along with glucose-6-phosphate and glucose are converted into lactate (El Rammouz et al., 2004a; Patterson et al., 2016). However, as energy sources are depleted, the pH drops as lactate and hydrogen ions from ATP hydrolysis accumulate during glycolysis (Bendall, 1973).

In general, the rate and extent of postmortem glycolysis, and subsequent drop of pH have a very crucial effect on meat quality (Berri et al., 2001). A rapid rate of pH fall may result in protein denaturation particularly if it is accompanied with high body temperature, as in the case of PSE meat (Solomon et al., 1998). However, a slow rate of pH drop may result in high ultimate pH and could produce a dark, firm, and dry (DFD) meat, particularly if glycogen is limited (El

Rammouz et al., 2004a). Postmortem poultry muscle normally exhibits a faster glycolysis rate compared with mammalian muscle; the reason is believed to be due to the type of poultry muscle fiber, heme protein concentrations and genetic differences (Rathgeber et al., 1999; Patterson et al., 2016). Postmortem metabolism stops in chicken breast muscle after approximately 6 h, and there is no significant difference in the metabolite levels between 6 h and 48 h postmortem (Savenije et al., 2002). However, the abnormal rapid glycolysis in poultry can compromise the meat quality.

Muscle fibers have different content of metabolites, and the abundance of metabolites at slaughter plays a major role in defining the rate and extent of postmortem glycolysis. For instance, glycogen levels in type II muscle fibers are typically greater than the levels in type I muscle fibers (Foegeding et al., 1996). A study on pork (Choe et al., 2008) showed that muscles with high levels of glycogen and lactate had a faster rate of postmortem metabolism and elevated protein denaturation compared with muscles with high glycogen and low lactate levels. Also, this study showed that muscles with low levels of glycogen and lactate had resulted in a moderate rate of postmortem metabolism and produced normal meat (Choe et al., 2008). Moreover, the metabolite abundances correlate with the type of muscle fibers, and both are believed to influence meat quality characteristics (Choe et al., 2008).

Glycogen is a polysaccharide consisting of multibranched subunits of glucose. It is the main form of glucose storage in animals, and it is mainly stored in the liver and skeletal muscle (Nelson & Cox, 2008). Levels of glycogen in the skeletal muscle depend on the type and amount of the feed consumed, physical activity, and metabolic rate as well as being influenced by several genetic aspects such as breed (Monin et al., 1987; Fernandez and Tornberg, 1991; Wasserman, 2009). Glycogen synthesis (glycogenesis) requires energy input, and it is synthesized from glucose monomers (Nelson & Cox, 2008). On the other hand, glycogenolysis is the breakdown of glycogen

by glycogen phosphorylase into glucose-1-phosphate (G1P) monomers which are then converted by phosphoglucomutase to glucose-6-phosphate (G6P) (Nelson & Cox, 2008). As the levels of glycogen decrease during postmortem metabolism, the lactate levels increase. This information can be used to determine the rate of glycolysis in which the muscles with rapid postmortem metabolism will show lower glycogen and higher lactate levels (Kylä-Puhju et al., 2004). Moreover, the glycogen content in muscle is a key determinant of the ultimate pH, color, cook loss, drip loss, and other properties of meat quality (Przybylski et al., 2016).

Glucose-6-phosphate (G6P) is a compound resulting from glycogen or glucose breakdown, and it has a critical position in the metabolism. G6P is positioned at the start of the pentose phosphate pathway and the glycolysis pathway, as well as the conversion into glycogen for storage or into glucose to be released in the bloodstream (Nelson & Cox, 2008). G6P levels are regulated by enzymes such as phosphoglucomutase which converts G1P to G6P; conversely, phosphoglucose isomerase converts G6P into fructose-6-phosphate and vice versa. In addition, hexokinase catalyzes the conversion of glucose to G6P, whereas glucose-6-phosphatase produces glucose from G6P (Nelson & Cox, 2008).

Glucose is a simple sugar, and it is the most widely used source of energy in the body for the processes of aerobic metabolism, or anaerobic metabolism (Bunn and Higgins, 1981). Glucose is oxidized through glycolysis to produce energy (ATP), water, and carbon dioxide. Glucose levels circulating through the bloodstream are regulated mainly by insulin. The excess glucose is usually stored as glycogen in the liver or skeletal muscles, and when circulating glucose levels decline, circulating glucose can be repleted by glycogen breakdown or dietary consumption of glucose, glycogen, or starch (Nelson & Cox, 2008).

Lactic acid (lactate) is an organic compound that is produced by the reduction of pyruvate by lactate dehydrogenase (LDH). This enzyme can convert lactate into pyruvate by oxidation in the presence of NAD^+ in the muscle, brain, and heart (Nelson & Cox, 2008), and lactate can serve as a substrate for gluconeogenesis to produce glucose in the liver. Accumulation of lactate in muscles can occur due to extreme oxygen demands such as intense physical activities in which the rate of lactate production is greater than the rate of lactate release into the bloodstream. Lactate accumulates during the postmortem period when there is no blood circulation, thereby increasing the acidity and decreasing the pH levels in the tissue (Nelson & Cox, 2008). Thus, the rate and extent of postmortem glycolysis are very important determinants of meat quality (Berri et al., 2001).

The glycolytic potential is an expression to indicate the status and the activity of glycolysis through the quantification of intermediate metabolites. Monin and Sellier (1985) established a method to estimate the glycolytic potential by measuring the levels of glycogen, glucose, glucose-6-phosphate, and lactate, then applying the following formula:

$$Glycolytic\ potential\ = 2\ (glycogen\ +\ G6P\ +\ glucose)\ +\ (lactate)$$

The glycolytic potential is the sum of the products resulting from the metabolism of glycogen into lactate, and it is estimated in the live animal by taking biopsies or by taking muscle samples as soon as practicable after the slaughter, and as late as 24 hours after slaughter (Maribo et al., 1999). The glycolytic potential is considered to be a major determinant of ultimate pH (pH_u), but it cannot fully predict pH_u (Van Laack, 2000). This is because glycolytic potential does not take into consideration the effects of specific enzymes such as AMP deaminase (AMPd) and phosphorylase (Van Laack, 2000). Moreover, it does not measure ATP, ADP, AMP, and IMP, which play many crucial roles including the feedback inhibition of glycolysis.

Adenosine triphosphate (ATP) is an organic compound that is the primary currency of energy in living organisms (Knowles, 1980). It is primarily produced through three major pathways; glycolysis, the citric acid cycle, and beta-oxidation (Rich, 2003). To use ATP for energy production during metabolic processes, ATP is hydrolyzed at the phosphodiester linkage yielding ADP. The process of dephosphorylation of ATP to produce ADP and AMP is followed by rephosphorylation of ADP and AMP back to produce ATP, and these processes occur repetitively during both aerobic and anaerobic metabolism (Tornroth-Horsefield and Neutze, 2008).

During the postmortem depletion of energy resources, two molecules of adenosine diphosphate (ADP) are re-phosphorylated by adenylate kinase to produce one adenosine monophosphate (AMP) and one ATP to maintain cellular homeostasis (Pearson, 1971). AMPd will convert AMP to inosine monophosphate (IMP) (Korzeniewski, 2006). This conversion is a crucial step in continuing synthesis of ATP since the accumulation of AMP will inhibit the reactivity of adenylate kinase (England et al., 2015).

IMP production is considered to be a terminator step in postmortem glycolysis since it is an end product which is unable to be used as a substrate for ATP synthesis (Bendall, 1973; Dalrymple and Hamm, 1975; Hamm, 1977; Greaser, 2001). Thus, AMPd plays a significant role in defining the extent of postmortem glycolysis and the ultimate pH of muscle (Van Laack et al., 2001; El Rammouz et al., 2004b). Several studies on chicken and pigs demonstrated a positive correlation between the levels of AMPd and the ultimate pH (Van Laack et al., 2001; El Rammouz et al., 2004b). Thus, even in the presence of glycogen, the pH decline could stop because of the conversion of AMP into IMP and the inactivation of glycolytic and glycogenolytic enzymes (El Rammouz et al., 2004b). Moreover, an earlier study found that AMPd activities can determine the ultimate pH in the presence of an abundance of glycogen (Scopes, 1971). It was concluded that if

the increase of AMPd levels or activity will elevate the ultimate pH, then the decrease of AMPd could extend anaerobic glycolysis and lower the ultimate pH (England et al., 2015).

Metabolism during the postmortem period has been extensively studied and has resulted in recommendations to improve meat quality such as reducing antemortem stress, changing feeding composition and improving slaughter strategies. However, there are still many unanswered questions concerning the role of postmortem biochemical mechanisms in determining meat quality and as a result, our ability to produce a consistent product of high quality remains limited (England et al., 2013).

1.3 MEAT QUALITY PARAMETERS

The American Meat Science Association (AMSA) defines meat quality based on physical and chemical composition and sensory evaluation of the product (AMSA, 2001). These factors include fat deposition, protein functionality, tenderness, lean to fat ratio, pH, color and appearance. Thus, the metabolite content and the type of muscle fiber have a major effect on meat quality characteristics (Choe et al., 2008).

Genetic selection plays an important role in meat quality by altering the meat composition of physical and chemical composition (Berri et al., 2001). In the case of turkeys, genetically selected birds grow faster and larger, have a higher feed conversion rate, and are more resistant to diseases. However, the increased size of turkey breast can obstruct the heat transfer delaying the chilling process which may cause negative consequences on meat quality (McKee and Sams, 1998).

One of the most important predictors of meat quality is pH, which is a measure of the concentration of hydrogen ions in a solution. In meat quality studies, pH is frequently measured at different time points after slaughter; e.g., the initial pH (within 15-45 min after slaughter), the

ultimate pH (24 h after slaughter), and sometimes one or more measures between the initial and ultimate pH (Braden, 2013). During the postmortem period, the initial pH drops mainly due to the accumulation of lactate from the anaerobic metabolism until it reaches the ultimate pH. The drop of pH is associated with the rate and extent of postmortem glycolysis, both in which are crucial to meat quality (Berri et al., 2001). For instance, the protein denaturation in PSE is due to the rapid decrease of pH while the body temperature is still relatively high (Owens et al., 2009; Braden, 2013; Malila et al., 2013).

Several studies show a significant correlation between the ultimate pH and meat quality of chickens and turkeys (Barbut, 1993, 1996; Fletcher, 1999; Van Laack et al., 2000). Moreover, the ultimate pH is affected by the genetic differences between the broiler breeds (El Rammouz et al., 2004b). Thus, pH is a significant determinant of meat quality, not only because it measures the acidity of the meat affecting the taste, but also, it affects the protein functionality parameters including drip loss, cook loss, marinade uptake, color, and other properties of the meat.

Protein functionality in meat is one of the most crucial factors in determining the attributes of meat quality. The term protein functionality includes "*any inherent or process-generated property of proteins that affect physical and sensory characteristics of raw and finished products*" (Xiong and Kenney, 1999). In industry, these attributes significantly affect the product stability, appearance, integrity, texture, and subsequently the consumer acceptance (Xiong and Kenney, 1999). In general, protein functionality in meat is affected by many factors such as pH, temperature, and ionic strength. The problem with protein denaturation is that it is usually irreversible and once it has occurred, the protein functionality will be affected reducing the properties of cohesiveness, color, and water-holding capacity of the meat product (McKee and Sams, 1998). Myosin and actomyosin are the most important functional proteins because they have

large and long fibrous structures, as well as a balanced hydrophilicity-hydrophobicity index (Xiong and Kenney, 1999). The hydrophilicity-hydrophobicity balance is a crucial index since it influences the protein solubility and thus alters the protein functionality. Disturbance of this balance can lead to conformational changes in meat proteins (Huff-Lonergan and Lonergan, 2005), reduction of the meat gelation properties (Li et al., 2015), changes of the characteristics and composition of the muscle water (exudate), as well as the water retention properties (Chan et al., 2011; Li et al., 2015). Protein functionality is measured by many techniques and using different instruments, and water-holding capacity, one of the most important functional properties in muscle protein, can be measured using drip loss, NMR, NIR, cook loss, and marinade uptake (Xiong and Kenney, 1999).

Drip loss is a traditional technique that measures the weight percentage of water or purge that exudes from the meat during postmortem storage. Purge is water that is loosely bound to meat protein or trapped in muscle tissue; it is used as a primary indicator of the water-holding capacity of the meat protein (Hamm, 1961; Kauffman et al., 1993; Honikel, 1998; Christensen, 2003; Kapper et al., 2014). In the muscle of the living animal, water is bound by proteins of the myofibrils. During the postmortem conversion of muscle to meat and subsequent aging process, myofibrils contract, cellular membranes undergo fragmention, and the subsequent gap formation facilitates water loss from the fibers accompanied by loss of soluble cytoplasmic proteins (Offer et al., 1989; Pearce et al., 2011). The exudate collected during aging can provide information about the meat composition, and can be used for predicting properties of the meat quality such as juiciness, meat color, tenderness and pH (Di Luca et al., 2013; Przybylski et al., 2016).

Studies on pork muscle show that quantification of sarcoplasmic proteins in the exudate is a reliable, rapid indicator of meat quality traits such as color, pH, and juiciness (Zelechowska et

al., 2012; Bowker et al., 2014; Przybylski et al., 2016). Moreover, studies in turkeys and chicken show a positive correlation between the drip loss and paleness or lightness (L*) which is very clear in the cases of PSE (Owens et al., 2000a; Garcia et al., 2010). The pale color results from disruption of the cell membrane which increases permeability and result increased water loss; the surface moisture increases light scattering resulting in lighter meat (Garcia et al., 2010). Quantification of drip loss coupled with analysis of the exudate contents can provide detailed information regarding the postmortem metabolism (Di Luca et al., 2013). Additionally, a study demonstrated the possibility of predicting glycolytic potential due to the relationship between glucose and lactate levels in the exudate with the muscle levels of glycolytic potential, lactate, glycogen, and meat quality traits (Przybylski et al., 2016).

Another traditional technique to measure meat quality is cook loss which evaluates the ability of cooked meat to retain water during and after cooking (Zhuang and Savage, 2013). Like drip loss, cook loss is inversely related to water-holding capacity in muscle tissue from red meats, poultry, and fish (Barbut, 1993; Skipnes et al., 2007; Zhuang and Savage, 2010). Likewise, marinade uptake is a complementary determinant of water-holding capacity and hence the meat quality. Marinade uptake measures the ability of muscle tissue to incorporate functional ingredients and flavors into meat via marination (Xiong and Kupski, 1999) in the processing of meat. This measurement is performed on minced meat by the addition of a saline solution, mixing, holding for a specific time, centrifuging, then weighing the meat to calculate the marinade uptake (NPPC, 2000).

Color is another essential quality factor that affects both meat quality and consumer acceptance. Color is determined by measuring the color space, L*, a*, and b*, which represents the CIE 1976 color space. L* represents the lightness or brightness, a* represents the red at positive

values and green at negative values, and b* represents the yellow at positive values and blue at negative values.

The L* parameter is generally much more useful than a* and b* as a meat quality determinant, and it is correlated with pH, denaturation of sarcoplasmic protein, and reduced meat quality as in the case of PSE meat (Bendall and Wismer□Pedersen, 1962; Lawrie, 1991; Swatland, 1993). Thus, some studies suggest using L* as a rapid test to identify meat that will become PSE (Owens et al., 2000a). As noted above, L* increases with protein denaturation due to the increase of the light scattering in the meat (Lawrie, 1991; Swatland, 1993).

High brightness values (L*) in turkey meat likely arise because of rapid early postmortem metabolism which results in elevated temperature and higher drip loss (McKee and Sams, 1998; Molette et al., 2003). These observations are supported by a study on chicken muscles that were maintained for four hours at 40°C. These muscles showed an increase in postmortem metabolism, leading to higher L* values and increased drip loss (Zhu et al., 2011). Another study on chicken showed that light scattering increases with protein denaturation and structure alteration, leading to a lighter, more pale meat surface (Swatland, 2008). Thus, L* levels are significantly affected by denaturation of myofibrillar and sarcoplasmic proteins with subsequent increase of exudate (Swatland, 2008). Genetics also plays a role in determining the meat color by the changes in the composition of muscle fiber. Most notably, there are differences in the content of heme pigments, mainly myoglobin, which contributes to approximately 90% of fresh meat color (Kranen et al., 1999; Mancini and Hunt, 2005).

Ultimately, the relationships between color, pH, drip loss, and myoglobin content influence the consumer appeal of meat products (Çelen et al., 2016). When meat proteins are substantially denatured, the resulting product exhibits poor cohesiveness, soft texture, and pale meat color,

coupled with accumulation of moisture purge in the package (McKee and Sams, 1998). Thus, these variations result in poor shelf life products with unacceptable quality and appearance of meat products to both the producers and consumers (Allen et al., 1998).

1.4 GENETIC SELECTION OF TURKEY

The continuous rise in the consumption of poultry over the past few decades (USDA-NASS, 2018) has pushed the industry to increase production to match consumer demand. The industry has responded by subjecting poultry to intense genetic selection of birds to produce higher body weight at a faster growth and greater muscle yield which results in higher profits (Solomon et al., 1998; Updike et al., 2005).

The intense selection of genetic traits has resulted in negative consequences for the muscle structure. These negative aspects include increased mortality, loss of connective tissue integrity, and fiber necrosis in a subset of turkeys which compromise the quality of the processed meat products (Sosnicki and Wilson, 1991; Solomon et al., 1998; Owens and Apple, 2010). Moreover, breast muscle from the fast-growing birds tends to have a higher shear force (indicating higher toughness) and lower water holding capacity (WHC) which is associated with reduced protein functionality and meat quality (Updike et al., 2005). Furthermore, genetic selection of birds has been accompanied by an increase in heat stress susceptibility (Owens and Apple, 2010). When subjected to thermal challenge, fast-growing turkeys exhibited higher body temperature and less thermoregulation efficiency compared with the slow-growing line, suggesting lower thermotolerance in the fast-growing birds (Mills et al., 1999). Additionally, broilers exposed to chronic thermal challenge showed less weight gain, more drip loss and paler meat in fast-growing broilers compared with the slow-growing broilers (Lu et al., 2007).

To study the effects of genetic selection of turkeys, researchers have developed a model consisting of two related lines. One is a slow-growing, random-bred control line (RBC2) representing a large-bodied turkey line established from random crosses of two commercial lines from late the 1960s (Nestor, 1977). RBC2 turkeys are similar to the turkey of the 1960s. The F-line is a fast-growing subline of RBC2 that has been selected for over 40 generations for only one trait: increased body weight at 16 weeks (Nestor, 1977; Nestor et al., 2008). F-line turkeys are similar to the modern commercial turkey with respect to their growth characteristics, but unlike commercial turkeys, they have not been subjected to selection for additional traits which could confound interpretation of growth studies.

1.5 THERMAL CHALLENGE AS A DETERMINANT OF MEAT QUALITY

A heat wave is defined by the National Weather Service (NWS) in USA as a duration of at least three consecutive days of air temperature above 103°F (39.4°C) in NWS heat index, which is based on both temperature and humidity. However, an alert will be issued by NWS when the heat index is expected to be above 105°F-110°F (40.5°C-43.3°C) for at least two days in sequence (National Weather Service, 2016). According to the Intergovernmental Panel on Climate Change (IPCC), the average temperature is rising globally, and it is predicted to continue rising. In North America, the average mean is projected to increase 4-11°F (2-6°C) more during the current century. In addition, models predict an increase in temperature variation, i.e., extremes of hot and cold (Romero-Lankao et al., 2014).

Typically, birds are more sensitive to heat stress is than than other animals because of the absence of sweat glands and the presence of feather coverage of the skin (Loyau et al., 2013). Thus, birds regulate their core temperature by panting, which may increase plasma pH, causing

respiratory alkalosis (Calder and Schmidt-Nielsen, 1966; Mongin, 1968; Yahav et al., 2005). When birds are exposed to chronic heat stress, they tend to consume less feed, and alter hormonal control of metabolissm; together, these responses lead to decreased growth (Geraert et al., 1996). Moreover, when turkey and chickens are exposed to chronic heat stress, they tend to exhibit accelerated postmortem metabolism which results in the production of PSE meat with reduced ultimate pH (McKee and Sams, 1997; Lu et al., 2007).

Thermal challenge has a significant impact in reducing meat quality in poultry. Turkeys that were thermally challenged (38°C at night/ 32°C at day) showed a rapid decline in pH, increased cook loss and drip loss, and paler color compared with control birds (McKee and Sams, 1997). Likewise, thermally challenged broilers (32°C for 3 wk) exhibited a 47% reduction in weight gain, and increases of 15% in abdominal fat deposition, 21% in subcutaneous fat, and 22% intermuscular fat (Ain Baziz et al., 1996). In another study with broilers, holding the temperature at 34°C during a 12 h feed withdrawal produced breast meat with lower pH and redness compared with meat from broilers held at 25°C (Petracci et al., 2001). In turkey, thermal challenge resulted in significant variations in marinade uptake, cook loss, and changes in thyroid hormone levels (T3 and T4) which were correlated with changes in meat quality (Chiang et al., 2008).

Meat quality is negatively affected by chronic heat stress due to the changes that occurr during glycolysis and aerobic metabolism resulting in the production of meat with pale color, low water holding capacity and increased drip loss and cook loss (Zaboli et al., 2018). Moreover, heat stress stimulates the activity of creatine kinase (CK) and glycolysis which could result in increased metabolic acidosis converting pyruvate to lactate in the early stages of postmortem conversion of muscle to meat (Mitchell and Sandercock, 2004).

1.6 PALE, SOFT, EXUDATIVE MEAT

Thermal challenge has been linked with the production of muscle abnormalities and low-quality meat. The PSE syndrome is one of the challenges facing the meat industry regarding meat quality which is mainly caused by antemortem thermal challenges and/or a genetic disorder. The incidence of PSE increases during hot weather up to 40% which subsequently results in dramatic loss to the meat industry (Owens et al., 2000a; Petracci et al., 2009).

In the PSE-susceptible animal, the postmortem metabolic rate accelerates during early conversion of muscle to meat, resulting in a rapid drop in pH while the body temperature is still warm (Owens et al., 2009). This will lead to denaturation of the protein causing abnormal light color (pale) of the meat with a soft texture, and exudation of water due to the reduced water-holding capacity (Owens et al., 2009; Malila et al., 2013).

PSE was first characterized in pork in the 1950s, and a similar syndrome was identified later in turkeys in the 1990s (Sosnicki and Wilson, 1991). The molecular basis for the development of much of the PSE pork was identified as a mutation in the ryanodine receptor (sarcoplasmic reticulum calcium channel) of stress-susceptible pigs and was associated with the development of some of the PSE pork (Fujii et al., 1991). Due to this mutation, the Ca^{2+} release activity of the ryanodine receptors is affected, altering Ca^{2+} homeostasis which leads to hypermetabolism resulting in excess heat and lactate production (Fujii et al., 1991; Wang et al., 1999). However, the detailed mechanism of PSE development in turkeys has not yet been determined.

Early studies on PSE turkey suggested that following slaughter, turkey muscle could be categorized into two groups: slow-glycolyzing muscle and unusually fast-glycolysis based on the percentage of initial ATP remaining 60 minutes after bleeding (Vanderstoep and Richards, 1974). Birds with a high rate of postmortem glycolysis produced meat with low water holding capacity,

lower cook yield, lower pH, and lighter in color (Pietrzak et al., 1997). Moreover, when turkeys are exposed to thermal challenge, they tend to deplete ATP faster and produce low quality meat (McKee and Sams, 1997). Initial studies to identify the cause of the PSE in poultry focused on a search for ryanodine receptor mutations. Ryanodine binding assays have been used as a biochemical method for detecting potential ryanodine mutations. In a study comparing fast-growing commercial turkeys with a slow-growing line (RBC2 line), muscle from commercial turkeys had a higher affinity of ryanodine binding compared to that of the RBC2 population, suggesting, an abnormality in calcium channel protein (Wang et al., 1999). However, PCR-based efforts to identify mutations in the turkey ryanodine receptor associated with PSE were unsuccessful (Chiang et al., 2004).

Subsequently, transcriptome analysis was used to compare the differential expression of genes in turkey skeletal muscle between normal and PSE turkey meat to determine whether differential expression of a key gene or genes could be responsible for accelerated postmortem muscle metabolism. In turkeys, transcriptome analysis was performed using both microarray and RNA deep sequencing approaches; both methods were validated using quantitative real-time PCR. Forty-nine differentially expressed transcripts were revealed by microarray analysis of RBC2 samples. Then, genes were selected for pathway analysis based on the false discovery rate and fold-change ranking. The RNA deep sequencing of RBC2 samples identified 494 differentially expressed transcripts that were used for pathway analysis. The results of both techniques showed that PDK4 expression was dramatically down-regulated in PSE turkey (Malila et al., 2013). Further quantification of PDK4 protein expression by immunoblot analysis showed a significant decrease in the protein product in PSE turkey breast muscle (Malila et al., 2014). As a critical

regulator of metabolism, differences in PDK4 protein in PSE turkey muscles might accelerate the shift to anaerobic metabolism as described below.

Protein denaturation and other PSE features lead to reduced protein functionalities such as higher drip loss, and cook loss and lower water holding capacity, and marinade uptake (Owens et al., 2009; Malila et al., 2013). This will result in inferior quality meat that can be managed in small kitchens, but it is highly undesirable in processing plants because it contributes to poor quality processed products, lower processing yields, increased costs, reduced shopper acceptability, and eventually financial loss to the turkey industry (Owens et al., 2009; Malila et al., 2013). However, the mechanism by which differential expression of genes leads to PSE meat remains unclear (Zhu et al., 2013).

1.7 PYRUVATE DEHYDROGENASE COMPLEX

Upon slaughter, delivery of nutrients and oxygen for cells stops. This begins the Delay phase in the conversion of muscle to meat. Aerobic metabolism continues as the body consumes reserves of oxygen bound to myoglobin. In poultry, the Delay phase takes approximately an hour before the beginning of rigor mortis (Aberle et al., 2001). As rigor development proceeds, glycogen and ATP are depleted. Moreover, the oxygen depletion will shift the body into anaerobic metabolism. This will lead to the formation of lactate and reduction of pH from 7.4 to about 5.6 in 2-3 h (Aberle et al., 2001). Heat is generated during the delay and rigor mortis phases which will raise the muscle temperature; however, in the development of PSE meat, early postmortem hypermetabolism results in increased temperatures (Aberle et al., 2001). Although the industry chills the carcasses by air chilling or water-immersion, the initial 15-20 minutes between slaughter and chilling are critical. In addition, the size of the muscle affects the heat transfer or chill rate.

The heat transfer is slower in larger muscle which is the main trait of the genetically selected birds leading to poor chilling rate.

Aerobic metabolism occurs in the live animal and and early in the delay phase after exsanguination until oxygen reserves are depleted. Aerobic metabolism has four stages: breakdown of glucose to pyruvate; conversion of pyruvate to acetyl-CoA, (catalyzed by the pyruvate dehydrogenase complex; PDC), metabolism of acetyl-CoA in the tricarboxylic acid (TCA) cycle; and oxidation via the Electron Transport chain (ETC) to produce ATP by phosphorylation of ADP (Fig. 1.1). In the absence of oxygen, ATP synthesis by ETC is blocked, anaerobic metabolism predominates, and pyruvate is converted to lactate (Nelson and Cox, 2008).

Pyruvate dehydrogenase complex (PDC) is a mitochondrial multienzyme complex that catalyzes the oxidative decarboxylation of pyruvate. PDC controls the synthesis of pyruvate and Coenzyme A (CoA) coupled with NAD^+ to produce Acetyl-CoA, NADH, and CO_2. This reaction is positioned at the junction of fatty acid metabolism, glucose metabolism and the TCA cycle (Sugden and Holness, 2003; Rardin et al., 2009; Zhang et al., 2014). This reaction serves as a link between two crucial pathways in metabolism: glycolysis and the TCA cycle and eventually the production of ATP. Overall, it is believed that the activity of PDC plays a major role in controlling the rate of aerobic catabolism of carbohydrate and energy production (Kolobova et al., 2001). Beside its role in controlling the aerobic metabolic flux, PDC has been under study in cancer biology due to its capability in some cancers to shift from oxidative metabolism to glycolysis (Patel and Korotchkina, 2006; Jeoung, 2015).

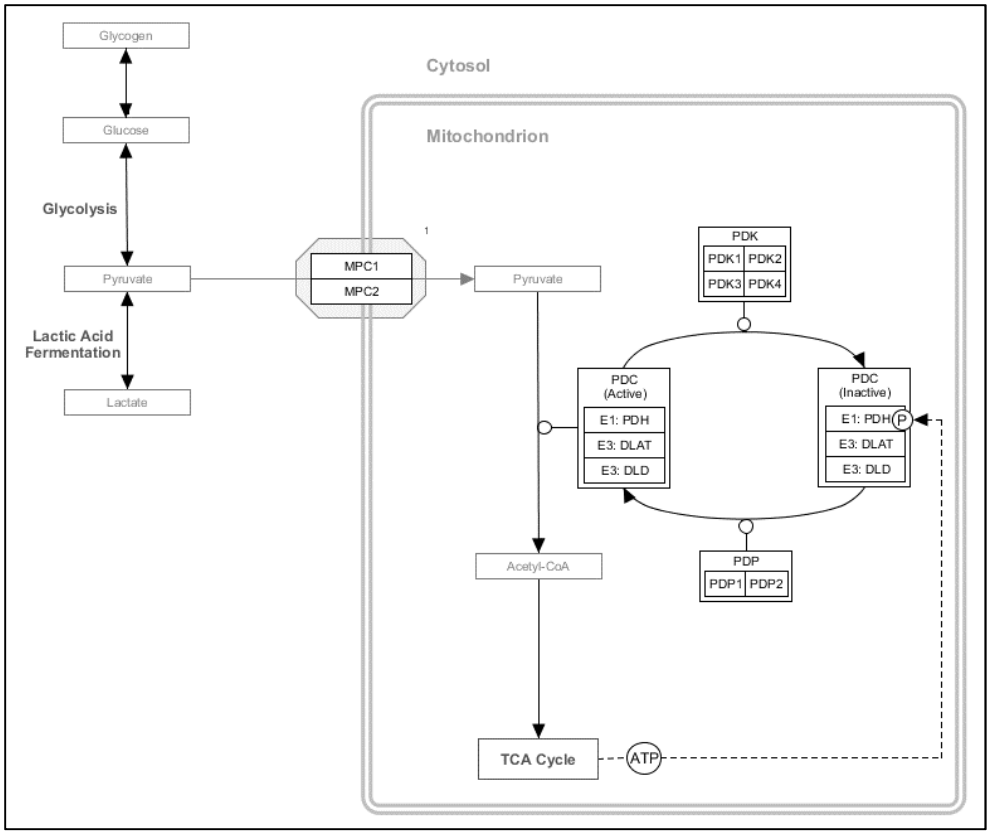

Figure 1.1 The fate of glucose in aerobic and anaerobic metabolism.

The pyruvate dehydrogenase complex (PDC) plays a pivotal role in regulating metabolic flux. PDC activity is regulated by pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP) to inhibit and activate PDC, respectively.

The PDC is composed of multiple subunits of three reactive enzymes (Fig. 1.2); E1: Pyruvate dehydrogenase (PDH), E2: Dihydrolipoyl transacetylase, and E3: Dihydrolipoyl dehydrogenase (Sugden and Holness, 2003; Rardin et al., 2009). PDH consists of two subunits: α & β, and these subunits are present in various copies depending on the species. E1α consists of 3 serine phosphorylation sites: Ser ^{293} (Site 1), Ser ^{300} (Site 2), and Ser ^{232} (Site 3).

The enzymatic activity of PDC is alternately regulated by phosphorylation and dephosphorylation. This regulation is achieved by two mitochondrial enzymes that inactivate and reactivate PDC depending on the organism's nutritional status and energy demand. The complex

is inactivated through phosphorylation of PDH by pyruvate dehydrogenase kinase (PDK) of which there are four different isoforms (PDK1, PDK2, PDK3, and PDK4). Inactivation is effected by phosphorylation of any one or more of the three sites of PDH.

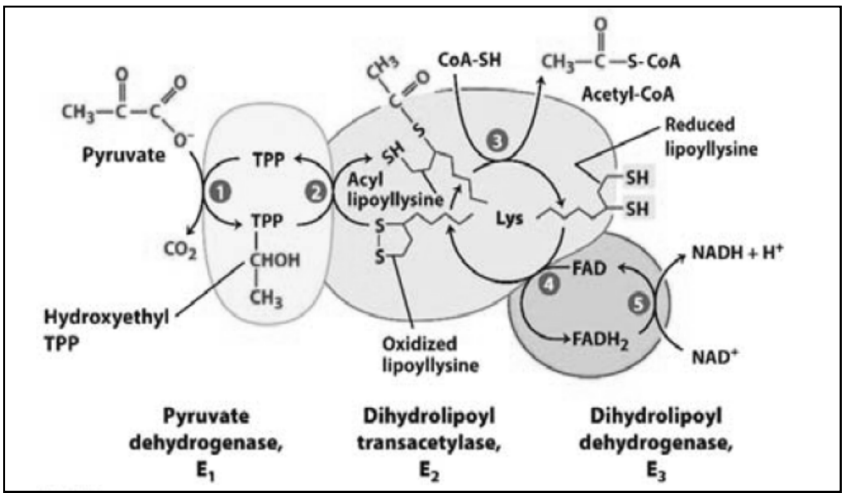

Figure 1.2 Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex (Nelson and Cox, 2008).

The PDC is activated by dephosphorylation of PDH by pyruvate dehydrogenase phosphatase (PDP), which is an enzyme that is located in the mitochondria (Kolobova et al., 2001; Patel and Korotchkina, 2006). Dephosphorylation of PDH by PDP reactivates the PDC, thereby reversing the PDK phosphorylation. There are two PDP isoforms: PDP1and PDP2 (Kolobova et al., 2001; Patel and Korotchkina, 2006). Despite the fact that the activity of PDP1 is higher than PDP2, both isoforms are capable of dephosphorylating all three sites, and the dephosphorylation rate is in the order site 2 > site 3 > site 1 (Patel and Korotchkina, 2006). The PDP1 isoform is expressed in heart, testis, and brain but it is predominantly expressed in muscle. The PDP2 isoform is expressed in kidney, brain, and heart and more abundantly adipose tissue and liver (Harris et al., 2001; Patel and Korotchkina, 2006; Rardin, 2008). The PDP isoforms are affected by the

surrounding metabolic conditions that eventually will affect the complex. For instance, the increase of pyruvate, AMP, NAD+, and CoA will favor the dephosphorylation decreasing PDK activity and activating PDC (Chen et al., 1996; Kolobova et al., 2001; Rardin et al., 2009).

1.8 PYRUVATE DEHYDROGENASE KINASE

Pyruvate dehydrogenase kinase is a critical metabolic enzyme which regulates PDH by phosphorylation. Inactivation of PDC will shift the fate of pyruvate from oxidation in mitochondria to anaerobic conversion to lactate in the cytoplasm (Harris et al., 2002; Baumgard and Rhoads, 2013).

The PDK family of enzymes comprises four different isoforms (PDK1 – PDK4) that differ in molecular mass; the MW for PDK1 is ~48 kDa, whereas the MWs for PDK2, PDK3, and PDK4 are ~45 kDa (Korotchkina and Patel, 2001). Phosphorylation of PDC at the three sites by the four isoforms occurs at different rates and with different specificity (Patel and Korotchkina, 2006). Site 1 and site 2 can be phosphorylated by all the isoforms; however, site 3 is only phosphorylated by PDK1 (Kolobova et al., 2001; Patel and Korotchkina, 2006; Rardin, 2008). Site 1 is the most rapidly phosphorylated followed by site 2 and then site 3 (Kolobova et al., 2001). Phosphorylation rates are site-specific, and the phosphorylation of one of the sites is enough to inactivate the complex (Kolobova et al., 2001). The four isoforms vary in their phosphorylation rate depending on the site, although they have much higher rates for site 1 phosphorylation than phosphorylation for sites 2 and 3 (Kolobova et al., 2001; Korotchkina and Patel, 2001). PDK1 phosphorylates site 3 slower than site 1 and faster than site 2. The isoforms PDK2, PDK3, and PDK4 phosphorylate site 1 faster than site 2 with phosphorylation rates of 19-, 6.2- and 2.5-fold, respectively (Kolobova et al., 2001).

Another complexity is that the expression of PDK isoforms is tissue-specific. PDK1 is abundant in heart and expressed to a smaller extent in skeletal muscle, liver, and pancreas. PDK2 is widely expressed in most tissues including liver, skeletal muscle, and heart with lower expression in lung and spleen. However, PDK3 is expressed mainly in testis and to a smaller extent in kidney, lung, spleen, brain, and heart. Lastly, PDK4 is expressed predominantly in skeletal muscle and the heart (Bowker-Kinley et al., 1998; Korotchkina and Patel, 2001; Rardin, 2008). Due to these complexities, it has been thought that the amount and percentage of each isoform in different tissues and under different physiological status will determine the activity of total PDK and will determine the regulating power over the PDC (Pilegaard and Darrell Neufer, 2004).

In purified form, PDK4 shows more activity with site 2 than the activities of PDK1-PDK3 (Korotchkina and Patel, 2001). Its activity is stimulated by NADH (an indicator of nutritional status), but it is not affected by the concentration change of acetyl-CoA. Also, this isoform is more resistant to inhibition by the increase of pyruvate concentration or inhibition by dichloroacetate (Bowker-Kinley et al., 1998).

The PDK4 expression is highly responsive to a variety of factors. Consumption of a high-fat diet, fasting, hyperthyroidism, induced diabetes, exercise, carnitine deficiency or other conditions with limited carbohydrate availability results in a significant increase in PDK4 level in skeletal muscle (Horiuchi et al., 1999; Wu et al., 1999; Holness et al., 2000; Sugden et al., 2000; Harris et al., 2001; Peters et al., 2001; Pilegaard et al., 2003; Pilegaard and Darrell Neufer, 2004). However, insulin treatment decreases levels of the PDK4 protein effectively in cases of diabetes and fasting (Harris et al., 2001). The expression of PDK4 in skeletal muscle is increased by NADH, acetyl-CoA, ATP and fatty acids and decreased by pyruvate, ADP, TPP (tetraphenylphosphonium), and insulin (Sugden and Holness, 2003; Connaughton et al., 2010).

However, PDK4 differs from other PDKs by being more resistant to the effect of high concentration of pyruvate (Holness et al., 2000).

PDK4 gene expression in muscles is regulated by number of other metabolites and hormones such as glucocorticoids, insulin, estrogen-related receptor alpha (ERRα), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), forkhead transcription factor (FoxO1), retinoic acid, free fatty acids, hepatic nuclear factor 4 (HNF4), peroxisome proliferator activated receptor α (PPARα), and prolactin (Huang et al., 2002; Kwon and Harris, 2004; Kwon et al., 2004; Zhang et al., 2006; Connaughton et al., 2010).

1.9 STUDIES ON IMPROVING THERMAL TOLERANCE OF POULTRY

Heat stress has been linked with the production of muscle abnormalities and low-quality meat such in the case of pale soft exudative (PSE) syndrome. Studies have been conducted since the 1970s to define the molecular mechanism of PSE development in poultry with the goal of eradicating or minimizing this incidence.

A variety of studies have been conducted in broilers to find an approach to overcome the adverse effect of the thermal challenge on the quality of meat. Yahav et al. (1996) studied the effects of a post-hatch thermal challenge on chickens by exposing birds to temperatures of 36°C for 24 h at age 5 days, or twice at age 5 and 7 days. These two groups and the control group were subsequently exposed at the age of 42 days to 35°C for 6 h. They found that the mortality rate was significantly lower in the thermally challenged birds than in the controls and they suggested that the early thermal exposure improved thermal tolerance. In another study on the the effects of post-hatch thermal challenge, Halevy et al. (2001) exposed chickens to 37.5°C for 24 h at age 3 days. This group and the control were raised to the age of 42 days at ambient temperature. The treated

group showed a significant increase in the breast muscle weight compared to control. Also, their results showed that heat exposure at an early age caused increased synthesis of satellite cell DNA followed by faster differentiation in the 3-day-old chicks.

Piestun et al. (2008) studied the effects of thermal manipulation during embryogenesis by incubating broiler eggs during the period between embryonic days 16 and 18 at 39.5°C for 3 h or 6 h / day. These two groups and the control group were raised to the age of 35 days post-hatch. The results showed that birds which had been thermally challenged as embryos produced an increased muscle growth, myofiber hypertrophy, and enhanced myogenin expression. In another study, eggs were incubated between embryonic day 7 and 16 at 39.5°C for 24 h or 12 h / day. Continuous embryonic exposure to thermal challenge (24h/d) affected embryo growth and development by lowering the weights of the embryo. However, the diurnal exposure lowered the metabolic rate and improved embryo relative weight (Piestun et al., 2009). The latter experiment was repeated with exposing the eggs to 39.5°C for 12 h/d, then exposure of the bird to 32°C for 12 h/d from day 21 post-hatch to day 35 (Piestun et al., 2011). They found that the treatment birds developed significantly larger breast muscle weight and fiber diameter than the control birds. Also, they found a reduced abdominal fat deposition compared to the control. Thus, they suggested that embryonic exposure to thermal manipulation had improved thermotolerance. A similar experiment was performed with 12 h/d exposure by Piestun et al. (2013), but the birds were raised to the market age of 70 days. This time they found that the treatment group exhibited lower body temperature accompanied by lower feed intake, greater breast muscle weight, and improved feed conversion ratio.

In general, all these studies concluded that these trials improved the thermal tolerance compared with the control birds (Yahav and Hurwitz, 1996; Halevy et al., 2001; Piestun et al.,

2008, 2011, 2013). The trials of finding a strategy to improve the thermotolerance were conducted using chickens, but it is hypothesized that similar responses would be observed with turkey due to genetic similarity with chicken.

CHAPTER 2: PHOSPHORYLATION STATE OF PYRUVATE DEHYDROGENASE AND METABOLITE LEVELS IN TURKEY SKELETAL MUSCLE IN NORMAL AND PSE MEATS

2.1 ABSTRACT

Annual turkey production increased dramatically since the 1970s as genetic selection has succeeded in increasing body weight and muscle yield to fulfill the increasing consumer demand for turkey meat. However, producing fast-growing, heavily muscled birds is linked to increased susceptibility to heat stress and to the production of pale, soft, exudative (**PSE**) meat. Previous studies in our laboratory indicated that pyruvate dehydrogenase kinase 4 (**PDK4**) is significantly reduced in PSE samples compared to normal meat samples, suggesting this as a candidate gene associated with the development of PSE meat. The objective of this study was to determine whether pre-market thermal challenge results in PSE meat as a result of differential expression of *PDK4*. Two genetic lines of turkeys were used in this study: 1) a reandombred control line 2 (**RBC2**) maintained without genetic selection since 1967, and 2) a commercial line. Birds were exposed to a pre-market thermal challenge of 12 h at 35°C followed by 12 h at 27°C for 5 days. Birds were slaughtered and processed according to industry standards. Pectoralis major samples were categorized into PSE or normal based on marinade uptake and cook loss indicators. In the first experiment, the relative expression of pyruvate dehydrogenase (**PDH**) and the phosphorylation state of PDH in normal and PSE turkey meat were analyzed by western blotting. In the second experiment, the same samples were used to measure metabolite levels at 5 min postmortem, comparing normal to the PSE samples. The results of the first experiment showed that PSE samples had significantly lower total PDH compared to normal meat samples. However,

there was no significant difference in the degree of phosphorylation of sites 1, 2, or 3. In the second experiment, there were no significant differences in the levels of glycogen, lactate, glycolytic potential, or ATP when comparing PSE to control samples. These results suggest that reduction in *PDK4* expression alone does not explain the development of PSE meat.

2.2 INTRODUCTION

Poultry meat is a noteworthy component of the human diet, contributing proteins, micronutrients, and energy. Moreover, it is a primary economic contributor throughout the world including the US. In the US, consumption of poultry has continuously risen since the 1950s compared with red meat (Daniel et al., 2011). Annual turkey production increased from 131.9 million birds, yielding 2.4 billion pounds of live weight with a value of approximately $0.68 billion in 1974 to 242 million birds, 7.5 billion pounds of live weight with a value of $4.8 billion in 2017 (USDA-NASS, 2018). Whereas turkey numbers have nearly doubled, the body weight per bird has more than tripled, demonstrating that today's turkeys are much larger at market age compared to the birds of the 1970s.

Poultry, like most meat animals, have been subjected to intense genetic selection for large size, rapid growth, and feed efficiency (Solomon et al., 1998; Owens and Apple, 2010). The economic driver is usually the basis for the selection traits used to produce higher body weight and more muscle yield (Solomon et al., 1998; Updike et al., 2005). However, intense genetic selection has frequently resulted in negative effects on muscle structure causing muscle abnormalities such as loss of connective tissue integrity and fiber necrosis (Sosnicki and Wilson, 1991; Solomon et al., 1998; Owens and Apple, 2010). In addition, genetic selection has been accompanied by an increased susceptibility to heat stress (Mills et al., 1999). Moreover, genetic selection is also linked

with decreased protein functionality in the meat, compromising the quality of the processed meat products (Updike et al., 2005; Owens and Apple, 2010).

Thermal challenge of the live bird increases the likelihood of reduced meat quality as measured by a faster decline in pH, higher cook loss and drip loss, and paler color (L*) compared with meat from non-stressed birds (McKee and Sams, 1997). Likewise, broilers subjected to chronic thermal challenge exhibited a reduction in weight gain, and an increase in abdominal, subcutaneous, and intermuscular fat deposition (Ain Baziz et al., 1996). Moreover, thermal challenge seems to affect the quality of meat from the fast-growing birds to a greater extent than from slow-growing birds. When slow-growing and fast-growing lines of turkeys were exposed to thermal challenge, the fast-growing turkeys exhibited higher body temperature and less thermoregulation efficiency (Mills et al., 1999). Likewise in broilers, the fast-growing line showed reduced weight gain and meat with a lighter color (pale) and greater drip loss compared to slow-growing broilers when exposed to chronic thermal challenge (Lu et al., 2007).

One of the challenges facing the meat (including turkey) industry is the PSE syndrome, which is mainly caused by antemortem stress that may or may not be coupled with a genetic predisposition. This syndrome was initially observed in pork (Briskey and Wismer-Pedersen, 1961; Briskey et al., 1962), and a similar myopathy was later found in turkeys (Sosnicki and Wilson, 1991). Pale soft exuadative meat is characterized by protein denaturation leading to poor protein solubility, poor binding in cooked products, pale color, soft texture, and reduced water-holding capacity. Because of the extensive use of turkey for processed meat, the use of PSE meat for the production of processed meat usually results in inferior quality products and reduced processing yields (Owens et al., 2000b). The incidence of PSE meat ranges from 5 to 30% and may reach 40% with the onset of hot weather. Thus, PSE results in severe financial losses to the

turkey industry of more than $200 million annually (McCurdy et al., 1996; Owens et al., 2000a, 2009).

Although the precise molecular basis underlying the development of PSE is not clear, one consistent observation is the unusually high rate of postmortem glycolysis (Pietrzak et al., 1997). This rapidly depletes the remaining oxygen and shifts the metabolism from aerobic to anaerobic for energy production, leading to lactate accumulation and pH drop. The combination of high temperature and rapid pH decline in the early postmortem conversion of muscle to meat is believed to result in protein denaturation and consequent production of PSE meat (Barbut et al., 2008).

In an effort to more specifically define the molecular mechanism of PSE development, Malila et al. (2013, 2014) conducted transcriptome analysis to test the hypothesis that differences in expression of key genes could contribute to differences in metabolic rates in PSE muscles compared with normal turkey muscle. This comparison found that both the transcriptional and translational expression of PDK4 are reduced in PSE muscles (Malila et al., 2014). Pyruvate dehydrogenase kinase 4 is a key regulator of metabolism; it regulates glucose metabolism by phosphorylating PDH resulting in inactivation of PDH, reducing the conversion of pyruvate to acetyl-CoA, that subsequently decreases glucose oxidation and the production of ATP (Baumgard and Rhoads, 2013). Pyruvate dehydrogenase kinase 4 was significantly down regulated in growth-selected turkey poults (F-line) compared to the randombred control line 2 (RBC2) under cold stress (31°C) and PDK4 was also down regulated in the F-line under heat stress (Barnes et al., 2019). Thus, PDK4 is a potentially significant candidate gene associated with the development of PSE meat. It is hypothesized that reduction of PDK4 results in the development of PSE in turkey meat by having less phosphorylated and more active PDH which will convert more pyruvate to acetyl-CoA. Under these conditions, aerobic metabolism would continue until the available oxygen is

depleted. These conditions would result in a shift to anaerobic metabolism, accumulation of lactate, and pH decline.

This knowledge gap represents an opportunity for the turkey meat industry to better understand the mechanism of development of PSE meat and reduce the incidence through management practices that will reduce economic losses for processors. The objective of the present study was to define a mechanistic role for decreased *PDK4* expression in the development of PSE meat in turkeys by comparing the phosphorylation state of pyruvate dehydrogenase and the metabolite levels in turkey skeletal muscle in normal and PSE meats.

2.3 MATERIALS AND METHODS

2.3.1 Sample Collection

Meat samples analyzed in this study were obtained from a previous study conducted by Chiang et al. (2008) at Michigan State University (MSU). Briefly, male and female turkeys from two different genetic lines: RBC2 and commercial turkeys (Comm) were used. RBC2 birds represent commercial turkeys during the 1960s and have been maintained at The Ohio State University (Wooster, OH) without subjection to genetic selection. The commercial birds were obtained from Michigan Turkey Cooperative (Wyoming, MI). Turkeys were raised from hatchlings at the MSU Poultry Teaching and Research Center (Chiang et al., 2008). Randombred control line 2 birds were raised to 22 wk of age and commercial birds to 16 wk of age. Before slaughter, both turkey lines were subjected to diurnal heat stress mimicking the conditions of a heat wave in Michigan (12 h at 35°C followed by 12 h at 27°C) for 0, 1, 3, or 5 days. In this study, the control (23°C) and 5 days heat stress samples were used. Breast muscle from one side from each bird was collected within 5 min after slaughter, snap-frozen in liquid nitrogen, and kept at -

80°C until further analysis. The carcass including the remaining breast muscle underwent standard commercial processing. Breast muscles were measured for pH at 15 min postmortem, and color, drip loss, cook loss, and marinade uptake at 24 h postmortem (Chiang et al., 2008). These samples were categorized as normal, or PSE mainly based on marinade uptake, then cook loss (Sporer et al., 2012). Normal meat samples showed high marinade uptake and low cook loss, but PSE samples showed the opposite. After categorization, 48 breast samples (2 lines x 2 treatments x 2 categories x 6 replicates) were used for further analyses.

2.3.2 Analysis of the Phosphorylation State of PDH in Normal and PSE Turkey Skeletal Muscles

2.3.2 (a) Materials

The following reagents and supplies were used: 4x Laemmli sample buffer, 10% Criterion™ TGX™ precast midi protein gel (12+2 well, 45 µL), Precision Plus Protein™ dual color standards (500 µL), running buffer: 10x tris/glycine/SDS, and nitrocellulose/filter paper sandwiches were obtained from Bio-Rad (Hercules, CA); NuPAGE® sample reducing agent, SimplyBlue™ SafeStain, NuPAGE® transfer buffer, and NuPAGE® antioxidant 20x were obtained from Invitrogen (Carlsbad, CA); phosphatase inhibitor cocktail, and protease inhibitor cocktail - EDTA-free, in DMSO were from Bimake (Houston, TX); alkaline phosphatase, from bovine intestinal mucosa, and bovine serum albumin, heat shock fraction, pH 7, were from Sigma-Aldrich (St. Louis, MO). Pierce™ BCA protein assay kit, and PDH monoclonal antibody (8D10E6) mouse Ab were from Thermo Fisher Scientific (Waltham, MA); Odyssey® blocking buffer (PBS), IRDye® 680RD goat anti-rabbit IgG (H + L), IRDye® 800CW goat anti-mouse IgG (H + L) and REVERT™ total protein stain were from LI-COR (Lincoln, NE). anti-PDH-E1α

(pSer[2□3]) rabbit pAb, anti-PDH-E1α (pSer[232]) rabbit pAb, and anti-PDH-E1α (pSer[3□□]) rabbit pAb were from Millipore), (Burlington, MA); and HEK293 whole cell lysate was from GeneTex (Irvine, CA).

2.3.2 (b) Levels of total PDH

Muscle samples were prepared using lysate preparation of Metcalfe et al. (2015). Briefly, frozen samples (2 g) were ground with a mortar and pestle using liquid nitrogen to make a powder. One-half gram of the powdered sample was homogenized at 4°C using a Dounce homogenizer with 5 vol (2.5 ml) of cell lysis buffer: 20 mM tris (pH 7.8), 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1% Triton X-100, 10% (w/v) glycerol, 1 mM EDTA, 1 mM dithiothreitol, supplemented with 1% protease and 5% phosphatase inhibitor cocktails. Homogenized samples were incubated for 1 h on ice, placed in the refrigerator and vortexed every 10 min. Samples were centrifuged for 10 min at 15,700 × *g* at 4°C (Beckman Coulter Allegra x-15r). The supernatant was divided into aliquots and kept at -80°C. Protein concentration of the samples was measured using the BCA method and following the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA).

Gel electrophoresis, transfer, and western blot procedures were performed following the protocols described in the protein blotting guide (Bio-Rad). Criterion™ precast midi-gels were loaded with 15 μg of protein per lane of cell lysis samples characterized as PSE or normal; one well was loaded with 2 μg of HEK293 cell lysate as a positive control. Electrophoresis was performed at 200 V for 43 min at room temperature. The sandwich of gel and the nitrocellulose membrane was prepared, and the transfer process was performed at 90 V for 100 min at 4°C.

Total PDH levels were measured by western blot analysis as follows. The membrane was rinsed with water after the transfer, dried, and rehydrated with water. The membrane was stained

with REVERT™ Total Protein Stain following the manufacturer's instructions (LI-COR) and imaged using the ODYSSEY Infrared Imaging System (LI-COR). The membrane was then washed with the washing solution,rinsed with tris-buffered saline (TBS) and treated with blocking buffer for 1 h at room temperature. The membrane was then incubated overnight at 4°C with continuous shaking with PDH-E1 alpha primary antibody (1:5000) to measure the total PDH levels. The next day, the membrane was washed 5 times with TBS containing 0.05% tween (TTBS) at pH 7.5 for 10 min each time, incubated with a secondary antibody anti-mouse IgG diluted in blocking buffer (1:3000). The incubation was performed for 1h at room temperature, followed by washing with TTBS buffer 5 times for 10 min each time. Immediate imaging of the membrane was performed using the ODYSSEY Infrared Imaging System.

For analysis of the immunoblot image, densitometry was performed using Image Studio Lite Ver 5.2 (LI-COR). Readings were taken, and band intensities were normalized to total protein content in the same lane.

2.3.2 (c) Phosphorylation state of PDH

Samples were prepared as described for total PDH analysis above. Following electrophoresis and transfer, the membrane was rinsed with water, dried, rehydrated with TBS buffer. The membrane was then treated with blocking buffer for 1 h at room temperature and incubated overnight at 4°C with continuous shaking with 2 primary antibodies: total PDH (1:5000) and an antibody for each phosphorylation site (1:2000). The following day, the membrane was washed with TTBS buffer 5 times for 10 min each, incubated with 2 secondary antibodies diluted in blocking buffer (1:3000), anti-rabbit IgG and anti-mouse IgG for detecting the site phosphorylation levels and the total PDH levels, respectively. The incubation was performed for

1 h at room temperature, followed by washing with TTBS buffer 5 times for 10 min each. Immediate imaging of the membrane was performed using the ODYSSEY Infrared Imaging System. Readings were taken, and the band intensities were normalized relative to the total PDH intensity of the same lane.

2.3.3 Metabolite Analysis in Normal and PSE Turkey Skeletal Muscles

2.3.3 (a) Materials

The following reagents and supplies were used: glucose-6-phosphate dehydrogenase (G6P-DH) from *Leuconostoc mesenteroides*, and hexokinase were obtained from Roche Diagnostics (Mannheim, Germany); L-lactic dehydrogenase from bovine heart, triethanolamine, glucose-6-phosphate (G6P), EDTA, and perchloric acid were from Sigma-Aldrich;. ATP: adenosine-5'-triphosphate disodium salt hydrate, ultrapure, and sodium L-lactate were from Alfa Aesar (Tewksbury, MA);. $MgCl_2$, dextrose and KOH pellets were from J.T. Baker (Phillipsburg, NJ); hydrazine hydrate, 55% (Hydrazine, 35%) was from Thermo Fisher Scientific. $NADP^+$: Nicotinamide adenine dinucleotide phosphate was from Cayman (Ann Arbor, MI). NAD: beta-Nicotinamide adenine dinucleotide - NAD, oxidised form was from VWR (Radnor, PA); glycine was from Invitrogen. and HCl (Millipore).

2.3.3 (b) Glycogen determination

Samples were powdered by grinding 2 g of frozen tissue with a mortar and pestle with liquid nitrogen. One hundred mg of the powder was mixed with 1 ml of 1.25 M HCl and incubated for 2 h at 90°C. The tubes were then centrifuged at 13,000 × *g* (Eppendorf centrifuge 5424) for 5

min at room temperature, and 500 µL of the supernatant was transferred to a new tube and neutralized with 500 µL of 1.25 M KOH (Patterson et al., 2016).

Glycogen concentration was determined by the method of Bergmeyer (1984) as modified by Hammelman (2003) for 96-well microplate enzymatic assay. In brief, 25µL of the sample and 75 µL of dH_2O were mixed in a glass tube with 1.5 mL of glycogen buffer (13.3 mM triethanolamine, 25 mM EDTA, 35 mM $MgCl_2$, 1.12 mM NADP). In a 96-well microplate, 200 µL of each sample was added to wells in triplicate. The same was done for the standard dilutions (150, 300, 600, 1200, 2400, and 4800 µM dextrose). Absorbance was measured at 340 nm using a Multi-label reader (2300 EnSpire, Perkin Elmer). A mixture of glucose-6-phosphate dehydrogenase (G6P-DH), hexokinase (HK), and ATP was added in the same glass tube; the contents were mixed and incubated at room temperature for 10 min. In another 96-well microplate, 200 µL of each sample was added to wells in triplicate, and absorbance was measured at 340 nm measuring NADPH. The first absorbance values were subtracted from the second values to determine the glycogen concentration (including the glucose and glucose-6-phosphate concentrations). This method is based on the enzymatic reactions on glycogen with simultaneous addition of both enzymes as listed below:

$$Glucose + ATP \xrightarrow{HK} glucose-6-P + ADP$$

$$Glucose-6-P + NADP \xrightarrow{G6P-DH} 6-P-gluconate + NADPH + H^+$$

2.3.3 (c) Lactate determination

Samples were powdered by grinding 2 g of frozen tissue with a mortar and pestle under liquid nitrogen. One hundred mg of the powder was mixed with 1 ml of 0.5 M perchloric acid on ice. The tubes were centrifuged after the incubation at 13,000 × g for 5 min at room temperature,

and 800 µL of the supernatant was transferred to a new tube and neutralized with 200 µL of 2 M KOH (Patterson et al., 2016).

Lactate concentration was measured by the method of Bergmeyer (1984) as modified by Hammelman for 96-well microplate enzymatic assay (2003). In brief, a 100 µL of sample was mixed in a glass tube with 1.5 mL of lactate buffer (0.429 mM hydrazine hydrate, 475.6 mM glycine, 0.35 mM NAD, 125 mM HCl). In a 96-well microplate, 200 µL of each sample was added to wells in triplicate. The same was done for the standard dilutions (150, 300, 600, 1200, 2400, and 4800 µM lactate). NADH was quantified by measuring absorbance at 340 nm for the plate using a Multi-label reader (2300 EnSpire, PerkinElmer). Lactate dehydrogenase (LDH) was added in the same glass tube, mixed and incubated at room temperature for 1 h. In another 96-well microplate, 200 µL of each sample was added in a well in triplicate, and the absorbance was again measured at 340 nm. Absorbance values were subtracted f to give the lactate concentration. This method is based on the following enzymatic reactions to measure NADH absorbance as representative of the lactate concentration.

$$Lactate + NAD^{+} \xrightarrow{LDH} pyruvate + NADH$$

2.3.3 (d) Glucose and Glucose-6-phosphate determination

Preparation of samples was as described for the lactate assay above. The glucose and glucose-6-phosphate concentrations were measured by enzymatic assay. In brief, 25 µL of the sample and 75 µL of dH_2O were mixed in a glass tube with 1.8 mL of glucose-6-phosphate buffer (62.5 mM triethanolamine, 25 mM EDTA, 35 mM $MgCl_2$, 1.12 mM NADP). In a 96-well microplate, 200 µL of each sample was added in a well in triplicate. The same was done for the standard dilutions (150, 300, 600, 1200, 2400, and 4800 µM glucose-6-phosphate). The first

absorbance was measured at 340 nm using a Multi-label reader (2300 EnSpire, PerkinElmer). G6P-DH was added in the same glass tube, and the contents were mixed; in another 96-well microplate, 200 µL of each sample was added in a well in triplicate and the absorbance was measured again at 340 nm. HK and ATP were added in the same glass tube, mixed and incubated at room temperature for 10 min. In another 96-well microplate, 200 µL of each sample was added to wells in triplicate, and the third absorbance (NADPH) was measured at 340 nm.

The first absorbance values were subtracted from the second absorbance to determine the glucose-6-phosphate concentrations. The first and second absorbance values were subtracted from the third absorbances to give glucose concentrations.

$$Glucose + ATP \xrightarrow{HK} glucose - 6 - P + ADP$$

$$Glucose - 6 - P + NADP \xrightarrow{G6P-DH} 6 - P - gluconate + NADPH + H^{+}$$

2.3.3 (e) ATP, ADP, AMP and IMP determination

Preparation of samples was as described for the lactate assay above. The samples were kept frozen at -80°C and shipped on dry ice to Dr. Eric England's laboratory at The Ohio State University (Columbus, OH). ATP, ADP, AMP and IMP levels were measured using the method of Bernocchi et al. (1994) as modified by Williams et. al (2008). In brief, ATP, ADP, AMP, and IMP were separated with an HP Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA) using an Accucore C18 2.6 µm 50 mm × 4.6 mm column (Thermo Scientific, Pittsburgh, PA). Absorbance of the peaks associated with each compound in chromatograms were were detected at 254 nm. For peak identification, standards were coeluted and the results compared using the standard curve for quantification (Bernocchi et al., 1994; Williams et al., 2008; England et al., 2015).

2.3.4 Statistical analysis

Results were analyzed statistically using SAS 9.4 (SAS Inst. Inc., Cary, NC). Metabolite levels, and the intensities of total PDH and the three phosphorylation sites were analyzed by a 3-way ANOVA using the Mixed procedure. The model included three factors: genetic lines (two levels: RBC2 and Commercial), days of treatment (two levels: 0 and 5 days), and quality status (two levels: Normal and PSE), plus all interaction terms. Pairwise comparisons of the result means were performed by Tukey's test using the LSMEANS statement of the Mixed procedure and an accepted type I error (α) of 5%.

2.4 RESULTS

2.4.1 Phosphorylation State of PDH in Normal and PSE Turkey Skeletal Muscles

Analysis of turkey meat samples (Figure 2.1) showed no significant difference between normal and PSE samples with respect to phosphorylation levels of site 1 ($pSer^{293}$), site 2 ($pSer^{300}$), or site 3 ($pSer^{232}$) on PDH. However, the total PDH levels in PSE samples were significantly lower ($P = 0.029$) compared with normal samples.

Comparing the two pre-market heat treatments, there were no significant differences between 0 and 5 d treatments in the total PDH levels and the phosphorylation levels of site 1, and site 2 on PDH (Figure 2.1). However, the phosphorylation levels of site 3 were significantly higher ($P < 0.0001$) in samples exposed to heat stress for 5 d compared with the control samples (0 d). On the other hand, the comparisons between the two genetic lines showed that samples from commercial turkeys had significantly lower levels of total PDH and lower levels of phosphorylation of site 1, and site 2, but higher phosphorylation levels of site 3 compared to RBC2 line. Statistical analysis showed that the meat category factor (PSE or normal) had no significant

interaction either with the genetic line factor or days of pre-market heat treatments in total PDH and phosphorylation of its sites.

Visually, the intensity levels at site 3 were weaker than at sites 1 and 2 when referenced to the levels of the HEK293 (positive control). However, these comparisons are not quantitative, and the intensity levels of these sites need to be measured in the same membrane for more accurate comparison.

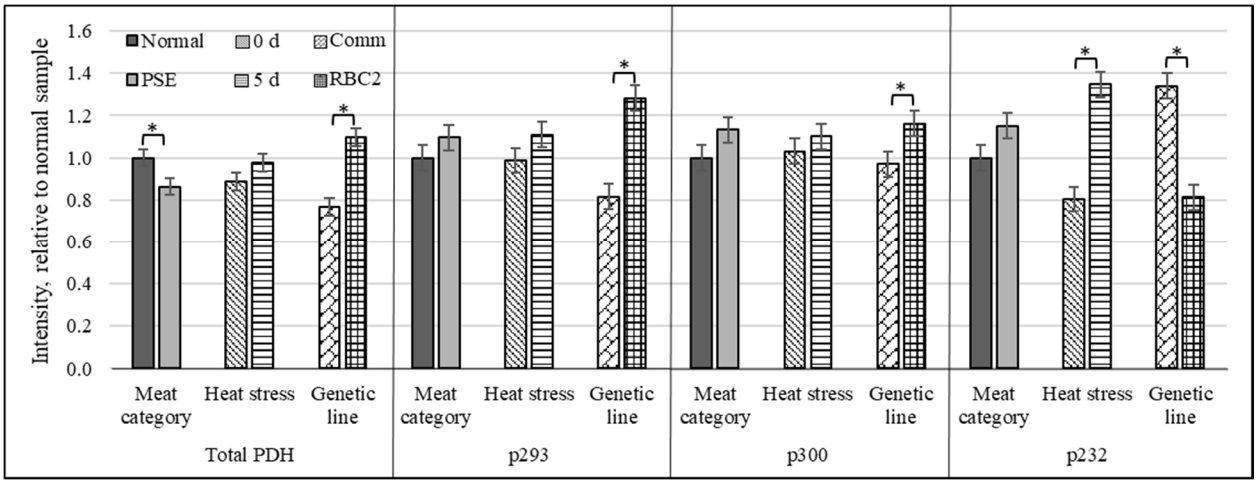

Figure 2.1 Total PDH levels and phosphorylation levels of the PDH sites assessed from western blotting. Intensities of the bands were normalized to the intensity of normal sample.

* Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

2.4.2 Metabolite Levels in Normal and PSE Turkey Skeletal Muscles

Pale soft exudative samples showed no significant differences compared with normal samples for the levels of glycogen, lactate, glycolytic potential, IMP, AMP, ADP, and ATP. However, there were significantly higher levels of glucose ($P = 0.029$) and G6P ($P = 0.018$) in PSE muscle samples (Table 2.1). Pre-market heat exposure for 5 d produced breast muscle samples with significantly lower glycogen ($P < 0.0001$), glucose ($P = 0.007$), glycolytic potential ($P =$

0.009), and AMP (P = 0.005) as shown in Table 2.1. However, there were no significant differences between 0 and 5 d treatments in the levels of G6P, lactate, IMP, ADP, and ATP.

The results of the comparisons between the two genetic lines (Table 2.1), showed no significant differences in the levels of glycogen, glucose, lactate, glycolytic potential, ADP, or ATP. However, samples from commercial turkeys had significantly higher levels of G6P (P = 0.014), and lower levels of IMP (P = 0.005), and AMP (P = 0.034).

There was no significant interaction between the meat category factor and the days of pre-market heat treatments in the measurement of metabolite levels. Moreover, the meat category factor showed no significant interaction with the genetic lines factor except in the AMP levels. The interaction of AMP levels showed that normal commercial samples had significantly lower AMP ($P < 0.04$) than PSE commercial and normal RBC2 samples. The interaction between the genetic lines and days of pre-market heat treatments showed significance in the levels of glycogen and glycolytic potential. For interactions of glycogen, samples from RBC2 muscle exposed to heat treatment had significantly lower levels of glycogen ($P < 0.049$) than in the unexposed samples from both genetic lines and the exposed commercial samples. However, interactions of glycolytic potential showed samples from RBC2 unexposed to heat treatment had significantly higher levels of glycogen ($P < 0.035$) than in the exposed samples from both genetic lines and the unexposed commercial samples.

Table 2.1 Metabolite levels in pectoralis major depending on the factor investigated: meat category, heat stress, and genetic line.

Meat category

Metabolites (μmol/g)	Normal	PSE	SE[1]	*P* value
Glycogen[2]	23.37	21.96	2.13	0.641
Glucose	3.60	4.24	0.20	0.029*
G6P	1.10	1.76	0.19	0.018*
Lactate	108.71	119.11	3.66	0.051
Glycolytic potential[3]	164.84	175.02	5.63	0.209
IMP	2.83	3.84	0.41	0.088
AMP	0.41	0.44	0.04	0.521
ADP	1.28	1.34	0.05	0.306
ATP	4.71	3.90	0.34	0.101

Heat stress

Metabolites (μmol/g)	0 d	5 d	SE	*P* value
Glycogen	28.99	16.35	2.13	0.0001*
Glucose	4.32	3.51	0.20	0.007*
G6P	1.29	1.57	0.19	0.293
Lactate	111.77	116.05	3.66	0.414
Glycolytic potential	180.95	158.91	5.63	0.009*
IMP	3.34	3.32	0.41	0.969
AMP	0.50	0.35	0.04	0.005*
ADP	1.36	1.26	0.05	0.102
ATP	4.23	4.38	0.34	0.748

Genetic lines

Metabolites (μmol/g)	Comm	RBC2	SE	*P* value
Glycogen	22.70	22.64	2.13	0.985
Glucose	3.70	4.14	0.20	0.126
G6P	1.77	1.09	0.19	0.014*
Lactate	110.02	117.8	3.66	0.141
Glycolytic potential	166.34	173.52	5.63	0.373
IMP	2.48	4.19	0.41	0.005*
AMP	0.37	0.48	0.04	0.034*
ADP	1.35	1.27	0.05	0.239
ATP	4.74	3.87	0.34	0.079

[1]SE: Standard error, [2]Glycogen results expressed as μmol of glucose per g of meat, [3]Glycolytic potential results expressed as μmol of lactate per g of meat, * Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

2.5 DISCUSSION

One of the distinguishing properties of PSE meat is the rapid glycolysis during the early postmortem conversion of muscle to meat that causes a rapid decrease in pH while body temperature is still high (Pietrzak et al., 1997). The expression levels of PDK4, which generally change in response to metabolic needs, were significantly lower in PSE samples (Malila et al., 2013). Hence, the PDK4 level is assumed to be a major contibuter in the PSE development and thus, the total PDH levels were hypothesized to show no difference between PSE and normal samples. Contrary to the hypothesis, PSE samples had significantly lower total PDH levels compared with normal samples. This could result in a reduced flux of pyruvate into the TCA cycle, resulting in the conversion of pyruvate into lactate rather than acetyl CoA; increasing accumulation of lactate would reduce the pH level of PSE meat. Although PDH protein levels were lower in PSE meat, PDH activity was not measured in the current work. PDH activity might be higher in PSE compared to normal samples to compensate for the lower total PDH levels to cope with the rapid glycolysis under the lower PDK4 levels.

The underlying hypothesis of PDH phosphorylation status was that down-regulation of *PDK4* in PSE turkey (Malila et al., 2013) would result in a significant reduction in PDH phosphorylation, specifically in sites 1, 2 or both (Kolobova et al., 2001). In this case, PDH in PSE muscle would be more active than in normal muscle resulting in more rapid conversion of pyruvate into acetyl-CoA entering the TCA cycle. Contrary to this hypothesis, no significant differences were observed in phosphorylation between PSE and normal samples. It is possible that an increase in the activity of PDK4 could have compensated for down-regulation in PSE samples. Also, phosphorylation levels of PDH could vary as a result of decreased pyruvate dehydrogenase phosphatase (PDP) levels or activities which would eventually minimize the dephosphorylation in

PSE samples.

Site 3 of PDH is only phosphorylated by PDK1 (Kolobova et al., 2001; Patel and Korotchkina, 2006; Rardin, 2008), and PDK1 is abundant in heart and expressed to a smaller extent in skeletal muscle, liver, and pancreas. In the current study, the phosphorylation level was not different at site 3 between samples from PSE and normal muscle. However, samples from commercial turkeys had a significantly higher phosphorylation level compared to the RBC2 line. Commercial birds are genetically selected for faster and heavier growth and are associated with increased risk for PSE meat. Thus, it is possible that phosphorylation of site 3 by PDK1 is linked to the development of PSE and considered as another side effect resulting from genetic selection.

The phosphorylation state of PDH is just one indicator of PDK4 activity. Having data on the level and activities of the other isoforms of PDK and PDP would provide better understanding of the metabolic difference between the genetic lines and their responses to thermal challenge.

This study hypothesized that down-regulation of PDK4 in PSE samples would shift metabolism to produce less lactate, more ATP, and faster glycogen consumption. However, the PSE samples showed no significant difference in the levels of glycogen, lactate, nor ATP. Also, the results do not agree with the outcomes presented by Pietrzak et al. (1997) that PSE samples showed lower muscle ATP and higher lactate levels. In this study, the samples were collected at 5 min postmortem which might be not enough time to show any difference in the metabolism and rate of glycogen consumption between normal and PSE samples.

The higher G6P levels in PSE samples could be attributed to the rapid glycogenolysis. Glycogenolysis commonly does not produce glucose molecules. In bovine, Young et al. (1988) found that glycogen could be uncommonly debranched into glucose due to the activity of two enzymes (EC 3.2.1.33; EC 2.4.1.25); however, this glucose could not be phosphorylated by

hexokinase leading to the accumulation of glucose molecules. Moreover, the higher levels of G6P will later produce higher ATP levels. However, there was no significant difference in IMP, AMP, ADP, and ATP levels in PSE samples compared with normal samples. This suggests a rapid shift of metabolism from aerobic to anaerobic in PSE samples compared to normal, increasing the lactate levels and lowering pH. Lactate levels were not significantly different between the PSE and normal samples but the pH of the PSE samples was significantly lower ($P = 0.023$, unpublished). However, pH was measured at 15 min postmortem, whereas the lactate level ($P = 0.051$) was measured at 5 min which could show a significantly different level in PSE when measured at 15 min postmortem.

The heat-exposed birds had significantly lower values of glycogen, glucose, and glycolytic potential which could be attributed to lower feed intake under heat stress as well as the amount of energy wasted during panting to regulate body temperature. This aligns with the findings in many earlier studies that showed reduction in feed intake in response to heat stress (Yahav and Hurwitz, 1996; Yahav and McMurtry, 2001; Lu et al., 2007; Azad et al., 2009; Piestun et al., 2011, 2013). Interestingly, commercial turkeys had significantly lower levels of IMP, and AMP than in RBC2. This suggests that commercial birds have a faster metabolic rate, that force them to use ADP to regenerate ATP and consequently accumulating AMP which is then converted into IMP declaring the end of metabolism for this molecule.

The interaction between genetic line and days of pre-market heat treatment (not presented here) showed significance in the levels of glycogen. In the interaction, samples from RBC2 turkeys exposed to heat treatment for 5 d had significantly lower levels of glycogen than both the heat-stressed commercial samples, and the control samples from both genetic lines. Control samples from RBC2 had significantly greater levels of glycolytic potential than the commercial bird

controls, and the heat-stressed samples from both genetic lines. This shows the different response of the genetic lines to heat exposure.

2.6 CONCLUSIONS

This study investigated the effects of pre-market thermal challenge in turkey skeletal muscle in normal and PSE meats. The significant decrease in PDK4 levels in PSE samples (Malila et al., 2013) did not result in a significant difference of the PDH phosphorylation levels between PSE and control samples. However, this study focused only on the relation between the levels of PDK4 and PDH. To complete this picture, it is recommended to measure the activities of both the PDK4 and PDH. Moreover, measurement of levels and activities of PDP isoforms, and the three other PDK isoforms would add more values and explanation. These would provide greater insight into the metabolic flux of pyruvate through PDH as well as the metabolic difference between the different lines and their responses to thermal challenge. Interestingly, PDH levels were significantly higher in RBC2 compared to commercial birds. This questions the role of PDH in the relation between the genetic line and the rate of the postmortem metabolism. This study did notmeasure both pyruvate and acetyl-CoA to evaluate the efficiency of PDH. Further research in fresh samples is needed. Measurement of metabolite levels and comparison between normal and PSE meat that exhibited a significant decrease in PDK4 levels (Malila et al., 2013) did not result in a significant difference of the glycogen, lactate, glycolytic potential and ATP levels between PSE and control samples. This was surprising because previous studies showed that PSE is characterized by lower pH and faster glycolytic rate in the early postmortem period (Pietrzak et al., 1997).

CHAPTER 3: USE OF EMBRYONIC THERMAL CHALLENGE TO IMPROVE TURKEY THERMOTOLERANCE AND MEAT QUALITY

3.1 ABSTRACT

Annual turkey production has increased dramatically since the 1970s as the industry has used genetic selection to increase body weight and muscle yield to meet increasing consumer demand for lean poultry products. However, a negative consequence of genetic selection for large, fast-growing birds is increased susceptibility to heat stress which was linked with the production of low-quality meat. Previous studies have shown that exposure of broiler embryos to thermal challenge during embryonic growth and development positively influenced the ability of birds to withstand pre-market heat stress and thereby improve overall meat quality. Thus, this study aimed to elucidate the effects of thermal manipulation during the embryonic development on the post-hatch thermotolerance and the consequent effects on turkey muscle structure, postmortem metabolism, and meat quality. Eggs from two turkey genetic lines were exposed to pre-hatch thermal challenge of 39.5°C for 3 h/d on embryonic days 21-25. After hatching, the birds were raised under for standard commercial conditions. At age 16 weeks, one-half of the birds were exposed to a pre-market thermal challenge of 32°C for 12h/d for 3 days followed by slaughter and processing of breast muscles. Muscle samples were collected at 15 min postmortem for histological analysis to determine perimysial space and fiber diameter. Metabolite levels were measured at two postmortem timepoints: 15 min and 24 h. Moreover, samples were analyzed for meat quality parameters including pH, color, drip loss, cook loss, and marinade uptake. Interestingly, the pre-hatch treatment did not improve the thermotolerance and showed no significant difference in the fiber diameter, perimysial space, and all measured parameters of meat

quality. Moreover, embryonic thermal challenge had no effect on the levels of metabolites from samples at 24 h postmortem, as well as the levels of glucose, G6P, lactate, glycolytic potential, and ADP at 15 min postmortem. However, the 15 min postmortem samples showed that levels of glycogen and ATP were significantly higher while the levels of IMP and AMP were significantly lower in the samples exposed to embryonic thermal manipulation compared to control when both exposed to the pre-market thermal challenge. These outcomes could be due the short time (3 h/d) of the pre-hatch thermal challenge. In conclusion, the embryonic thermal manipulation used in this study did not improve the thermotolerance of turkys nor did it improve the breast meat qulity.

3.2 INTRODUCTION

The consumption of poultry commodities in the US, including chicken and turkey, increased dramatically and continuously compared with red meat since the 1950s (Daniel et al., 2011). According to the National Agricultural Statistics Service (2018), annual turkey production increased from approximately 131.9 million birds and 2.4 billion pounds live weight in 1974 to 242 million birds and 7.5 billion pounds in 2017. The breast muscle is the most valuable component of the turkey because of its versatility in value-added processed meat products (Updike et al., 2005). Thus, economics is the primary driver for the use of genetic selection to produce higher body weight and greater muscle yield in a reduced period of time (Solomon et al., 1998; Updike et al., 2005). However, this intense selection has resulted in unintended consequences that compromise the quality of the processed meat products. These negative effects include decreased protein functionality, the appearance of PSE-like meat, and muscle abnormalities such as loss of connective tissue integrity and fiber necrosis (Sosnicki and Wilson, 1991; Barbut, 1997; Solomon et al., 1998; Updike et al., 2005; Owens and Apple, 2010).

Selection for faster growth rate and increased size of turkeys has been accompanied by increased susceptibility of turkey to thermal challenge (Mills et al., 1999). In contrast, slow-growing broilers displayed greater thermotolerance compared to a fast-growing strain (Lu et al., 2007).. Thermal challenge is associated with decreased meat quality characteristics such as pale color (L*), faster pH decline, and greater drip loss and cook loss compared with the meat of non-challenged birds (McKee and Sams, 1997). The effects of the thermal challenge on the meat quality are amplified in the intensely selected, fast-growing turkeys (Mills et al., 1999). Thus, thermal challenge typically leads to the production of inferior meat products which subsequently results in economic losses to the meat producers and processors.

There is some evidence that exposure of poultry to thermal challenge in the early stages of growth and development may positively influence the ability of birds to withstand pre-market heat stress and thereby improve overall meat quality. Studies on broilers suggest that acute exposure to thermal manipulation in the latter stages of embryonic development or immediately post-hatch may enable birds to adapt to temperature variation and thus, improve thermotolerance (Yahav and Hurwitz, 1996; Ain Baziz et al., 1996; Piestun et al., 2008). These studies demonstrated that thermal manipulation resulted in reduced mortality rate (Yahav and Hurwitz, 1996), increased food conversion rate (Ain Baziz et al., 1996; Piestun et al., 2013), increased muscle yield and muscle growth (Halevy et al., 2001; Piestun et al., 2008, 2011, 2013). Together, these studies concluded that early thermal challenge improved thermal tolerance compared with the control birds.

Although effects of early thermal challenge have not been studied in turkeys, it is hypothesized that turkeys will respond to early thermal challenge similarly to chickens. Moreover, there is a paucity of data on the effects of embryonic thermal manipulation or post-hatch thermal challenge on meat quality in either broilers or turkeys. The molecular response in muscle exposed

to thermal challenge is expected to differ with the development stage and its genetic traits for adaptability to the thermal challenge and the molecular mechanisms of the avian response to thermal challenge may or may not result in the production of inferior meat products.

Current models of climate variation predict an increasing frequency of temperature extremes which will likely have negative consequences for meat quality. Thus, the goal of this study is to develop a strategy of improving the thermotolerance of turkeys that will mitigate the deleterious effects of climate variation. The success of this strategy depends on a detailed understanding of the physiological response to the thermal challenge. The present study focuses on elucidating the effects of thermal manipulation during the embryonic stage on the pre-market thermotolerance and its consequent effects on the muscle structure, postmortem metabolism, and meat quality.

3.3 MATERIALS AND METHODS

3.3.1 Sample Collection

To investigate the effect of the genetic selection on thermotolerance, two turkey lines were utilized: the randombred control line 2 (RBC2) and F-line. RBC2 turkeys represent the commercial turkeys during the 1960s which have not been subjected to genetic selection. The F-line birds were genetically selected from the RBC2 line for greater body weight at 16 weeks. Eggs from both lines were obtained from The Ohio State University (Wooster, OH) and they were transported to the Poultry Teaching and Research Center at Michigan State University (MSU) where they were incubated at 38°C. After ten days, eggs were candled, and infertile eggs were discarded. The eggs were divided into 2 groups per line. One group from each line was maintained at 38°C for the entire incubation period. The other two groups were exposed to a pre-hatch thermal challenge by

incubating at 39.5°C for 3 h/d on embryonic days 21 to 25 (Piestun et al., 2011). On day 28, all hatched birds were sexed. The male birds from the four groups were placed in a brooder at 35°C for 2 wk, and then transferred to pens where they were maintained at conventional temperature (20°C ± 2). At 16 wk of age, half of the birds from each of the four groups representing the control group for the pre-market thermal challenge were transporteded 4.1 miles to the MSU Meat Laboratory to be slaughtered on March 20, 2018. Three days later, all the remaining birds from the four groups were exposed to 3 days of a diurnal thermal challenge consisting of 12 h at 36°C followed by 12 h at 32°C, after which birds were transported to the MSU Meat Laboratory to be slaughtered. At this point, there were 8 experimental groups (2 lines x 2 pre-hatch treatment x 2 pre-market treatment) with replicates ranging from 12 to 19 birds per group and 123 birds in total. Both turkey lines were given ad libitum access to the same commercial feed (Home Fresh Multi-flock, Kent Feeds, Inc. Muscatine, IA, USA) until 16 h before the slaughter. They were raised using conventional husbandry practices common for commercial turkeys. All methods applied were approved by the Institutional Animal Care and Use Committee (IACUC#: 01/17-003-00).

On the day of slaughter, the live bird weights were recorded immediately prior to slaughter. Birds were subjected to electrical stunning at 60 Hz for 5 s using an electrical knife at a setting of 7 (Model VS200, Midwest Processing Systems, Minneapolis, MN 55410), followed by cutting the carotid artery. After bleeding out, carcasses were immersed in a scalding tank at 60°C for alternating intervals of 6 s in and 6 s out for a total of 60 s. Carcasses were then defeathered, eviscerated, and carcass weight was recorded. The pectoralis major from the left breast was removed, weighed, placed in a bag for further analysis and kept at 4°C.

3.3.2 Analysis of Muscle Structural Characteristics

3.3.2 (a) Sample preparation

After evisceration, the skin was removed from the carcass, and a sample was taken 15 min postmortem from the cranial end of the right pectoralis major muscle for histological analysis for all birds (n=123) as described by Velleman et al. (2003). Briefly, a sample was carefully dissected to a size of approximately 0.5 cm wide, 0.5 cm deep, and 3 cm long parallel to the orientation of the muscle fibers. The muscle sample was tied to a wooden stick before removal to prevent muscle contraction. Each sample was placed in 10-12 ml of 10% (vol/vol) formalin, pH 7.0 and kept at 4°C. The samples were transported to Dr. Velleman's laboratory at The Ohio State University (Wooster, Ohio) for analysis according to the procedure of Jarrold et al. (1999). The samples were dehydrated in a series of dilute ethanol solutions and cleared in Pro Par Clearant (Anatech, Battle Creek, MI). The samples were embedded in paraffin, processed, and the resulting blocks were cross-sectioned to 5 μm thickness. Each section was adhered to charged Starfrost Adhesive slides (Mercedes Medical, Sarasota, FL), with each slide containing 4 sections, and then the sections were stained with hematoxylin and eosin (Jarrold et al., 1999; Velleman and Clark, 2015).

3.3.2 (b) Muscle fiber diameter

Muscle fiber diameter was measured on four viewing fields from one section per bird by measuring the diameter of a minimum 20 fibers within each viewing field (Velleman and Clark, 2015; Clark and Velleman, 2017). The diameter of the myofibers was measured using ImageJ (US National Institutes of Health, Bethesda, MD). The measurement of muscle fiber diameter was done by measuring the shortest distance across the geometric center of each myofiber (Clark and Velleman, 2017).

3.3.2 (c) Perimysial space

Photomicrographs were captured with CellSens Imaging software on an Olympus IX70 fluorescent microscope and QImaging digital camera (Clark et al., 2017). Images then were analyzed for perimysial space using ImageJ (US National Institutes of Health. Average perimysial space was calculated by measuring the mean perimysial width at 4 distinct locations along every perimysium present within the field of view.

3.3.3 Determination of Metabolite Levels

The samples used for the analysis of metabolite levels were taken at two different postmortem times: 15 min and 24 h. Six samples were randomly chosen from each group using the random number generator in the Google Chrome browser resulting in 48 samples in total (2 lines x 2 pre-hatch treatment x 2 pre-market treatment x 6 replicates). For the 15 min postmortem time point, samples were taken from the middle side of the pectoralis major at the right breast, whereas samples for the 24 h postmortem were taken from pectoralis major on the left breast. Samples were snap frozen in liquid nitrogen and kept at -80°C.

3.3.3 (a) Materials

The following reagents and supplies were used: glucose-6-phosphate dehydrogenase (G6P-DH) from *Leuconostoc mesenteroides*, and hexokinase were from Roche Diagnostics (Mannheim, Germany); L-lactic dehydrogenase from bovine heart, triethanolamine, glucose-6-phosphate, EDTA, and perchloric acid were from Sigma-Aldrich (St. Louis, MO). ATP: adenosine-5'-triphosphate disodium salt hydrate, ultrapure, and sodium L-lactate were from Alfa Aesar (Tewksbury, MA); $MgCl_2$, dextrose and KOH pellets were from J.T. Baker (Phillipsburg, NJ); hydrazine hydrate, 55% (Hydrazine, 35%) was from Thermo Fisher Scientific (Waltham, MA).

$NADP^+$: Nicotinamide adenine dinucleotide phosphate was from Cayman (Ann Arbor, MI); NAD: beta-Nicotinamide adenine dinucleotide - NAD, oxidised form was from VWR (Radnor, PA); glycine was from Invitrogen, (Carlsbad, CA); and HCl was from Millipore (Bedford, MA).

3.3.3 (b) Glycogen determination

Samples were powdered by grinding 2 g of frozen tissue with a mortar and pestle with liquid nitrogen. One hundred mg of the powder was mixed with 1 ml of 1.25 M HCl and incubated for 2 h at 90°C. The tubes were then centrifuged at 13,000 × *g* (Eppendorf centrifuge 5424) for 5 min at room temperature, and 500 µL of the supernatant was transferred to a new tube and neutralized with 500 µL of 1.25 M KOH (Patterson et al., 2016).

Glycogen concentration was determined by the method of Bergmeyer (1984) as modified by Hammelman (2003) for 96-well microplate enzymatic assay. In brief, 25µL of the sample and 75 µL of dH_2O were mixed in a glass tube with 1.5 mL of glycogen buffer (13.3 mM triethanolamine, 25 mM EDTA, 35 mM $MgCl_2$, 1.12 mM NADP). In a 96-well microplate, 200 µL of each sample was added to wells in triplicate. The same was done for the standard dilutions (150, 300, 600, 1200, 2400, and 4800 µM dextrose). Absorbance was measured at 340 nm using a Multi-label reader (2300 EnSpire, Perkin Elmer). A mixture of glucose-6-phosphate dehydrogenase (G6P-DH), hexokinase (HK), and ATP was added in the same glass tube; the contents were mixed and incubated at room temperature for 10 min. In another 96-well microplate, 200 µL of each sample was added to wells in triplicate, and absorbance was measured at 340 nm measuring NADPH. The first absorbance values were subtracted from the second values to determine the glycogen concentration (including the glucose and glucose-6-phosphate

concentrations). This method is based on the enzymatic reactions on glycogen with simultaneous addition of both enzymes as listed below:

$$Glucose + ATP \xrightarrow{HK} glucose-6-P + ADP$$

$$Glucose-6-P + NADP \xrightarrow{G6P-DH} 6-P-gluconate + NADPH + H^{+}$$

3.3.3 (c) Lactate determination

Samples were powdered by grinding 2 g of frozen tissue with a mortar and pestle under liquid nitrogen. One hundred mg of the powder was mixed with 1 ml of 0.5 M perchloric acid on ice. The tubes were centrifuged after the incubation at 13,000 × *g* for 5 min at room temperature, and 800 µL of the supernatant was transferred to a new tube and neutralized with 200 µL of 2 M KOH (Patterson et al., 2016).

Lactate concentration was measured by the method of Bergmeyer (1984) as modified by Hammelman for 96-well microplate enzymatic assay (2003). In brief, a 100 µL of sample was mixed in a glass tube with 1.5 mL of lactate buffer (0.429 mM hydrazine hydrate, 475.6 mM glycine, 0.35 mM NAD, 125 mM HCl). In a 96-well microplate, 200 µL of each sample was added to wells in triplicate. The same was done for the standard dilutions (150, 300, 600, 1200, 2400, and 4800 µM lactate). NADH was quantified by measuring absorbance at 340 nm for the plate using a Multi-label reader (2300 EnSpire, PerkinElmer). Lactate dehydrogenase (LDH) was added in the same glass tube, mixed and incubated at room temperature for 1 h. In another 96-well microplate, 200 µL of each sample was added in a well in triplicate, and the absorbance was again measured at 340 nm. Absorbance values were subtracted f to give the lactate concentration. This method is based on the following enzymatic reactions to measure NADH absorbance as representative of the lactate concentration.

$$Lactate + NAD^{+} \xrightarrow{LDH} pyruvate + NADH$$

3.3.3 (d) Glucose and Glucose-6-phosphate determination

Preparation of samples was as described for the lactate assay above. The glucose and glucose-6-phosphate concentrations were measured by enzymatic assay. In brief, 25 µL of the sample and 75 µL of dH_2O were mixed in a glass tube with 1.8 mL of glucose-6-phosphate buffer (62.5 mM triethanolamine, 25 mM EDTA, 35 mM $MgCl_2$, 1.12 mM NADP). In a 96-well microplate, 200 µL of each sample was added in a well in triplicate. The same was done for the standard dilutions (150, 300, 600, 1200, 2400, and 4800 µM glucose-6-phosphate). The first absorbance was measured at 340 nm using a Multi-label reader (2300 EnSpire, PerkinElmer). G6P-DH was added in the same glass tube, and the contents were mixed; in another 96-well microplate, 200 µL of each sample was added in a well in triplicate and the absorbance was measured again at 340 nm. HK and ATP were added in the same glass tube, mixed and incubated at room temperature for 10 min. In another 96-well microplate, 200 µL of each sample was added to wells in triplicate, and the third absorbance (NADPH) was measured at 340 nm.

The first absorbance values were subtracted from the second absorbance to determine the glucose-6-phosphate concentrations. The first and second absorbance values were subtracted from the third absorbances to give glucose concentrations.

$$Glucose + ATP \xrightarrow{HK} glucose - 6 - P + ADP$$

$$Glucose - 6 - P + NADP \xrightarrow{G6P-DH} 6 - P - gluconate + NADPH + H^{+}$$

3.3.3 (e) ATP, ADP, and IMP determination

Preparation of samples was as described for the lactate assay above. The samples were kept frozen at -80°C and shipped on dry ice to Dr. Eric England's laboratory at The Ohio State University (Columbus, OH). ATP, ADP, AMP and IMP levels were measured using the method of Bernocchi et al. (1994) as modified by Williams et. al (2008). In brief, ATP, ADP, AMP, and IMP were separated with an HP Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA) using an Accucore C18 2.6 μm 50 mm × 4.6 mm column (Thermo Scientific, Pittsburgh, PA). Absorbance of the peaks associated with each compound in chromatograms were were detected at 254 nm. For peak identification, standards were coeluted and the results compared using the standard curve for quantification (Bernocchi et al., 1994; Williams et al., 2008; England et al., 2015).

3.3.4 Determination of Meat Quality Parameters

3.3.4 (a) pH

The pH of the breast muscles was determined at 15 min and 24 h postmortem according to Bendall and Bourne (1973) by using 6 samples from each of the 8 groups (n=48). The samples for measuring the pH at 15 min were taken from the middle side of the pectoralis major of the right breast, while the samples for measuring the pH at 24 h were taken from the caudal end of the pectoralis major of the left breast. Samples were snap frozen in liquid nitrogen and kept at -80°C.

Samples were prepared by grinding 2-3 g of frozen tissue with a mortar and pestle under liquid nitrogen to make a powder. One gram of the powder was mixed with 10 ml of a blend of 150 mM KCl and 5 mM sodium iodoacetate (pH 7.0), and then the mixture was vortexed for 30 s. The pH meter was standardized at the beginning and after every 6 readings using pH 4, 7, and 10

standards. The measurement was done in duplicate at room temperature, and the two readings were recorded and averaged.

3.3.4 (b) Drip loss

Following slaughter, evisceration, and chilling to 4°C, the pectoralis major muscles on the left breast of 123 birds were removed, weighed, placed individually in bags and suspended for drip loss determination at 4°C. After 24 h, each muscle was removed from the bag, blotted dry and weighed. The drip loss percentage was calculated based on the weight of the muscle before and after the 24 period (NPPC, 2000).

3.3.4 (c) Cook loss

The samples were taken 24 h postmortem from the middle of the pectoralis major of the left breast. The cook loss measurement of the muscles was done using the same samples used for color measurements for all samples (n=123). Samples were trimmed to 12 cm x 10 cm, weighed then kept at 4°C. All samples were placed in cooking bags and cooked in a commercial smoker at the MSU Meat Laboratory to an internal temperature of 76°C. The samples were placed on trays to chill in a cold room (4°C) until the internal temperature reached 23°C. The samples were removed from the cooking bags, blotted dry and weighed. The cook loss percentage was calculated based on the weight of the muscle before and after cooking (NPPC, 2000).

3.3.4 (d) Marinade uptake

The marinade uptake for all samples (n=123) was determined at 48 h postmortem. Samples were taken from the cranial end of the pectoralis major of the left breast and kept at 4°C. Six grams

(±0.2 g) of muscle sample were chopped in a food blender (Waring Model 1120, Waring Products Division, New Hartford, Conn.) for 30 s (5 s on followed by 5 s off), then placed in 50 ml centrifuge tubes. Ten ml of 3.5% (w/v) NaCl solution was added to each tube and the tubes were shaken gently, then vortexed vigorously for 10-15 sec at room temperature. Subsequently, the tubes were incubated in a water bath at 25°C for 30 min. Next, the tubes were centrifuged at 13°C for 20 min at 3000 × *g* using a Beckman Coulter Allegra X-15 R swinging bucket rotor (Beckman Coulter Inc., Brea, CA). The supernatant was removed, and the marinade uptake percentage was calculated based on the weight of the sample before and after (NPPC, 2000).

3.3.4 (e) Color

The color measurement of the muscles was done for all samples (n=123) at 24 h postmortem. The samples were taken from the middle of the pectoralis major of the left breast, and the muscle was sliced horizontally and left to bloom for 15 min at 12°C. Then, the CIELAB color values; lightness (L*), redness (a*) and yellowness (b*), were measured in two different locations on the muscle using a HunterLab MiniScan XE Plus Colorimeter (Hunterlab, Reston, VA, USA), and the readings were recorded and averaged.

3.3.5 Statistical analysis

The results were analyzed statistically using SAS 9.4 (SAS Inst. Inc., Cary, NC). The results of muscle fiber diameter, perimysial space, metabolite levels, and the meat quality parameters were analyzed by a 3-way ANOVA using the Mixed procedure. The model included three factors: genetic lines (two levels, RBC2 and F-line), pre-hatch treatment (two levels, Control and Treated), and pre-market treatment (two levels, Control and Treated) and all their interactions.

Pairwise comparisons of the result means were performed by Tukey's test using the LSMEANS statement of the Mixed procedure and the accepted type I error (α) was 5%.

3.4 RESULTS

3.4.1 Effects of Embryonic Thermal Manipulation and Pre-market Thermal Challenge on Muscle Structure

Muscle fiber diameter and perimysial spacing were measured to determine the effects of embryonic thermal manipulation and premarket thermal challenge on muscle structure. The histology results (Table 3.1) show that the interaction between the pre-hatch thermal manipulation (thermotolerance strategy) and pre-market thermal treatment had no significant effect on both muscle fiber diameter and perimysial space of the breast muscle samples. In addition, the pre-hatch treatment had no significant effect on both measurements of the breast muscle samples compared with control samples. However, the samples from birds exposed to pre-market thermal manipulation treatment showed significantly larger perimysial space ($P = 0.008$) but no significant difference of muscle diameter compared with control samples. Finally, there was no significant effect of genetic lines on the perimysial space, although the F-line samples showed larger muscle diameter ($P = 0.001$) compared with the RBC2 samples.

Table 3.1 Effects of genetic line, pre-hatch thermal manipulation, pre-market thermal challenge, and interaction of thermal treatments on fiber diameter and perimysial width of pectoralis major.

Genetic line

	F-line	SE	RBC2	SE	*P* value
Fiber Diameter (μm)	46.7	0.9	43.0	0.6	0.001*
Perimysial space (μm)	26.8	1.5	28.7	1.3	0.350

Pre-hatch treatment

	Control	SE	Treated	SE	*P* value
Fiber Diameter (μm)	45.5	0.8	44.3	0.8	0.249
Perimysial space (μm)	26.1	1.1	29.4	1.6	0.097

Pre-market treatment

	Control	SE	Treated	SE	*P* value
Fiber Diameter (μm)	44.5	0.8	44.3	0.8	0.454
Perimysial space (μm)	30.4	1.4	25.0	1.4	0.008*

Thermotolerance

	CT[1]	SE[2]	TT[3]	SE	*P* value
Fiber Diameter (μm)	45.4	1.1	45.3	1.1	0.945
Perimysial space (μm)	22.4	2.3	27.7	2.3	0.059

[1] CT: birds exposed only to pre-market thermal challenge; [2] SE: Standard error; [3] TT: birds exposed to both pre-hatch thermal manipulation and pre-market thermal challenge; * Significant difference ($P < 0.05$) between the turkey samples within the investigated factor

3.4.2 Effects of Embryonic Thermal Manipulation and Pre-market Thermal Challenge on Postmortem Metabolite Levels in Muscle

Pre-market thermal challenge increases the probability of PSE meat quality which results from accelerated postmortem metabolism. Exposure of the late-stage embryo to a mild thermal manipulation has been proposed as mitigation strategy designed to improve thermotolerance in the pre-market turkey. Accordingly, the levels of several key compounds which serve as markers of postmortem metabolism were determined in breast muscle samples from birds from the two genetic lines exposed to a pre-market thermal challenge, a pre-hatch thermal manipulation, both pre-hatch and pre-market treatments, and in samples from control birds not exposed to either thermal treatment. The results (Table 3.2) showed that the pre-hatch treatment had no significant

effect on the breast muscle compared with control samples for levels of glycogen, glucose, G6P, lactate, and the glycolytic potential in pectoralis major samples taken at both 15 min and 24 h postmortem. Samples from the pre-hatch treatment group showed significantly lower levels of IMP ($P = 0.01$) and AMP ($P = 0.003$) at 15 min postmortem. Moreover, they showed significantly higher levels of ADP ($P = 0.015$) and ATP ($P = 0.019$) at 15 min postmortem. However, there were no significant differences in the levels of IMP, AMP, ADP, and ATP between the pre-hatch and normal samples at 24 h postmortem.

Table 3.2 Effect of pre-hatch thermal manipulation on metabolite levels in pectoralis major at 15 min and 24 h postmortem.

Metabolites (μmol/g)	15 min postmortem				24 h postmortem			
	Control	Treated	SE[1]	*P* value	Control	Treated	SE	*P* value
Glycogen[2, ‡]	7.39	8.49	0.67	0.251	-1.35	-1.17	0.23	0.61
Glucose	3.24	3.11	0.23	0.693	3.53	3.14	0.24	0.27
G6P	1.83	2.22	0.29	0.351	2.19	2.08	0.33	0.83
Lactate	116.18	114.14	2.49	0.567	122.08	120.76	2.11	0.66
Glycolytic potential[3]	141.09	141.76	3.35	0.893	130.82	128.87	2.56	0.59
IMP	5.36	4.44	0.24	0.01*	9.03	8.72	0.14	0.14
AMP	0.77	0.52	0.06	0.003*	0.40	0.35	0.03	0.25
ADP	0.78	0.96	0.05	0.015*	0.34	0.34	0.02	0.94
ATP	1.51	2.50	0.29	0.019*	0.10	0.10	0.01	0.96

[1] SE: Standard error; [2] Glycogen results expressed as μmol of glucose per g of meat; [‡] Glycogen results at 24 h are negative due to the correction factor; [3] Glycolytic potential results expressed as μmol of lactate per g of meat; * Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

Breast samples from birds exposed to pre-market thermal challenge (Table 3.3) showed significantly higher values of glycogen ($P < 0.0001$), G6P ($P = 0.0002$), glycolytic potential ($P < 0.0001$), IMP ($P = 0.003$), and AMP ($P < 0.0001$) at 15 min postmortem, and lower levels of ADP ($P < 0.03$) and ATP ($P < 0.01$). There was no significant difference of lactate levels in the samples from treated and normal birds at both 15 min and 24 h postmortem. Moreover, the samples from

the treated group at 24 h post mortem showed higher levels of glycogen ($P < 0.0001$), glucose ($P < 0.0001$), G6P ($P = 0.001$), AMP ($P < 0.0001$), and ADP ($P < 0.0001$), but they showed lower IMP ($P = 0.0003$) and ATP ($P < 0.0001$) levels.

Table 3.3 Effect of pre-market thermal challenge on metabolite levels in pectoralis major at 15 min and 24 h postmortem.

Metabolites (μmol/g)	15 min postmortem				24 h postmortem			
	Control	Treated	SE[1]	*P* value	Control	Treated	SE	*P* value
Glycogen[2, ‡]	3.05	12.83	0.67	<.0001*	-2.02	-0.50	0.23	<.0001*
Glucose	3.20	3.14	0.23	0.868	2.62	4.05	0.24	<.0001*
G6P	1.19	2.86	0.29	0.0002*	1.32	2.95	0.33	0.001*
Lactate	114.41	115.9	2.49	0.674	122.46	120.39	2.11	0.493
Glycolytic potential[3]	129.29	153.56	3.35	<.0001*	126.30	133.39	2.56	0.057
IMP	4.36	5.44	0.24	0.003*	9.27	8.48	0.14	0.0003*
AMP	0.48	0.82	0.06	<.0001*	0.21	0.55	0.03	<.0001*
ADP	0.95	0.79	0.05	0.03*	0.23	0.45	0.02	<.0001*
ATP	2.55	1.45	0.29	0.01*	0.15	0.04	0.01	<.0001*

[1] SE: Standard error; [2] Glycogen results expressed as μmol of glucose per g of meat; [‡] Glycogen results at 24 h are negative due to the correction factor; [3] Glycolytic potential results expressed as μmol of lactate per g of meat; * Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

The interaction between the pre-hatch and pre-market treatments showed that the proposed thermotolerance strategy had no significant effects on all nine metabolite indicators at 24 h postmortem (Table 3.4). The results also showed no significant effects on the levels of glucose, G6P, lactate, glycolytic potential, and ADP at 15 min postmortem. However, the breast muscle samples from birds exposed to pre-hatch thermal treatment had significantly higher levels of glycogen ($P = 0.026$) and ATP ($P = 0.001$), and significantly lower levels of IMP ($P = 0.0004$) and AMP ($P = <0.001$) at 15 min postmortem.

Table 3.4 Effect of pre-hatch thermal manipulation on metabolite levels in pectoralis major at 15 min and 24 h postmortem of turkeys exposed to pre-market thermal challenge.

Metabolites (μmol/g)	15 min postmortem				24 h postmortem			
	CT[1]	TT[2]	SE[3]	*P* value	CT	TT	SE	*P* value
Glycogen[4, ‡]	11.28	14.37	0.94	0.026*	-0.65	-0.35	0.33	0.533
Glucose	3.54	2.75	0.33	0.101	4.18	3.93	0.34	0.610
G6P	2.68	3.04	0.41	0.545	2.81	3.09	0.47	0.674
Lactate	119.53	112.27	3.52	0.153	121.41	119.36	2.99	0.630
Glycolytic potential[5]	154.54	152.58	4.99	0.784	134.09	132.69	3.63	0.787
IMP	6.36	4.52	0.34	0.0004*	8.55	8.40	0.20	0.613
AMP	1.02	0.61	0.08	0.001*	0.60	0.50	0.04	0.108
ADP	0.69	0.89	0.07	0.059	0.46	0.45	0.02	0.934
ATP	0.40	2.50	0.41	0.001*	0.04	0.03	0.01	0.770

[1] CT: birds exposed only to the pre-market thermal challenge; [2] TT: embryos exposed to pre-hatch thermal manipulation and pre-market thermal challenge; [3] SE: Standard error; [4] Glycogen results expressed as μmol of glucose per g of meat; ‡ Glycogen results at 24 h are negative due to the correction factor; [5]Glycolytic potential results expressed as μmol of lactate per g of meat; * Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

Analysis of the genetic line differences (Table 3.5) showed no significant difference in the levels of lactate, AMP and ATP at both postmortem periods. F-line samples taken at 15 min postmortem showed higher values of glycogen ($P = 0.011$), glucose ($P = 0.013$), G6P ($P = 0.001$), glycolytic potential ($P = 0.03$), and ADP ($P = 0.024$), but lower levels of IMP ($P < 0.0001$) compared to RBC2 birds. At 24 h postmortem, samples taken from F-line birds showed higher levels of glucose ($P = 0.007$), G6P ($P < 0.0001$), and glycolytic potential ($P = 0.034$), but lower levels of glycogen ($P < 0.0001$) and IMP ($P = 0.0003$) compared to RBC2.

Table 3.5 Metabolite levels in pectoralis major by turkey line (F-line and RBC2) at 15 min and 24 h postmortem.

Metabolites (μmol/g)	15 min postmortem				24 h postmortem			
	F-line	RBC2	SE[1]	*P* value	F-line	RBC2	SE	*P* value
Glycogen[2]	9.20	6.68	0.67	0.011*	-2.05	-0.47	0.23	<.0001*
Glucose	3.60	2.74	0.23	0.013*	3.83	2.85	0.24	0.007*
G6P	2.73	1.32	0.29	0.001*	3.15	1.12	0.33	<.0001*
Lactate	115.98	114.34	2.49	0.6446	124.00	118.85	2.11	0.093
Glycolytic potential[3]	147.04	135.81	3.35	0.03*	133.83	125.86	2.56	0.034*
IMP	4.18	5.62	0.24	<.0001*	8.47	9.27	0.14	0.0003*
AMP	0.68	0.61	0.06	0.372	0.38	0.38	0.03	0.9535
ADP	0.96	0.79	0.05	0.024*	0.33	0.35	0.02	0.3932
ATP	2.27	1.73	0.29	0.192	0.10	0.09	0.01	0.401

[1] SE: Standard error; [2] Glycogen results expressed as μmol of glucose per g of meat; ‡ Glycogen results at 24 h are negative due to the correction factor; [3] Glycolytic potential results expressed as μmol of lactate per g of meat; * Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

3.4.3 Meat Quality Parameters from Turkeys Exposed to Embryonic Thermal Manipulation and Pre-market Thermal Challenge

There was no significant effect of pre-hatch treatment (Table 3.6) on the weights of live birds, carcass weights, and pectoralis major weights, nor on pH values at 15 min and 24 h, L*, and b* values. The samples from treated groups showed significantly higher drip loss ($P = 0.034$) and cook loss ($P = 0.021$), as well as lower marinade uptake ($P = 0.001$) and a* ($P = 0.009$) values.

Table 3.6 Effect of pre-hatch thermal manipulation on body weight, carcass weight, breast muscle weight, and meat quality parameters.

	Control	Treatment	SE[1]	*P* value
Live weight (kg)	11.71	11.91	0.21	0.491
Carcass weight (kg)	9.02	9.01	0.16	0.961
P. major weight (kg)	0.95	0.95	0.03	0.835
pH 15 (min)	6.25	6.28	0.03	0.507
pH 24 (h)	6.03	6.03	0.03	0.939
Drip Loss (%)	0.70	0.95	0.08	0.034ǂ
Cook Loss (%)	21.99	22.82	0.25	0.021ǂ
Marinade uptake (%)	54.52	44.34	2.04	0.001ǂ
L*	48.75	49.81	0.44	0.1
a*	6.04	5.41	0.17	0.009ǂ
b*	11.08	11.09	0.11	0.963

[1] SE: Standard error; ǂ Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

The pre-market thermal manipulation had no significant effect on the weights of live birds, carcasses, and pectoralis major nor of muscle pH at 15 min and 24 h postmortem, a*, cook loss, marinade uptake (Table 3.7). However, these samples showed significantly higher values in the results of L* ($P = 0.009$), lower b* ($P = 0.001$), and lower drip loss ($P = 0.0002$) compared to control.

Table 3.7 Effect of pre-market thermal challenge on body weight, carcass weight, breast muscle weight, and meat quality parameters.

	Control	Treatment	SE[1]	*P* value
Live weight (kg)	11.63	11.98	0.21	0.247
Carcass weight (kg)	9.00	9.03	0.16	0.899
P. major weight (kg)	0.92	0.98	0.02	0.068
pH 15 (min)	6.28	6.25	0.03	0.471
pH 24 (h)	6.07	5.99	0.03	0.051
Drip Loss (%)	1.04	0.61	0.03	0.0002ǂ
Cook Loss (%)	22.39	22.41	0.25	0.941
Marinade uptake (%)	50.44	48.43	2.03	0.487
L*	48.43	50.13	0.45	0.009ǂ
a*	5.88	5.57	0.17	0.194
b*	11.35	10.81	0.11	0.001ǂ

[1] SE: Standard error; ǂ Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

The statistical analysis of the interactions between the pre-hatch and pre-market treatments showed that the pre-hatch thermal manipulation resulted in a significant increase in the drip loss ($P = 0.001$) and a significant decrease in the marinade uptake ($P = 0.017$). However, the pre-hatch treatment resulted in no significant difference in pH at both 15 min and 24 h postmortem as well as all the rest of the tested meat quality parameters (Table 3.8).

Analysis of genetic lines for drip loss, and pH at 15 min postmortem showed that there were no significant line effects (Table 3.9). Samples from F-line showed significantly higher values of the weights of live birds ($P < 0.0001$), carcass weight ($P < 0.0001$), and pectoralis major weight ($P < 0.0001$), as well as breast muscle cook loss ($P < 0.0001$), L* ($P < 0.0001$), b* ($P < 0.0001$). However, the samples from F-line birds showed significantly lower pH at 24 h postmortem ($P = 0.001$), marinade uptake ($P = 0.04$) and a* ($P < 0.0001$).

Table 3.8 Effect of pre-hatch thermal manipulation on thermotolerance of turkeys subjected to pre-market thermal challenge assessed by differences in body weight, carcass weight, breast muscle weight, and meat quality parameters.

	CT[1]	TT[2]	SE[3]	*P* value
Live weight (kg)	11.87	12.10	0.3	0.584
Carcass weight (kg)	9.05	9.01	0.23	0.921
P. major weight (kg)	0.98	0.98	0.04	0.987
pH 15 (min)	6.21	6.28	0.04	0.256
pH 24 (h)	5.99	6.00	0.04	0.780
Drip Loss (%)	0.50	0.72	0.04	0.001ǂ
Cook Loss (%)	22.14	22.69	0.35	0.272
Marinade uptake (%)	53.36	43.49	2.85	0.017ǂ
L*	50.08	50.18	0.62	0.906
a*	5.86	5.29	0.23	0.086
b*	11.01	10.61	0.15	0.075

[1] CT: exposed only to the pre-market treatment; [2] TT: exposed to both the pre-hatch and the pre-market treatments; [3] SE: Standard of error; ǂ Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

Table 3.9 Comparison of live weights, carcass weights, P. major weights and meat quality parameters between two genetic lines of turkeys (F-line and RBC2).

	F-line	RBC2	SE[1]	*P* value
Live weight (kg)	15.70	7.92	0.2	<.0001ǂ
Carcass weight (kg)	11.96	6.07	0.16	<.0001ǂ
P. major weight (kg)	1.30	0.60	0.01	<.0001ǂ
pH 15 (min)	6.29	6.24	0.03	0.203
pH 24 (h)	5.96	6.10	0.03	0.001ǂ
Drip Loss (%)	0.79	0.86	0.08	0.517
Cook Loss (%)	23.15	21.66	0.23	<.0001ǂ
Marinade uptake (%)	46.44	52.42	1.93	0.04ǂ
L*	52.05	46.51	0.53	<.0001ǂ
a*	5.07	6.38	0.16	<.0001ǂ
b*	11.45	10.71	0.11	<.0001ǂ

[1] SE: Standard of error; ǂ Significant difference ($P < 0.05$) between the turkey samples within the investigated factor.

3.5 DISCUSSION

3.5.1 Muscle Structural Characteristics in Different Thermal Manipulation Conditions

The turkey breast muscle (pectoralis major) is a fast-twitch anaerobic muscle composed mainly of glycolytic type IIb muscle fibers (Bandman et al., 1982; Remignon et al., 1995). The primary energy sources for type IIb myofibers are glucose and glycogen. Anaerobic respiration in the glycolytic muscle results in glycogen metabolism and lactic acid formation. The circulatory system delivers nutrients to the fibers and removes metabolic wastes such as lactic acid and carbon dioxide (Bangsbo et al., 1991). The blood capillaries are positioned between muscle fibers (endomysial space) and between muscle fiber bundles (perimysial space). Thus, an increase of myofiber diameter can decrease the endomysial space and the perimysial space which decreases the presented space for blood circulation (Wilson et al., 1990; Dransfield and Sosnicki, 1999; Velleman et al., 2003). The reduced surface to area could result in reduced blood supply in the live animal, increasing retention of lactic acid which in turn results in decreased pH, and consequent damage to muscle fibers and production of PSE meat (Velleman et al., 2003).

The pre-hatch thermal manipulation treatment had no significant effect on the muscle fiber diameter and perimysial space of the breast muscle samples compared with control samples. This could be due to the short incubation period (3 h). These results are consistent with the study on broilers by Clark et al. (2017) which indicated that the 3 h incubation treatment did not show a significant change in fiber width. Our previous work (unpublished data) compared three durations (3, 6, and 12 h/d) of embryonic thermal manipulation, without pre-market exposure. The results of that study showed that the use of 3 h/d produced slightly but not significantly better results than 12 h/d.

There was a significantly reduced perimysial space in the samples exposed to pre-market heat stress. However, there was no significant effect in the interaction between the pre-hatch and pre-market treatments in both fiber diameter and perimysial space of the breast muscle samples.

Comparison of genetic lines showed no significant difference in the perimysial space, but the F-line samples showed larger muscle fiber diameter (hypertrophy) compared with the RBC2 samples. It was expected that F-line would have larger muscle diameter since this line was selected for larger growth traits. This aligns with the work of Velleman et al (2003) that showed that genetic selection for faster growing turkeys has resulted in larger muscle fiber diameter of the *pectoralis major*. However, the larger fiber diameter did not change the perimysial space, which is not consistent with the results of studies on turkeys (Velleman et al., 2003). Likewise, studies on *pectoralis superficialis* of broilers (Hoving-Bolink et al., 2000) indicated that larger fiber size reduced the space for circulation in the breast muscle of fast-growing birds. This differences could be due to the different developmental stage when the samples were collected, embryonic day 25 (Velleman et al., 2003) and day 35 posthatch (Hoving-Bolink et al., 2000).

3.5.2 Effects of Pre-hatch Thermal Manipulation on the Turkey Metabolite Levels and Meat Quality

Based on previous studies in broilers, it was hypothesized that early thermal challenge would improve the thermotolerance and produce improved meat quality when birds are exposed to the pre-market thermal challenge. However, in the current study, the interaction analysis between pre-hatch and pre-market thermal treatments showed no significant differences in comparisons of all nine of the tested metabolite indicators and the pH at 24 h postmortem, nor the levels of glucose, G6P, lactate, glycolytic potential, ADP, and pH at 15 min postmortem. The pre-

hatch thermal manipulation treatment showed no significant difference in pH at both 15 min and 24 h postmortem as well as all the other meat quality parameters tested. The pre-hatch treatment used in this study was 3 h/d for five days, and it is possible that this amount of time might not be sufficient to affect the development of thermotolerance of the pre-market bird. However, contrary to the hypothesis, the strategy actually resulted in reduced quality indicators including a significant increase in the drip loss and a significant decrease in the marinade uptake. This could be due to changes in levels of thyroid hormone in response to thermal challenge which negatively influenced the processing quality, including higher drip loss, and lower marinade uptake (Chiang et al., 2008; Piestun et al., 2011).

The metabolite analysis showed that the embryonic thermal manipulation strategy resulted in samples with significantly higher levels of glycogen and that consequently led to significantly higher levels of ATP compared to that of samples at 15 min postmortem from birds exposed only to the pre-market thermal challenge. This could mean that the strategy partially succeeded in building thermotolerance, through reduced energy consumption and increased synthesis of glycogen and ATP whereas birds exposed only to pre-market thermal challenge might increase rate of panting which requires energy and utilizes glycogen and ATP. As a result of the high levels of ATP at 15 min postmortem, there was less total energy demand which would require synthesis of ATP by using two ADP to produce ATP and AMP. Thus, there was significantly lower IMP and AMP at 15 min postmortem from birds exposed to both treatments when compared to samples from birds only exposed to the pre-market thermal challenge.

3.5.3 Effect of Pre-hatch and Pre-market Treatments and Genetic Line Differences on the Breast Muscle of Turkeys.

Thermal manipulation of turkey embryos had effects only on the samples collected at 15 min postmortem, which showed significantly lower levels of IMP and AMP, whereas levels of ADP and ATP were significantly higher compared to control. The higher levels of ATP were not a result of glycogen abundance nor glucose but could be explained by faster metabolic flux which resulted in accumulation of ATP, sparing the use of ADP, and leading to lower IMP and AMP levels at 15 min in samples exposed to the pre-hatch treatment. Moreover, thermal manipulation of the embryos resulted in reduced meat quality as indicated by significantly higher levels of drip loss and cook loss, and significantly lower marinade uptake. These findings are contrary to those of Loyau et al. (2013) who showed that the pre-hatch treatment (incubation condition) of broilers had no major effect on the breast meat quality indicators.

In contrast to pre-hatch treatment, pre-market thermal challenge resulted in reduced drip loss and increased lightness compared to control samples. Our findings of turkey breast lightness (L*) agree with the findings of McKee et al. (1997) who showed that heat-stressed turkeys exhibited a significantly higher L* values than the controls. However, contrary to the same study their results showed a significant increase of drip loss in the samples of heat-stressed turkeys. This difference could be attributed to the fact that the turkeys in our study were exposed to cold temperatures during transport from the Poultry Teaching and Research Center at MSU where they were housed to the MSU Meat Laboratory where they were kept in lairage on the truck and were exposed to ambient outdoor temperatures while awaiting slaughter.

Samples from the birds subjected to pre-market thermal challenge showed significantly higher glycogen levels at 15 min postmortem compared with normal, but glucose levels were not

significantly different. This could be due to the early postmortem time point at which measurements were taken when glycogen was being hydrolyzed to glucose. Consistent with the lack of differences in glucose at this time point, levels of lactate and pH were also not significantly different than levels in normal samples. However, even with the high glycogen levels, the ATP was significantly lower compared to normal.

Samples exposed to pre-market treatment and collected at 24 h postmortem also showed glycogen levels that were significantly higher and consequently resulted in higher levels of the glucose. Nevertheless, levels of lactate and pH were not significantly different from levels in control samples. This was elucidated by El Rammouz et al. (2004a) who found that the level of glycogen in turkey meat did not fully explain the ultimate pH of the meat and suggested that poultry postmortem metabolism is influenced by multiple factors in addition glycogen levels. Moreover, ATP continued to be significantly lower compared to control sample. This could be due to reduced enzymatic activity in glycolysis that led to decline in the production of pyruvate and subsequent reduction in both ATP and lactate with the excess glycogen. Thus, pyruvate measurement could offer a more complete picture of the metabolic profile that would explain the persistence no differences in ATP levels in both cases of existence and absence of significant difference of glucose levels.

In comparing the genetically selected F-line with the unselected RBC2 line, there was significantly heavier weight of the live bird, the carcass, and the pectoralis major muscle. However, F-line samples showed reduced protein functionality, significantly higher percentage of cook loss and significantly lower marinade uptake compared to RBC2 samples. Moreover, these samples were significantly lighter in color (L*) with significantly lower pH levels. According to Updike (2005), the fast-growing birds tend to have breast muscle with lower water holding capacity which

is associated with reduced protein functionality and the meat quality. Moreover, Lu et al. (2007) found that fast-growing broilers showed more drip loss and paler meat in compared with the slow-growing broilers when exposed to the chronic thermal challenge.

3.6 CONCLUSIONS

The goal of this work was to determine whether embryonic thermal manipulation could be used as a strategy to improve thermotolerance of turkeys. The histological analysis showed that there was no effect of the proposed strategy, and that the fiber diameter and the perimysial space results were not correlated. At 15 min postmortem, the proposed strategy resulted in significant higher levels of glycogen and ATP and lower levels of IMP and AMP compared to control. Nonetheless, these levels were not significantly different at the 24 h postmortem time point. Moreover, the proposed strategy did not result in significant differences for any of the eight metabolites level nor the glycolytic potential at 24 h postmortem compared to the controls . The proposed strategy resulted in increased drip loss and reduced marinade uptake while the other meat quality characteristics were not significantly different compared to control samples. These outcomes could be due the short time (3 h/d) of the embryonic thermal manipulation. Thus, future studies will be conducted with thermal manipulation for 12 h/d and then compared the changes in the meat characteristics after exposing turkeys to pre-market thermal challenges. Moreover, it will be interesting to perform the study on samples taken from 3 and/or 6 h at time points in addition to the 15 min and 24 h postmortem points.

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

In this project, there were two studies focused on the effects of the thermal challenge on the quality of turkey meat. The first study (*chapter 2*) was focused on investigating the underlying mechanism of PSE development in turkey meat. The previous observation that PDK4 levels were reduced in PSE samples compared to normal meat samples (Malila et al., 2013, 2014) formed the basis for the hypothesis that phosphorylation of PDH would be reduced in PSE samples. However, there were no significant differences of PDH phosphorylation levels between PSE and normal samples. Moreover, the PDH levels in PSE meat samples were significantly lower than in normal samples. However, in the first part of this study, the focus was only on the relation between PDK4 and PDH. To complete this picture, it is recommended to measure the levels of other metabolites including pyruvate and acetyl-CoA which will provide a more detailed understanding of the response of PDH to thermal challenge in turkeys. Also, it is recommended that the levels and activities of PDP isoforms and the three other PDK isoforms (PDK1-3) be analyzed, in addition to measuring the PDH activity. This would provide greater insight into the metabolic flux of pyruvate through the PDH getaway to the TCA cycle as well as providing a deeper understanding of the metabolic differences between the different lines and their responses to the different thermal challenges. Interestingly, we found that PDH levels were significantly higher in the RBC2 line compared to the samples from commercial birds (genetically selected). This might raise a question regarding the role of the PDH in the relation between the different genetic lines and the rate of the postmortem metabolism.

The results of metabolite analysis in the second part of the first study (*chapter 2*) comparing normal and PSE meats demonstrated that a significant decrease in PDK4 levels in PSE samples did not result in a significant difference of the glycogen, lactate, glycolytic potential and ATP

levels between PSE and normal samples. Moreover, the significantly higher levels of glucose and G6P in PSE samples did not result in any differences in the levels of IMP, AMP, ADP, and ATP compared to the levels in normal samples. This was not expected since the PSE is characterized by lower pH and faster glycolytic rate. However, it should be noted that the samples used for this study were taken at 5 min postmortem which may not be enough time to show significant differences in the postmortem metabolism between the PSE and normal samples. Further research in fresh samples is required to find if the new results are aligning with presented results showing the differences PSE and control samples. Also, it is recommended to get samples from two postmortem time points such as 15 min and 3 h. This will provide more details about the effect of the thermal challenge on the rate of the glycolysis and how fast the metabolism shifts from aerobic to anaerobic.

The second study (*chapter 3*) focused on the potential use of embryonic thermal manipulation as a strategy to improve thermotolerance of turkeys. Based on previous studies in broilers, a brief, mild thermal manipulation of late-stage embryos resulted in improved resistance to the effects of thermal stress. Accordingly, beginning at embryonic day 21, turkey embryos were exposed to 3 hours per day for 5 days of a 1.5°C increase of temperature. Birds were then exposed to pre-market thermal challenge, and the breast muscle meat quality characteristics were evaluated to determine the success of this strategy. Firstly, the histological analysis showed that both the fiber diameter and the perimysial space were not affected by the proposed strategy for improving the thermotolerance. In addition, the results showed no correlation between the fiber diameter and the perimysial space. Samples exposed to pre-market heat stress had smaller perimysial space, but there was no difference in the fiber diameter. The F-line turkeys had larger fiber diameter, but there was no difference in the perimysial space. Secondly, the metabolite analysis showed no

significant difference between the samples exposed to pre-hatch thermal treatment compared to control when both were exposed to the pre-market thermal treatment at 24 h postmortem. At 15 min postmortem, the levels of glycogen and ATP were significantly higher while the levels of IMP and AMP were significantly lower in the samples exposed to embryonic thermal manipulation compared to control when both exposed to the pre-market thermal challenge. Lastly, the thermotolerance strategy showed lower meat quality by having significantly higher drip loss and lower marinade uptake. This could be attributed to the changes in the levels of thyroid hormone in response to the embryonic thermal exposure which might led to the negative processing quality of the meat. The rest of the measured meat quality characteristics of samples from birds exposed to early thermal challenge were not significantly different compared to samples from control when both exposed to the pre-market thermal challenge. These outcomes could be due the short time (3 h/d) of the embryonic thermal manipulation. A previous study in our laboratory (unpublished) compared three levels of embryonic thermal manipulation (3, 6, and 12 h/d), without pre-market exposure, showed that the use of 3 h/d produced slightly but not significantly better results than 12 h/d. Given the results of this study, it is recommended to expose the turkey embryos to thermal manipulation for 12 h/d and then compare the changes in the meat characteristics after exposing turkeys to pre-market thermal challenges. Moreover, it will be interesting to perform the study on samples taken from 3 and/or 6 h beside the 15 min and 24 h postmortem. This will cover more time points showing more details about the rate of postmortem metabolism and the changes in the metabolite levels between the time points.

The second study (*chapter 3*) was faced with infrastructure and workforce limitations in MSU. The first limitation was the capacity of poultry farm which cannot divide birds and hold them into two different thermal conditions at the same time, based on the number of birds used in

this study. Thus, half of the birds (control) had to be sent to MSU Meat Laboratory to be slaughtered while the other half were held at the farm for three days prior to the pre-market thermal challenge for 3 days before slaughter. This means that the second group was 6 days older than the first group (control), and the pre-market thermal challenge did not take place with a contemporaneous control. Other challenges included the transportation, waiting time, and weather temperature. The birds were under stress during loading, transportation, waiting and unloading. The loading started at 5 am, and the temperature was approximately -6°C (21°F). The birds were held outside the MSU Meat Laboratory to be unloaded gradually based on the speed of the slaughtering process. The last birds were removed from the truck at approximately 10 am when the temperature was approximately 2°C (35.6°F). This could add another mitigating factor affecting the metabolism of the birds as well as the possibility of reducing the effect of pre-market thermal challenge.

APPENDIX

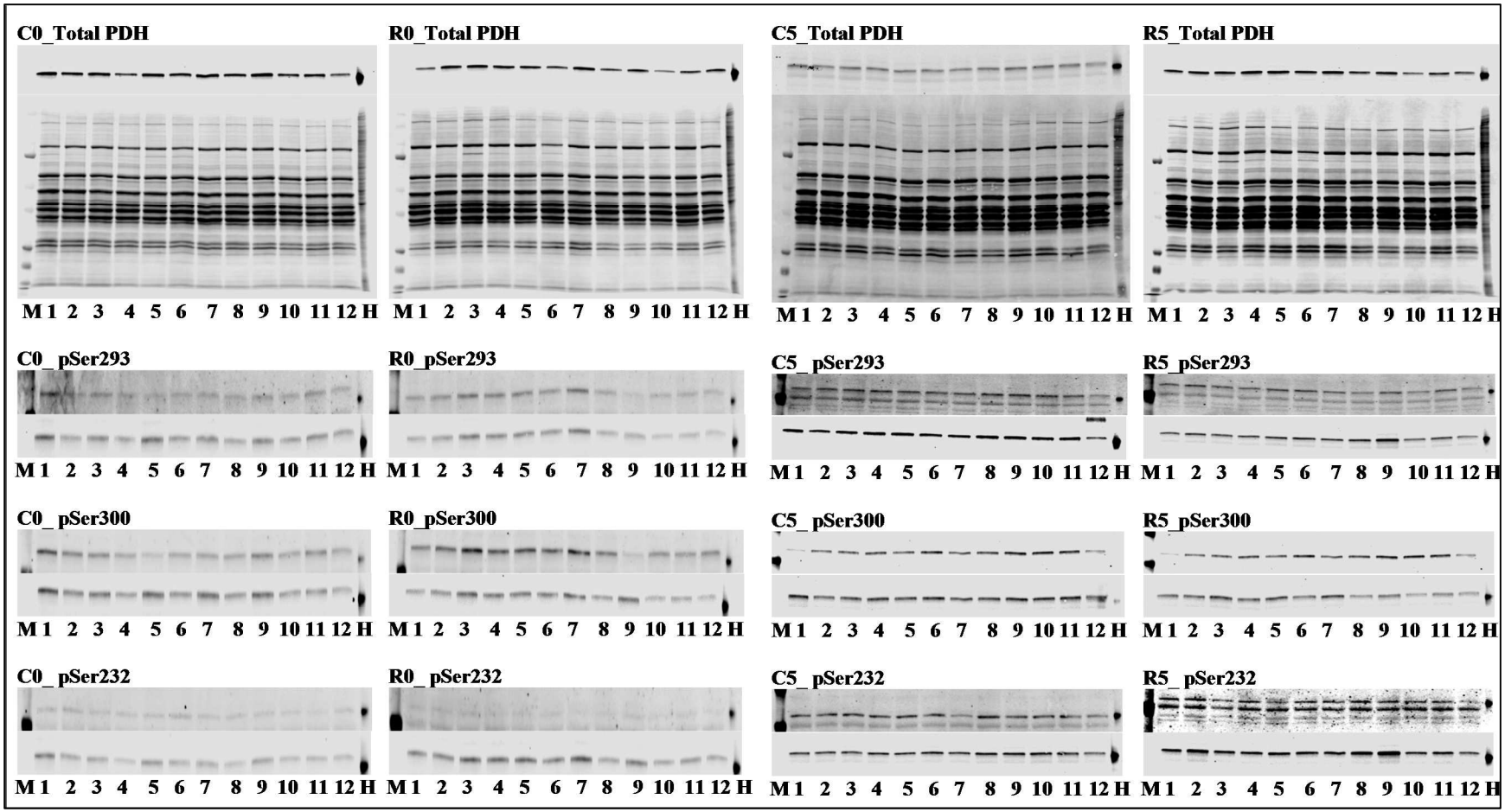

Figure A.1 PDH levels and phosphorylation levels of the sites.

Western blots show PDH levels or PDH phosphorylation abundance in normal (n = 6) and PSE (n = 6) turkey meat. Analysis of an individual bird presented in each lane. Total protein stain or total PDH was used as an endogenous control. Starting from the left, odd number samples were for normal samples while evens for PSE samples. The upper picture in each group is used to measure the target while the lower is for normalization. The samples from 5 days treatment are not included in this figure. C: Commercial turkeys; R: Random-bred line 2; 0: 0 days of thermal challenge; 5: 5 days of thermal challenge; M: Molecular weight marker; H: HEK293 cell lysate.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2001. Principles of Meat Science. 4th ed. Kendall Hunt Pub Co, Dubuque, IA.

Aberle, E. D., and T. S. Stewart. 1983. Growth of fiber types and apparent fiber number in skeletal muscle of broiler-and layer-type chickens. Growth 47:135–144.

Ain Baziz, H., P. A. Geraert, J. C. Padilha, and S. Guillaumin. 1996. Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. Poult. Sci. 75:505–513.

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.

AMSA. 2001. Meat evaluation handbook. 1st ed. American Meat Science Association, Savoy, IL.

Azad, M. A. K., M. Kikusato, T. Maekawa, H. Shirakawa, and M. Toyomizu. 2009. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. Comp. Biochem. Physiol. Part A 155:401–406.

Bandman, E., R. Matsuda, and R. C. Strohman. 1982. Developmental appearance of myosin heavy and light chain isoforms in vivo and in vitro in chicken skeletal muscle. Dev. Biol. 93:508–518.

Bangsbo, J., P. D. Gollnick, T. E. Graham, and B. Saltin. 1991. Substrates for muscle glycogen synthesis in recovery from intense exercise in man. J. Physiol. 434:423–440.

Barbut, S. 1993. Colour 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. British Poultry Science Problem of pale soft exudative meat in broiler chickens Problem of pale soft exudative meat in broiler chickens. Br. Poult. Sci. 38:355–358.

Barbut, S., A. A. Sosnicki, S. M. Lonergan, T. Knapp, D. C. Ciobanu, L. J. Gatcliffe, E. Huff-Lonergan, and E. W. Wilson. 2008. Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. Meat Sci. 79:46–63.

Barnes, N. E., K. M. Mendoza, G. M. Strasburg, S. G. Velleman, and K. M. Reed. 2019. Thermal challenge alters the transcriptional profile of the breast muscle in Turkey poults. Poult. Sci. 98:74–91.

Baumgard, L. H., and R. P. Rhoads. 2013. Effects of heat stress on postabsorptive metabolism and energetics. Annu. Rev. Anim. Biosci. 1:311–337.

Bendall, J. R. 1973. Postmortem changes in Muscle.Pages 244–306 in The Structure and Function of Muscle. Bourne, G.H., ed. 2nd ed. Academic Press, New York.

Bendall, J. R., and J. Wismer□Pedersen. 1962. Some Properties of the Fibrillar Proteins of Normal and Watery Pork Muscle. J. Food Sci. 27:144–159.

Bergmeyer, H. U. 1984. Methods of enzymatic analysis (HU Bergmeyer, J Bergmeyer, and M Grabl, Eds.). Third Ed. Verlag Chemie, Weinheim.

Bernocchi, P., C. Ceconi, A. Cargnoni, P. Pedersini, S. Curello, and R. Ferrari. 1994. Extraction and assay of creatine phosphate, purine, and pyridine nucleotides in cardiac tissue by reversed-phase high-performance liquid chromatography. Anal. Biochem. 222:374–379.

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.

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.

Bowker-Kinley, M. M., W. I. Davis, P. Wu, R. A. Harris, and K. M. Popov. 1998. Evidence for existence of tissue-specific regulation of the mammalian pyruvate dehydrogenase complex. Biochem. J. 329 (Pt 1:191–6.

Bowker, B. C., J. S. Eastridge, and M. B. Solomon. 2014. Measurement of muscle exudate protein composition as an indicator of beef tenderness. J. Food Sci. 79:C1292–C1297.

Braden, K. W. 2013. Converting Muscle to Meat: The Physiology of Rigor.Pages 79–98 in The Science of Meat Quality. Kerth, C.R., ed. 1'st. John Wiley & Sons, Inc., Oxford, UK.

Briskey, E. J., R. N. Sayre, and R. G. Cassens. 1962. Development and Application of an Apparatus for Continuous Measurement of Muscle Extensibility and Elasticity Before and During Rigor Mortis. J. Food Sci. 27:560–566.

Briskey, E. J., and J. Wismer-Pedersen. 1961. Biochemistry of Pork Muscle Structure. 1. Rate of Anaerobic Glycolysis and Temperature Change versus the Apparent Structure of Muscle Tissue. J. Food Sci. 26:297–305.

Brooke, M. H., and K. K. Kaiser. 1970. Muscle fiber types: How many and what kind? Arch. Neurol. 23:369–379.

Bunn, H. F., and P. J. Higgins. 1981. Reaction of monosaccharides with proteins: possible evolutionary significance. Science (80-.). 213:222–224.

Calder, W. A., and K. Schmidt-Nielsen. 1966. Evaporative cooling and respiratory alkalosis in the

pigeon. Proc. Natl. Acad. Sci. U. S. A. 55:750–756.

Çelen, M. F., B. Söğüt, Ö. Zorba, H. Demirulus, A. Tekeli, M. F. Çelen, B. Söğüt, Ö. Zorba, H. Demirulus, and A. Tekeli. 2016. Comparison of normal and PSE turkey breast meat for chemical composition, pH, color, myoglobin, and drip loss. Rev. Bras. Zootec. 45:441–444.

Chan, J. T. Y., D. A. Omana, and M. Betti. 2011. Application of high pressure processing to improve the functional properties of pale, soft, and exudative (PSE)-like turkey meat. Innov. Food Sci. Emerg. Technol. 12:216–225.

Chen, G., L. Wang, S. Liu, C. Chuang, and T. E. Roche. 1996. Activated function of the pyruvate dehydrogenase phosphatase through Ca2+-facilitated binding to the inner lipoyl domain of the dihydrolipoyl acetyltransferase. J. Biol. Chem. 271:28064–28070.

Chiang, W., C. P. Allison, J. E. Linz, and G. M. Strasburg. 2004. Identification of two αRYR alleles and characterization of αRYR transcript variants in turkey skeletal muscle. Gene 330:177–184.

Chiang, W., A. Booren, and G. M. Strasburg. 2008. The effect of heat stress on thyroid hormone response and meat quality in turkeys of two genetic lines. Meat Sci. 80:615–622.

Choe, J. H., Y. M. Choi, S. H. Lee, H. G. Shin, Y. C. Ryu, K. C. Hong, and B. C. Kim. 2008. The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. Meat Sci. 80:355–362.

Christensen, L. B. 2003. Drip loss sampling in porcine m. longissimus dorsi. Meat Sci. 63:469–477.

Clark, D. L., and S. G. Velleman. 2017. Spatial influence on breast muscle morphological structure, myofiber size, and gene expression associated with the wooden breast myopathy in broilers. Poult. Sci. 95:2930–2945.

Clark, D. L., K. G. Walter, and S. G. Velleman. 2017. Incubation temperature and time of hatch impact broiler muscle growth and morphology. Poult. Sci. 96:4085–4095.

Connaughton, S., F. Chowdhury, R. R. Attia, S. Song, Y. Zhang, M. B. Elam, G. A. Cook, and E. A. Park. 2010. Regulation of pyruvate dehydrogenase kinase isoform 4 (PDK4) gene expression by glucocorticoids and insulin. Mol. Cell. Endocrinol. 315:159–167.

Dalrymple, R. H., and R. Hamm. 1975. Postmortem glycolysis in prerigor ground bovine and rabbit muscle. J. Food Sci. 40:850–853.

Daniel, C. R., A. J. Cross, C. Koebnick, and R. Sinha. 2011. Trends in meat consumption in the USA. Public Health Nutr. 14:575–83.

Dransfield, E., and A. A. Sosnicki. 1999. Relationship between muscle growth and poultry meat quality. Poult. Sci. 78:743–746.

England, E. M., S. K. Matarneh, T. L. Scheffler, C. Wachet, and D. E. Gerrard. 2015. Altered AMP deaminase activity may extend postmortem glycolysis. Meat Sci. 102:8–14.

England, E. M., T. L. Scheffler, S. C. Kasten, S. K. Matarneh, and D. E. Gerrard. 2013. Exploring the unknowns involved in the transformation of muscle to meat. Meat Sci. 95:837–843.

Fernandez, X., and E. Tornberg. 1991. A review of the causes of variation in muscle glycogen content and ultimate ph in pigs. J. Muscle Foods 2:209–235.

Fletcher, D. L. 1999. Broiler breast meat color variation, pH, and texture. Poult. Sci. 78:1323–1327.

Foegeding, E. A., T. C. Lanier, and H. O. Hultin. 1996. Characteristics of edible muscle tissues.Pages 879–942 in Food Chemistry. Fennema, O.R., ed. 3rd ed. Marcel Dekker, Inc., New York.

Fujii, J., K. Otsu, F. Zorzato, S. de Leon, V. K. Khanna, J. E. Weiler, P. J. O'Brien, and D. H. MacLennan. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253:448–451.

Furuichi, Y., N. Goto-Inoue, Y. Manabe, M. Setou, K. Masuda, and N. L. Fujii. 2014. Imaging mass spectrometry reveals fiber-specific distribution of acetylcarnitine and contraction-induced carnitine dynamics in rat skeletal muscles. Biochim. Biophys. Acta - Bioenerg. 1837:1699–1706.

Garcia, R., L. de. Freitas, A. W. Schwingel, R. M. Farias, F. R. Caldara, A. M. a. Gabriel, J. D. Graciano, C. D. Komiyama, and I. C. L. Almeida Paz. 2010. Incidence and physical properties of PSE chicken meat in a mommercial processing plant. Brazilian J. Poult. Sci. 12:233–237.

Geraert, P. A., J. C. F. Padilha, and S. Guillaumin. 1996. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens : biological and endocrinological variables. Br. J. Nutr. 75:205–216.

Greaser, M. L. 2001. Postmortem muscle chemistry.Pages 21–37 in Meat Science and Applications. Hui, YH, Nip, WK, Rogers, RW, Young, O., ed. Marcel Dekker, Inc., New York.

Guernec, A., C. Berri, B. Chevalier, N. Wacrenier-Cere, E. Le Bihan-Duval, and M. J. Duclos. 2003. Muscle development, insulin-like growth factor-I and myostatin mRNA levels in chickens selected for increased breast muscle yield. Growth Horm. IGF Res. 13:8–18.

Halevy, O., A. Krispin, Y. Leshem, J. P. Mcmurtry, and S. Yahav. 2001. Early-age heat exposure affects skeletal muscle satellite cell proliferation and differentiation in chicks. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 281:302–309.

Hamm, R. 1961. Biochemistry Of Meat Hydration. Adv. Food Res. 10:355–463.

Hamm, R. 1977. Postmortem breakdown of ATP and glycogen in ground muscle: A review. Meat

Sci. 1:15–39.

Hammelman, J. E., B. C. Bowker, A. L. Grant, J. C. Forrest, A. P. Schinckel, and D. E. Gerrard. 2003. Early postmortem electrical stimulation simulates PSE pork development. Meat Sci. 63:69–77.

Harris, R. A., M. M. Bowker-Kinley, B. Huang, and P. Wu. 2002. Regulation of the activity of the pyruvate dehydrogenase complex. Adv. Enzyme Regul. 42:249–259.

Harris, R. A., B. Huang, and P. Wu. 2001. Control of pyruvate dehydrogenase kinase gene expression. Adv. Enzyme Regul. 41:269–288.

Holness, M. J., A. Kraus, R. A. Harris, and M. C. Sugden. 2000. Targeted upregulation of pyruvate dehydrogenase kinase (PDK)-4 in slow-twitch skeletal muscle underlies the stable modification of the regulatory characteristics of PDK induced by high-fat feeding. Diabetes 49:775–781.

Honikel, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49:447–457.

Horiuchi, M., K. Kobayashi, M. Masuda, H. Terazono, and T. Saheki. 1999. Pyruvate dehydrogenase kinase 4 mRNA is increased in the hypertrophied ventricles of carnitine-deficient juvenile visceral steatosis (JVS) mice. BioFactors 10:301–309.

Hoving-Bolink, A. H., R. W. Kranen, R. E. Klont, C. L. M. Gerritsen, and K. H. De Greef. 2000. Fibre area and capillary supply in broiler breast muscle in relation to productivity and ascites. Meat Sci. 56:397–402.

Huang, B., P. Wu, M. M. Bowker-Kinley, and R. A. Harris. 2002. Regulation of Pyruvate Dehydrogenase Kinase Expression by Peroxisome Proliferator–Activated Receptor-α Ligands, Glucocorticoids, and Insulin. Diabetes 51:276–283.

Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71:194–204.

Jarrold, B. B., W. L. Bacon, and S. G. Velleman. 1999. Expression and localization of the proteoglycan decorin during the progression of cholesterol induced atherosclerosis in Japanese quail: implications for interaction with collagen type I and lipoproteins. Atherosclerosis 146:299–308.

Jeoung, N. H. 2015. Pyruvate dehydrogenase kinases: Therapeutic targets for diabetes and cancers. Diabetes Metab. J. 39:188–197.

Kapper, C., C. J. Walukonis, T. L. Scheffler, J. M. Scheffler, C. Don, M. T. Morgan, J. C. Forrest, and D. E. Gerrard. 2014. Moisture absorption early postmortem predicts ultimate drip loss in fresh pork. Meat Sci. 96:971–976.

Karlsson, A. H., R. E. Klont, and X. Fernandez. 1999. Skeletal muscle fibres as factors for pork

quality. Livest. Prod. Sci. 60:255–269.

Kauffman, R. G., W. Sybesma, F. J. M. Smulders, G. Eikelenboom, B. Engel, R. L. J. M. Van Laack, A. H. Hoving-Bolink, P. Sterrenburg, E. V. Nordheim, P. Walstra, and P. G. van der Wal. 1993. The effectiveness of examining early post-mortem musculature to predict ultimate pork quality. Meat Sci. 34:283–300.

Kerth, C. R. 2013. Muscle Metabolism and Contraction.Page 312 in The Science of Meat Quality. Kerth, C.R., ed. 1'st. John Wiley & Sons, Inc., Oxford, UK.

Knowles, J. R. 1980. Enzyme-Catalyzed Phosphoryl Transfer Reactions. Annu. Rev. Biochem. 49:877–919.

Kolobova, E., A. Tuganova, I. Boulatnikov, and K. M. Popov. 2001. Regulation of pyruvate dehydrogenase activity through phosphorylation at multiple sites. Biochem. J. 358:69–77.

Korotchkina, L. G., and M. S. Patel. 2001. Site specificity of four pyruvate dehydrogenase kinase isoenzymes toward the three phosphorylation sites of human pyruvate dehydrogenase. J. Biol. Chem. 276:37223–37229.

Korzeniewski, B. 2006. AMP deamination delays muscle acidification during heavy exercise and hypoxia. J. Biol. Chem. 281:3057–3066.

Kranen, R. W., T. H. Van Kuppevelt, H. A. Goedhart, C. H. Veerkamp, E. Lambooy, and J. H. Veerkamp. 1999. Hemoglobin and myoglobin content in muscles of broiler chickens. Poult. Sci. 78:467–476.

Kwon, H.-S., and R. A. Harris. 2004. Mechanisms responsible for regulation of pyruvate dehydrogenase kinase 4 gene expression. Adv. Enzyme Regul. 44:109–121.

Kwon, H.-S., B. Huang, T. G. Unterman, and R. A. Harris. 2004. Protein Kinase B-α Inhibits Human Pyruvate Dehydrogenase Kinase-4 Gene Induction by Dexamethasone Through Inactivation of FOXO Transcription Factors. Diabetes 53:899–910.

Kylä-Puhju, M., M. Ruusunen, R. Kivikari, and E. Puolanne. 2004. The buffering capacity of porcine muscles. Meat Sci. 67:587–593.

Van Laack, R. L. J. M. 2000. Determinants of ultimate pH of meat and poultry.Pages 74–75 in 53 rd Annual Reciprocal Meat Conference.

Van Laack, R. L. J. M., C. H. Liu, M. O. Smith, and H. D. Loveday. 2000. Characteristics of pale, soft, exudative broiler breast meat. Poult. Sci. 79:1057–1061.

Van Laack, R. L. J. M., J. Yang, and E. Spencer. 2001. Determinants of ultimate pH of pork.

Larzul, C., L. Lefaucheur, P. Ecolan, J. Gogue, A. Talmant, P. Sellier, P. Le Roy, 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.

Lawrie, R. A. 1991. Meat science. 5th ed. Pergamum Press, oxford. UK, New York, NY.

Li, K., Y. Y. Zhao, Z. L. Kang, P. Wang, M. Y. Han, X. L. Xu, and G. H. Zhou. 2015. Reduced functionality of PSE-like chicken breast meat batter resulting from alterations in protein conformation. Poult. Sci. 94:111–122.

Listrat, A., B. Lebret, I. Louveau, T. Astruc, M. Bonnet, L. Lefaucheur, B. Picard, and J. Bugeon. 2016. How muscle structure and composition influence meat and flesh quality. Sci. World J. 2016:1–14.

Loyau, T., C. Berri, L. Bedrani, S. Métayer-Coustard, C. Praud, M. J. Duclos, S. Tesseraud, N. Rideau, N. Everaert, S. Yahav, S. Mignon-Grasteau, and A. Collin. 2013. Thermal manipulation of the embryo modifies the physiology and body composition of broiler chickens reared in floor pens without affecting breast meat processing quality 1. J. Anim. Sci 91:3674–3685.

Lu, Q., J. Wen, and H. Zhang. 2007. Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. Poult. Sci. 86:1059–1064.

Di Luca, A., G. Elia, A. M. Mullen, and R. M. Hamill. 2013. Monitoring post mortem changes in porcine muscle through 2-D DIGE proteome analysis of Longissimus muscle exudate. Proteome Sci. 11:9.

MacIntosh, B. R., P. F. Gardiner, and A. J. McComas. 2006. Muscle architecture and muscle fiber anatomy.Pages 3–21 in Skeletal muscle: form and function. Robertson, L., ed. 2nd ed. Human Kinetics, Champaign, IL.

Malila, Y., K. M. Carr, C. W. Ernst, S. G. Velleman, K. M. Reed, and G. M. Strasburg. 2014. Deep transcriptome sequencing reveals differences in global gene expression between normal and pale, soft, and exudative (PSE) turkey meat. J Anim Sci 92:1250–1260.

Malila, Y., R. J. Tempelman, K. R. B. Sporer, C. W. Ernst, S. G. Velleman, K. M. Reed, and G. M. Strasburg. 2013. Differential gene expression between normal and pale, soft, and exudative turkey meat. Poult. Sci. 92:1621–1633.

Mancini, R. A., and M. C. Hunt. 2005. Current research in meat color. Meat Sci. 71:100–121.

Maribo, H., S. Støier, and P. F. Jørgensen. 1999. Procedure for determination of glycolytic potential in porcine m. longissimus dorsi. Meat Sci. 51:191–193.

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

McKee, S. R., E. M. Hirschler, and A. R. Sams. 1997. Physical and biochemical effects of broiler breast tenderization by aging after pre-rigor deboning. J. Food Sci. 62:959–962.

McKee, S. R., and A. R. Sams. 1997. The effect of seasonal heat stress on rigor development and the incidence of pale, exudative turkey meat. Poult. Sci. 76:1616–1620.

McKee, S. R., and A. R. Sams. 1998. Rigor mortis development at elevated temperatures induces pale exudative turkey meat characteristics. Poult. Sci. 77:169–174.

Metcalfe, R. S., F. Koumanov, J. S. Ruffino, K. A. Stokes, G. D. Holman, D. Thompson, and N. B. J. Vollaard. 2015. Physiological and molecular responses to an acute bout of reduced-exertion high-intensity interval training (REHIT). Eur. J. Appl. Physiol. 115:2321–2334.

Mills, L. J., M. A. Mitchell, and M. Mahon. 1999. Susceptibility to heat stress in fast and slow growing turkey lines. Poult. Sci 78:247.

Mitchell, M. A., and D. A. Sandercock. 2004. Spontaneous and stress induced myopathies in modern meat birds: A cause for quality and welfare concerns.Pages 100–107 in Proc. Aust. Poult. Sci. Symp.

Molette, C., H. Rémignon, and R. Babilé. 2003. Maintaining muscles at a high post-mortem temperature induces PSE-like meat in turkey. Meat Sci. 63:525–532.

Mongin, P. 1968. Role of Acid-Base Balance in the Physiology of Egg Shell Formation. Worlds. Poult. Sci. J. 24:200–230.

Monin, G., A. Mejenes-Quijano, A. Talmant, and P. Sellier. 1987. Influence of breed and muscle metabolic type on muscle glycolytic potential and meat pH in pigs. Meat Sci. 20:149–158.

National Weather Service. 2016. Heat Safety. Available at https://www.weather.gov/ (verified 31 December 2016).

Nelson, D. L., and M. M. Cox. 2008. Lehninger Principles of Biochemistry. 5th ed. Sara Tenney, New York.

Nestor, K. E. 1977. The use of a paired mating system for the maintenance of experimental populations of turkeys. Poult. Sci. 56:60–65.

Nestor, K. E., J. W. Anderson, R. A. Patterson, and S. G. Velleman. 2008. Genetics of growth and reproduction in the turkey. 17. Changes in genetic parameters over forty generations of selection for increased sixteen-week body weight. Poult. Sci. 87:1971–1979.

NPPC. 2000. Pork composition and quality assessment procedures (EP Berg, Ed.). National Pork Producers Council, Des Moines, IA.

Offer, G., P. Knight, R. Jeacocke, R. Almond, T. Cousins, J. Elsey, N. Parsons, A. Sharp, R. Starr, and P. Purslow. 1989. The structural basis of the water-holding, appearance and toughness of meat and meat products. Food Microstruct. 8,:151–170.

Owens, C. M., C. Z. Alvarado, and A. R. Sams. 2009. Research developments in pale, soft, and exudative turkey meat in North America. Poult. Sci. 88:1513–1517.

Owens, C. M., and J. K. Apple. 2010. Pale, Soft, and Exudative Poultry Meat.Pages 515–531 in Handbook of Poultry Science and Technology. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Owens, C. M., E. M. Hirschler, S. R. McKee, R. Martinez-Dawson, and A. R. Sams. 2000a. The characterization and incidence of pale, soft, exudative turkey meat in a commercial plant. Poult. Sci. 79:553–558.

Owens, C. M., S. R. Mckee, N. S. Matthews, and A. R. Sams. 2000b. The development of pale, exudative meat in two genetic lines of turkeys subjected to heat stress and its prediction by halothane screening. Poult. Sci. 79:430–435.

Patel, M. S., and L. G. Korotchkina. 2006. Regulation of the pyruvate dehydrogenase complex. Biochem. Soc. Trans. 34:217.

Patterson, B. A., S. K. Matarneh, K. M. Stufft, E. M. England, T. L. Scheffler, R. H. Preisser, H. Shi, E. C. Stewart, S. Eilert, and D. E. Gerrard. 2016. Pectoralis major muscle of turkey displays divergent function as correlated with meat quality. Poult. Sci.:1–12.

Pearce, K. L., K. Rosenvold, H. J. Andersen, and D. L. Hopkins. 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes - A review. Meat Sci. 89:111–124.

Pearson, A. M. 1971. The science of meat and meat products.Pages 208–229 in The science of meat and meat products. Price, J.F., Schiweigert, B.S., eds. 2nd ed. WH Freeman and Co., San Francisco, CA.

Peter, J. B., R. J. Barnard, V. R. Edgerton, C. A. Gillespie, and K. E. Stempel. 1972. Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. Biochemistry 11:2627–2633.

Peters, S. J., R. A. Harris, P. Wu, T. L. Pehleman, G. J. Heigenhauser, and L. L. Spriet. 2001. Human skeletal muscle PDH kinase activity and isoform expression during a 3-day high-fat/low-carbohydrate diet. Am. J. Physiol. Endocrinol. Metab. 281:E1151–E1158.

Petracci, M., M. Bianchi, and C. Cavani. 2009. The European perspective on pale, soft, exudative conditions in poultry. Poult. Sci. 88:1518–1523.

Petracci, M., D. L. Fletcher, and J. K. Northcutt. 2001. The effect of holding temperature on live shrink, processing yield, and breast meat quality of broiler chickens. Poult. Sci. 80:670–675.

Picard, B., C. Jurie, M. P. Duris, and G. Renand. 2006. Consequences of selection for higher growth rate on muscle fibre development in cattle. Livest. Sci. 102:107–120.

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. J. 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. 94:273–268.

Piestun, Y., M. Harel, M. Barak, S. Yahav, and O. Halevy. 2008. 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.

Pietrzak, M., M. L. Greaser, and A. A. Sosnicki. 1997. Effect of rapid rigor mortis processes on protein functionality in pectoralis major muscle of domestic turkeys. J. Anim. Sci. 75:2106–2116.

Pilegaard, H., and P. Darrell Neufer. 2004. Transcriptional regulation of pyruvate dehydrogenase kinase 4 in skeletal muscle during and after exercise. Proc. Nutr. Soc. 63:221–226.

Pilegaard, H., B. Saltin, and P. D. Neufer. 2003. Effect of short-term fasting and refeeding on transcriptional regulation of metabolic genes in human skeletal muscle. Diabetes 52:657–662.

Przybylski, W., B. Sionek, D. Jaworska, and V. Santé-Lhoutellier. 2016. The application of biosensors for drip loss analysis and glycolytic potential evaluation. Meat Sci. 117:7–11.

El Rammouz, R., R. Babilé, and X. Fernandez. 2004a. Effect of ultimate pH on the physicochemical and biochemical characteristics of turkey breast muscle showing normal rate of postmortem pH fall. Poult. Sci. 83:1750–1757.

El Rammouz, R., C. Berri, E. Le Bihan-Duval, R. Babilé, and X. Fernandez. 2004b. Breed differences in the biochemical determinism of ultimate pH in breast muscles of broiler chickens - A key role of AMP deaminase? Poult. Sci. 83:1445–1451.

Rardin, M. J. 2008. Reversible phosphorylation in mitochondria. Electron. Theses Diss.

Rardin, M. J., S. E. Wiley, R. K. Naviaux, A. N. Murphy, and J. E. Dixon. 2009. Monitoring phosphorylation of the pyruvate dehydrogenase complex. Anal. Biochem. 389:157–64.

Rathgeber, B. M., J. A. Boles, and P. J. Shand. 1999. Rapid postmortem pH decline and delayed chilling reduce quality of turkey breast meat. Poult. Sci. 78:477–484.

Rehfeldt, C., M. Henning, and I. Fiedler. 2008. Consequences of pig domestication for skeletal muscle growth and cellularity. Livest. Sci. 116:30–41.

Remignon, H., M. F. Gardahaut, G. Marche, and F. H. Ricard. 1995. Selection for rapid growth increases the number and the size of muscle fibres without changing their typing in chickens. J. Muscle Res. Cell Motil. 16:95–102.

Remignon, H., L. Lefaucheur, J. C. Blum, and F. H. Ricard. 1994. Effects of divergent selection for body weight on three skeletal muscles characteristics in the chicken. Br. Poult. Sci. 35:65–76.

Rich, P. R. 2003. The molecular machinery of Keilin's respiratory chain. Biochem. Soc. Trans. 31:1095–1105.

Romero-Lankao, P., J. B. Smith, D. J. Davidson, N. S. Diffenbaugh, P. L. Kinney, P. Kirshen, P. Kovacs, and L. Villers Ruiz. 2014. North America.Pages 1439–1498 in Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Barros, V.R., Field, C.B., Dokken, D.J., Mastrandrea, M.D., Mach, K.J., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.. O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.. R., White, L.L., eds. Cambridge University Press, New York, NY, USA.

Ryu, Y. C., and B. C. Kim. 2005. The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. Meat Sci. 71:351–357.

Savenije, B., F. J. G. Schreurs, H. A. Winkelman-Goedhart, M. A. Gerritzen, J. Korf, and E. Lambooij. 2002. Effects of feed deprivation and electrical, gas, and captive needle stunning on early postmortem muscle metabolism and subsequent meat quality. Poult. Sci. 81:561–571.

Schiaffino, S., L. Gorza, S. Sartore, L. Saggin, S. Ausoni, M. Vianello, K. Gundersen, and T. Lømo. 1989. Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. J. Muscle Res. Cell Motil. 10:197–205.

Schiaffino, S., and C. Reggiani. 1996. Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol. Rev. 76:371–423.

Scopes, R. K. 1971. The biochemistry of post mortem glycolysis.Pages 14–20 in Proceedings of the 17th European meeting of Meat Research Workers. Bristol, UK.

Skipnes, D., M. L. Østby, and M. E. Hendrickx. 2007. A method for characterising cook loss and water holding capacity in heat treated cod (Gadus morhua) muscle. J. Food Eng. 80:1078–1085.

Solomon, M. B., R. L. J. M. Van Laack, and J. S. Eastridge. 1998. Biophysical basis of pale, soft, exudative (pse) pork and poultry muscle: a review. J. Muscle Foods 9:1–11.

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

Sporer, K. R. B., H.-R. Zhou, J. E. Linz, A. M. Booren, and G. M. Strasburg. 2012. Differential expression of calcium-regulating genes in heat-stressed turkey breast muscle is associated with meat quality. Poult. Sci. 91:1418–24.

Sugden, M. C., and M. J. Holness. 2003. Recent advances in mechanisms regulating glucose oxidation at the level of the pyruvate dehydrogenase complex by PDKs. Am. J. Physiol. Endocrinol. Metab. 284:E855–E862.

Sugden, M. C., H. S. Lall, R. A. Harris, and M. J. Holness. 2000. Selective modification of the pyruvate dehydrogenase kinase isoform profile in skeletal muscle in hyperthyroidism: Implications for the regulatory impact of glucose on fatty acid oxidation. J. Endocrinol. 167:339–345 Available at http://www.ncbi.nlm.nih.gov/pubmed/11054649 (verified 6 December 2016).

Swatland, H. J. 1993. Pork Quality: Genetic and Metabolic Factors.Pages 1–21 in CAB International, Wallingford, UK. Puolanne, E., Demeyer, D.I., Demeyer, D.I., Puolanne, E., Ruusunen, M., Ellis, S., eds. C.A.B. International.

Swatland, H. J. 2008. How pH causes paleness or darkness in chicken breast meat. Meat Sci. 80:396–400.

Tornroth-Horsefield, S., and R. Neutze. 2008. Opening and closing the metabolite gate. Proc. Natl. Acad. Sci. 105:19565–19566.

Updike, M. S., H. N. Zerby, J. C. Sawdy, M. S. Lilburn, G. Kaletunc, and M. P. Wick. 2005. Turkey breast meat functionality differences among turkeys selected for body weight and/or breast yield. Meat Sci. 71:706–12.

USDA-NASS. 2018. Data and statistics/quick stats. Available at https://quickstats.nass.usda.gov/results/B101DF26-6FF6-3D8D-8A53-4F52F6EC1EE5?pivot=short_desc (verified 5 January 2017).

Vanderstoep, J., and J. F. Richards. 1974. Post-Mortem Glycolytic and Physical Changes in Turkey Breast Muscle. Can. Inst. Food Sci. Technol. J. 7:120–124.

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 D. L. Clark. 2015. Histopathologic and myogenic gene expression changes associated with wooden breast in broiler breast muscles. Avian Dis. 59:410–418.

Wang, L. J., T. M. Byrem, J. Zarosley, A. M. Booren, and G. M. Strasburg. 1999. Skeletal muscle calcium channel ryanodine binding activity in genetically unimproved and commercial turkey populations. Poult. Sci. 78:792–797.

Wasserman, D. H. 2009. Four grams of glucose. Am. J. Physiol. Endocrinol. Metab. 296:E11-21.

Williams, J. H., S. E. Vidt, and J. Rinehart. 2008. Measurement of sarcoplasmic reticulum Ca2+ ATPase activity using high-performance liquid chromatography. Anal. Biochem. 372:135–139.

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

Wu, P., K. Inskeep, M. M. Bowker-Kinley, K. M. Popov, and R. A. Harris. 1999. Mechanism

responsible for inactivation of skeletal muscle pyruvate dehydrogenase complex in starvation and diabetes. Diabetes 48:1593–1599.

Xiong, Y. L., and P. B. Kenney. 1999. Functionality of proteins in meat products.Pages 67–69 in 52nd Annual Reciprocal Meat Conference.

Xiong, Y. L., and D. R. Kupski. 1999. Time-dependent marinade absorption and retention, cooking yield, and palatability of chicken filets marinated in various phosphate solutions. Poult. Sci. 78:1053–1059.

Yahav, S., and S. Hurwitz. 1996. Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. Poult. Sci. 75:402–6.

Yahav, S., and J. P. McMurtry. 2001. Thermotolerance acquisition in broiler chickens by temperature conditioning early in life. The effect of timing and ambient temperature. Poult. Sci. 80:1662–1666.

Yahav, S., D. Shinder, J. Tanny, and S. Cohen. 2005. Sensible heat loss: the broiler's paradox. Proc. Nutr. Soc. 61:419–434.

Young, O. A., S. M. Humphrey, and D. J. C. Wild. 1988. Effects of sugars on post-mortem glycolysis in bovine muscle mince. Meat Sci. 23:211–225.

Zaboli, G., X. Huang, X. Feng, and D. U. Ahn. 2018. How can heat stress affect chicken meat quality ? – a review. Poult. Sci.:1–6.

Zelechowska, E., W. Przybylski, D. Jaworska, and V. Santé-Lhoutellier. 2012. Technological and sensory pork quality in relation to muscle and drip loss protein profiles. Eur. Food Res. Technol. 234:883–894.

Zhang, S., M. W. Hulver, R. P. McMillan, M. A. Cline, and E. R. Gilbert. 2014. The pivotal role of pyruvate dehydrogenase kinases in metabolic flexibility. Nutr. Metab. (Lond). 11:10.

Zhang, Y., K. Ma, P. Sadana, F. Chowdhury, S. Gaillard, F. Wang, D. P. McDonnell, T. G. Unterman, M. B. Elam, and E. A. Park. 2006. Estrogen-related receptors stimulate pyruvate dehydrogenase kinase isoform 4 gene expression. J. Biol. Chem. 281:39897–39906.

Zhu, X., M. Ruusunen, M. Gusella, M. Ylä-Ajos, X. Xu, G. Zhou, and E. Puolanne. 2013. High early post-mortem temperature induces activation of AMP-activated protein kinase and development of pale, soft and exudative characteristics in turkey muscles. Meat Sci. 93:600–606.

Zhu, X., M. Ruusunen, M. Gusella, G. Zhou, and E. Puolanne. 2011. High post-mortem temperature combined with rapid glycolysis induces phosphorylase denaturation and produces pale and exudative characteristics in broiler Pectoralis major muscles. Meat Sci. 89:181–188.

Zhuang, H., and E. M. Savage. 2010. Comparisons of sensory descriptive flavor and texture

profiles of cooked broiler breast fillets categorized by raw meat color lightness values. Poult. Sci. 89:1049–1055.

Zhuang, H., and E. M. Savage. 2013. Comparison of cook loss, shear force, and sensory descriptive profiles of boneless skinless white meat cooked from a frozen or thawed state. Poult. Sci. 92:3003–3009.