PHYSICO-BIOCHEMICAL CHARACTERIZATION OF BREAST MUSCLE IN FAST AND SLOW GROWING CHICKENS

by

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(Under the Direction of Louise Wicker)

ABSTRACT

With increasing consumption of poultry products, it is important for consumers, retailers, and poultry industry to concern on consistent high quality. Growth rate and water holding capacity (WHC) are important meat quality parameters. Selection for growth has affected meat attributes. Pale, soft, and exudative meat has poor WHC which results in an economic loss of processors, as well as a decrease in consumer acceptance. Our goal was to find the relationship in changes of muscles from two chicken populations with varying growth rate and WHC through online monitoring method and proteomic analysis. Two chicken populations were established; a slow growing sub-population (SG) with an average growth rate of 229 g/wk and a fast growing sub-population (FG) with an average growth rate of 319 g/wk. The initial and final pH was higher in the FG compared to the SG population. The SG had significantly higher L*, b*, drip loss (DL), and cook yield when compared to the FG. From spectrum of raw breast chicken muscles by Raman spectroscopy, there were 8 wavenumbers (P<0.05) differed in growth population: 538, 582, 682, 691, 1367, 1625, 1704, 1743 cm^{-1} and 5 wavenumbers (P<0.05) in WHC population: 1270, 1277, 1354, 1653, 1737 cm^{-1}. For proteomic analysis, protein markers that are associated with growth and WHC from chicken muscle were investigated. Water soluble

protein (WSP) and non-WSP extracts were prepared from breast muscle of chickens that differed in growth rate or WHC. A total of 22 selected protein spots were excised from two-dimensional gel electrophoresis and analyzed by in-gel tryptic digestion and MALDI-TOF mass spectrometry. The mass spectra of 20 protein spots significantly matched to the on-line database (protein score > 83; $p<0.05$). In non-WSP extract, there were unique proteins that were present only in FG population: gi|118099530; gi|20664362; gi|71895043; gi|114794125; gi|297343122; gi|296214263. Overall conclusions were: FG showed better technological yields than SG chickens; color parameters and Raman spectroscopy could be used to segregate muscle for further processing and online monitoring; information from proteomics could be used to identify functional candidate genes for meat quality traits in chickens.

INDEX WORDS: Poultry, chicken, protein, growth, water holding capacity, meat quality attribute, Raman spectroscopic, 2DE, muscle proteome, proteomics, MALDI

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DEDICATION

I have been through so much to get to this point. I have got some scars from the battle; however, I am wiser and stronger for it. The enlightenment, discipline, stability and persistent I have gained are invaluable resource. I have noticed that I was experimenting with different things in my life. All of these experiences fed my spirit and offered me knowledge for the future. This dissertation is dedicated to my dear family. The great support from my parents, my sisters, my aunts, my teachers and my friends gave me the strength and courage to finish this work.

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CHAPTER 1

INTRODUCTION

The production of broiler poultry has substantially increased. The US per capita consumption of broiler meat has risen from 36 kg in 2000 to 42 kg in 2007 (USDA, 2010). The broiler production in the state of Georgia has also risen from 1.38 billion broilers in 2006 generating $2.73 billion to 1.40 billion broilers in 2008 generating $3.36 billion accounting for 16% of the national production. The increase in poultry meat production is the result of intense selection for growth. Several factors influence consumer perception of poultry meat quality, especially quality attributes which results from changes to the physical or biochemical properties of the muscle and consequently affect appearance, eating quality and nutritive value. Continuous economic viability of the poultry meat industry will depend on improving appearance (color), eating quality (texture, flavor) and nutritional quality.

Breast chicken meat is usually of most interest in the US, where white meat is preferred over dark meat (Fanatico, Pillai, Emmert, & Owens, 2007). Breast meat color is strongly related to muscle pH (Allen, Russell, & Fletcher, 1997; Boulianne & King, 1998; Fletcher, 1999; Qiao, Fletcher, Smith, & Northcutt, 2001) and is affected by the state of haem pigments, myoglobin content and muscle pH (Froning, 1995). Muscle pH has also been associated with tenderness (Bouton, Carroll, Fisher, Harris, & Shorthose, 1973; Fjelkner-Modig & Ruderus, 1983; Purchas, 1990), water holding capacity (WHC) (Dransfield & Sosnicki, 1999; Honikel, Kim, Hamm, & Roncales, 1968; Le Bihan-Duval, Berri, Baeza, Millet, & Beaumont, 2001), juiciness and shelf life (Le Bihan-Duval, Berri, Baeza, Millet, & Beaumont, 2001). Le Bihan-Duval et al. (2008)

showed that breast meat with lighter color had higher drip loss. Higher drip loss results unsatisfied texture which is an important quality factor associated with consumer satisfaction in the eating quality of poultry. The intense genetic selection for growth did not affect muscle fiber types (Le Bihan-Duval, Debut, Berri, Sellier, Santé-Lhoutellier, Jégo, & Beaumont, 2008), but led to structural changes and changes in meat composition and pH (Le Bihan-Duval, Debut, Berri, Sellier, Santé-Lhoutellier, Jégo, & Beaumont, 2008). Most parameters used to measure meat quality such as water holding capacity, shear force (tenderness), color, pH, drip loss, etc., were developed over 30 years ago and these methods are inefficient, laborious, time consuming, expensive, and do not allow on-line testing and real time adaptation of corrective strategies. Development of online rapid monitoring tool has gained interest for the industry when dealing with cut-up parts or deboned meat, strategies such as identifying and separating pale soft exudative (PSE) meat, diverting it to no or low moisture-added products, and including additives to compensate for the poor water holding and texture can be beneficial.

To date, a few genomic segments and genes relating to poultry meat quality have been identified (Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004; Teltathum & Mekchay, 2009). In the post-genomic era, proteomics tools offer us unique innovative techniques to study muscle protein functions and characteristics that directly relate to meat quality. These proteomics techniques and meat technological parameters will allow us to develop new innovative tools for assessing poultry meat quality. These technologies will maintain Georgia's lead in supplying poultry meat of the highest quality to the consumer and maintain profitability.

Goal: To find relationship in changes of muscle and meat physico-biochemical property traits through online monitoring method and proteomic analysis

General Objective:

To develop a method to test for association between the genetic and protein bio-markers and poultry meat physico-biochemical properties, and to determine and identify protein relate to meat physico-biochemical properties.

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CHAPTER 2

LITERATURE REVIEW

Xiong (1999) stated "quality characteristics of meat include a range of physical and chemical attributes of the muscle food and its products". The quality of poultry and poultry products (Duclos, Berri, & Le Bihan-Duval, 2007) can be described in terms of sensory attributes (color, tenderness, flavor, juiciness) and physical attributes (muscle yield, water holding capacity, cooking loss).

Meat quality attributes are important to consumer acceptance and profitability for the meat industry. Two main factors (Xiong, Ho, & Shahidi, 1999); intrinsic and extrinsic, influence meat quality. Examples of intrinsic factors (Berri, 2004) include muscle structure and chemical composition, age, strain, and sex. Examples of extrinsic factors (Berri, 2004) include environmental condition, nutritional condition, ante-mortem stress, post-mortem handling, and storage conditions.

Environmental conditions

As consumer interest in the organic market increases, many experiments assess the impact of genotype and outdoor access on growth rate and carcass yield (Fanatico, Pillai, Cavitt, Owens, & Emmert, 2005; Wang, Shi, Dou, & Sun, 2009). The outdoor or free-range birds had decreased growth performance, abdominal fat, and tibia strength without an effect on carcass traits and meat quality such as eviscerated carcass, breast, thigh, and wing weights, nutrient composition (water, protein, and fat), water-holding capacity, shear force, or pH of the muscle (Wang, Shi, Dou, & Sun, 2009). Indoor-raised birds had higher body weight and weight gain

than the free-range birds (Wang, Shi, Dou, & Sun, 2009). Breast meat from chickens fed a low-nutrient diet yielded higher protein and α-tocopherol, but was lower in fat than the fast growing bird which had higher nutrient diet (Fanatico, Pillai, Emmert, & Owens, 2007).

Ante-mortem stress

Stress affected color (Santos, Sakomura, Freitas, Fortes, & Carrilho, 2005). No significant effects of stresses were found on chickens from fast-growing line and slow-growing line on breast meat characteristics (pH, color, drip loss and processing yield) but on thigh meat by decreasing of the ultimate pH and lighter color and lower curing-cooking yield (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003). However, heat stress resulted in higher L* and a*, of breast meat from both slow and fast growing chicken genotypes (Molette, Serieye, Rossignol, Babile, Fernandez, & Remignon, 2006). In thigh meat, heat stress caused a decrease in the ultimate pH which resulted in paler color and lower curing-cooking yield (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003). Transportation caused an increase in the ultimate pH (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003). If long durations of wing flapping occurred on the shackle line, then the initial pH was influenced by time on the shackle (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003). Acutely stressed quails from the high-fear line had higher levels of plasma creatine kinase and a smaller increase in corticosterone levels, and the ultimate pH value of the breast meat and drip-loss were higher (Berri, 2004).

Genotype

In some studies, a slow growing chicken genotype and a fast growing chicken genotype were raised to achieve a similar final body weight at a processing plant (Abdullah, Al-Beitawi, Rjoup, Qudsieh, & Ishmais, 2010; Fanatico, Pillai, Cavitt, Owens, & Emmert, 2005; Fanatico, Pillai, Emmert, & Owens, 2007). However, at the weight fixed by the market, the animal may have a lower degree of maturity (Pascual & Pla, 2008). Different chicken genotypes resulted in different overall average daily weight gain, most efficient feed conversion ratio (Abdullah, Al-Beitawi, Rjoup, Qudsieh, & Ishmais, 2010) and meat features (Jaturasitha, Srikanchai, Kreuzer, & Wicke, 2008): meat color (Fanatico, Pillai, Emmert, & Owens, 2007; Molette, Serieye, Rossignol, Babile, Fernandez, & Remignon, 2006), texture (Abdullah, Al-Beitawi, Rjoup, Qudsieh, & Ishmais, 2010; Jaturasitha, Srikanchai, Kreuzer, & Wicke, 2008), body weight at slaughter (Jaturasitha, Srikanchai, Kreuzer, & Wicke, 2008), curing-cooking yield of thigh meat, drip loss (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003; Fanatico, Pillai, Emmert, & Owens, 2007), and fat content (Abdullah, Al-Beitawi, Rjoup, Qudsieh, & Ishmais, 2010; Jaturasitha, Srikanchai, Kreuzer, & Wicke, 2008).

In five different chicken genetic strains, chosen for differences in yield, no consistent relationship between meat quality and breast yield was shown (Mehaffey, Pradhan, Meullenet, Emmert, McKee, & Owens, 2006). Higher b* was found in slow growing chicken genotype (Fanatico, Pillai, Emmert, & Owens, 2007; Molette, Serieye, Rossignol, Babile, Fernandez, & Remignon, 2006). The slow growing birds had poorer water-holding capacity (Fanatico, Pillai, Emmert, & Owens, 2007), lower drip processing yield, higher drip loss of breast meat (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval,

2003) but were more tender than the fast growing birds (Fanatico, Pillai, Emmert, & Owens, 2007).

Age

Older birds have a darker color from an increase in the haem-iron content of muscle meat. Less mature birds are generally selected for commercial sale because they tend to have juicier and more tender meat but less intense flavor than older birds (Berri, 2004).

Sex

Male birds had higher overall body weight (Fanatico, Pillai, Cavitt, Owens, & Emmert, 2005; Fanatico, Pillai, Emmert, & Owens, 2007), average daily gain, feed intake, leg cut percentage, cooking loss than female birds but had lower breast cut percentage and lower abdominal fat yield than females (Abdullah, Al-Beitawi, Rjoup, Qudsieh, & Ishmais, 2010; Santos, Sakomura, Freitas, Fortes, & Carrilho, 2005). Different growth curve was reported between males and female broiler chickens (Scheuermann, 2003). Growth rate maturity parameter and maximal growth rate for body weight and breast weight in broiler chicken were slower in male birds than female birds, but male birds had higher muscle fiber than female birds (Scheuermann, 2003).

Growth

Many factors that could impact growth are genotype, age, sex, diet, density, environment, exercise, and pasture intake (Fanatico, Pillai, Emmert, & Owens, 2007). Many researchers have considered body weight and breast meat yield of six-week-old chickens for changes that could improve bird growth performance and muscle development (Berri, 2004).

In term of production, the advantages of a fast growing chicken process include less feed and lower maintenance costs compared to a slow growing chicken process (Fanatico, Pillai, Emmert, & Owens, 2007). Hossain, Bulbul, Nishibori, & Islam (2008) estimated 60-70 % of the

total production cost is connected to diet cost. Commercial poultry producers are trying different approaches for better growth and economic broiler meat production (Hossain, Bulbul, Nishibori, & Islam, 2008). However, fast growing chickens have also been reported to have some negative effects, such as inducing larger fiber diameters, higher glycolytic fibers, faster rigor mortis, lower proteolytic potential causing paler color and decrease of water holding capacity (Dransfield & Sosnicki, 1999). On the other hand, slow growing chickens were also reported some negative effects, such as having more struggles during shackling and accelerated post-mortem glycolysis, which affects the quality of breast meat (Fanatico, Pillai, Emmert, & Owens, 2007).

Many studies have determined effects of growth rate on meat quality and most of the studies were done on porcine, cattle, rabbit, and poultry meats. Dietary-induced changes of muscle growth rate increased growth rate as a result of increased protein turnover. The change of muscle growth rate affected post-mortem tenderness development (Dransfield & Sosnicki, 1999; Kristensen, Therkildsen, Riis, Sorensen, Oksbjerg, Purslow, & Ertbjerg, 2002). Very high growth rate in poultry showed decrease activity of proteolytic enzymes (μ-calpain, cathepsin) and had excess inhibitors. These reduction of proteolytic potential in faster growing chicken lines resulted in decrease in tenderization (Dransfield & Sosnicki, 1999). Curing-cooking yield was higher in fast growing chicken breast meat than in slow growing chicken breast meat, which resulted from less rapid pH decline (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003). There was a conclusion that fast growing birds could be used for industrial transformation in further-processed products (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003).

Muscle protein

Proteins are the main contributor to the structural and functional components in many food systems including muscle foods. Quality of end products can be predicted from functional properties of proteins. Proteins can be separated based on functional properties, structures, or solubilities (Baeza, 2004; Herrero, 2008b). Protein interaction in poultry meat products can be divided into 3 categories: protein-water interactions, protein-fat interactions, and protein-protein interactions (Smith D. M., 2001).

Protein structures can be divided to: primary (amino acid sequence), secondary (repeated local structures stabilized by hydrogen bonds: α-helix, β-sheet, random coil), tertiary (spatial relationship of the secondary structures to one another), and quaternary (structure that results from the interaction of subunits of a protein). Stabilization of protein structure result from covalent and hydrogen bonds, hydrophobic interactions, electrostatic and Van der Waals forces (Herrero, 2008b).

Protein in skeletal muscle can be separated into 3 groups based on their solubility (Chiang, Byrem, & Strasburg, 2007): sarcoplasmic, myofibrillar, and stromal. Sarcoplasmic proteins are 30-35% of muscle total protein and are soluble in water or low ionic strength solution and are present in the cytoplasm (Smith D. M., 2001). Examples of sarcoplasmic protein include glycolytic (glycolysis and glycogenolytic) enzymes; pentose shunt enzymes, certain isozymes of the tricarboxylic acid cycle, and ancillary enzymes, such as creatine kinase and AMP deaminase, proteinases, and the oxygen storage protein myoglobin, which are involved in the regulation of cell and energy homeostasis (Chiang, Byrem, & Strasburg, 2007).

Myofibrillar proteins are salt soluble protein and comprise 50-56% of total skeletal muscle protein. Myofibrillar proteins can be grouped into contractile proteins, regulatory

proteins, and cytoskeletal proteins (Smith D. M., 2001). Examples of myofibrillar protein are myosin and actin which account for 40% of the total protein in skeletal muscle (Chiang, Byrem, & Strasburg, 2007). Myofibrillar proteins can be solubilized in salt solutions (e.g. 0.3 M NaCl) whereas in muscle tissue the salt concentration is about 0.15 M NaCl. Major myofibrillar proteins in skeletal muscle are listed in Table 2.1. The behavior of myofibrillar proteins is important in fresh meat and processed meat products because the extent of contraction and rigor-mortis development are critical in the successful conversion of muscle to meat (Chiang, Byrem, & Strasburg, 2007). When carcass chilling is delayed, denaturation of myofibrillar protein occurs (Rathgeber, Boles, & Shand, 1999).

Actin and tropomyosin are thin filaments (Smith D. M., 2001). Actin is a ubiquitous cytoskeletal protein which consists of two forms, globular and filamentous (Bárány, Bárány, & Giometti, 1995). The isoelectric point of actin is about 4.8 (Smith D. M., 2001). Actin binds the thick filament protein, myosin, during muscle contraction, forming crossbridges between the two filaments. Actin also binds to tropomyosin and troponin. Tropomyosin and troponin regulate contraction in response to changes in calcium concentration by switching on or off actin's ability to bind myosin. Tropomyosin (MW=65,000) consists of two α-helical subunits. One tropomyosin molecule spans seven actin monomers of the thin filament (Chiang, Byrem, & Strasburg, 2007).

Myosin is about 43% of the muscle myofibril (Chiang, Byrem, & Strasburg, 2007). The isoelectric point of myosin is about 5.3 (Smith D. M., 2001). Myosin is the thick filaments and has a large molecular weight (MW=521,000). Myosin has 6 polypeptide chains: 2 heavy chains of molecular weight 223,000, and 4 light chains of molecular weight ranging from 15,000 to 20,000. Myosin is an important structural constituent of muscle. Myosin acts as a molecular

motor that produces contractile force that splits ATP. During contraction, myosin heads are able to link to actin molecules of the thin filament and form crossbridges (Chiang, Byrem, & Strasburg, 2007). Myosin is in pre-rigor muscle. In post-rigor muscle, the globular head of myosin binds to actin to form a complex known as actomyosin. This cross-linking between actin and myosin in post-rigor muscle influences meat tenderness in intact muscle (Smith D. M., 2001). Higher degradation of myosin heavy chain (as observed from several bands on gel electrophoresis) for muscle kept at high temperature (40 ºC) was found when compared with the muscle held at chilled temperature (4 ºC) (Dransfield & Sosnicki, 1999). Lower myosin heavy chain-I (lower slow oxidative fiber) influenced texture of the meat to be more tough and hold water lesser than meat that present higher myosin heavy chain-I (Ramírez, Oliver, Pla, Guerrero, Ariño, Blasco, Pascual, & Gil, 2004).

Stromal proteins are insoluble in either water or salt solutions and are only 3-6% of the total muscle protein content (Smith D. M., 2001). The stromal proteins are correlated to meat toughness by forming fibrous connective tissues (Chiang, Byrem, & Strasburg, 2007; Pascual & Pla, 2008). However, the toughness of meat also depends on protein solubility and cross-linking (Pascual & Pla, 2008). Two major proteins in stromal protein are collagen (90%) and elastin (10%) (Chiang, Byrem, & Strasburg, 2007). Dark meat has more collagen (Alvarado, 2007). Examples of soluble non-protein nitrogen in muscle are creatine phosphate, amino acids, nucleic acids and nucleotides (e.g. ATP, GTP). Creatine phosphate and ATP are key components in the development of rigor mortis (Chiang, Byrem, & Strasburg, 2007).

Protein extraction

Protein extractability is described as the amount of protein that is released or dissociated from the organized myofibrillar structure during processing (Smith D. M., 2001). Solubility

depends on the distribution of hydrophobic and hydrophilic amino acids on the surface of a protein and on the thermodynamics of the protein-water interactions. Muscle protein extractability and solubilities are affected by pH, salt concentration, type of salt and temperature (Smith D. M., 2001).

Pale soft and exudative (PSE) muscles tend to exhibit lower protein extraction i.e. myosin and solubility than normal meat (Barbut, Zhang, & Marcone, 2005; Molette, Serieye, Rossignol, Babile, Fernandez, & Remignon, 2006; Pietrzak, Greaser, & Sosnicki, 1997). Lower protein extractability was found in fast glycolysing muscle than normal glycolysing muscle (Sosnicki, Greaser, Pietrzak, Pospiech, & Sante, 1998). Protein solubility affects some of the physical properties of the meat (Barbut & Findlay, 1989; Bendall & Swatland, 1988; Camou & Sebranek, 1991; Gordon & Barbut, 1992). In pork, myofibrillar and sarcoplasmic protein solubility are highly correlated with water retention measurements, such as drip loss and moisture uptake.

Conversion of muscle into meat (aging)

Muscle is converted into meat by activity of endogenous proteolytic enzyme in muscle tissues (Chiang, Byrem, & Strasburg, 2007). After death, as the development of rigor mortis advances, the likelihood of paler color and reduced water holding capacity and poorer quality of further processed products increases. Reduced proteolytic potential likely increases the toughness of poultry meats (Dransfield & Sosnicki, 1999). Handling carcass after slaughter is important to rigor mortis development. Carcass with rapid rigor should be chilled quickly to reduce protein denaturation, and slower rigor should be chilled more slowly to reduce their toughness (Dransfield & Sosnicki, 1999).

Creatine kinase is an enzyme of significance in both living muscle tissue and in post-mortem conversion to meat. Creatine kinase is used by the muscle cell to maintain stable levels

of ATP (Chiang, Byrem, & Strasburg, 2007). The two most relevant energy reserves in muscle under post-mortem anaerobic conditions are creatine phosphate (CrP) and glycogen (Chiang, Byrem, & Strasburg, 2007). Glycogen is the greatest resource for the regeneration of ATP but CrP is more readily available. Glycogen must first be cleaved by glycogen phosphorylase into monomeric hexose units (glucose-1-P) that enters the glycolytic cascade to yield ATP and pyruvate. Then pyruvate, in the absence of oxygen, is further converted to lactic acid to regenerate a cofactor required to continue glycolysis (Chiang, Byrem, & Strasburg, 2007). Although the decline in pH is primarily due to the generation of hydrogen ions during the hydrolysis of ATP, the rate of decline in pH closely reflects the accumulation of lactic acid and, hence the rate of glycolysis in post-mortem muscle (Chiang, Byrem, & Strasburg, 2007). There are several proteinases in muscle sarcoplasm that are probably responsible for protein turnover in muscle growth and maintenance such as calpains or calcium-dependent proteinases (Chiang, Byrem, & Strasburg, 2007). Aging is the final phase in the conversion of muscle to meat and associated with increased sarcomere length, extensibility, and meat tenderness (Chiang, Byrem, & Strasburg, 2007). Rapid glycolysis (fast twitch, white muscle) has faster aging rate than oxidative type (slow twitch, red muscle) and is also lower in calpain content. Calpain is involved in post-mortem proteolysis of myofibrillar and associated proteins (Huff-Lonergan & Lonergan, 2005).

Quality attributes of muscle

Meat quality could be determined by color, water holding capacity, cook yield, drip loss, tenderness and so on. Water holding capacity and tenderness are considered to be the most important factors in determining poultry quality, company aspect and consumer acceptance (Fletcher, 1999).

Post-mortem metabolism of the muscle tissue influences the characteristics of the meat. After bleeding, cessation of oxygen supply modifies muscular metabolism during the initiation of rigor mortis. The muscle relies on the anaerobic glycolytic pathway to use the glycogen stores for ATP regeneration, which causes to the accumulation of lactic acid and protons. Therefore, acidification depends upon the amount of glycogen stores and the rate of the glycolysis (Duclos, Berri, & Le Bihan-Duval, 2007).

Normal post-mortem metabolism in skeletal muscle yields meat with a lower pH (5.5 vs. 7.4). The reduction in pH or pH decline has both positive and negative effects on the characteristics of meat. Acidic pH will retard microbial growth and extend shelf-life. As a consequence of the reduction in net charge and electrostatic repulsion, myofibrils shrink and lose much of their water-holding capacity. Lower ultimate pH results a pale appearance, whereas meat with a high ultimate pH and dry surface will have a dark appearance (Chiang, Byrem, & Strasburg, 2007).

pH

Different strains in chicken influence muscle pH (Santos, Sakomura, Freitas, Fortes, & Carrilho, 2005). Additionally, pH may be influenced by others internal factors such as muscle type, and individual characteristics and external factors such as feed, fasting, electrical stimulation and chilling, as well (Santos, Sakomura, Freitas, Fortes, & Carrilho, 2005). pH in the muscle is around 5.5-5.6 in broiler meat at ultimate pH (Abdullah, Al-Beitawi, Rjoup, Qudsieh, & Ishmais, 2010), 5.6-5.7 in Gushi chicken under indoor and outdoor raising system (Wang, Shi, Dou, & Sun, 2009). Rate of pH decline was associated with the activities of several proteolytic and lipolytic enzymes in porcine meat differing in stress susceptibility and in breeding (Claeys, De Smet, Demeyer, Geers, & Buys, 2001) and also among chicken genetic lines and between

individual birds (Dransfield & Sosnicki, 1999). Breast muscle is more sensitive to fast rate of pH decline than thigh meat (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003) because breast muscle contains more white fiber which requires more time to undergo rigor mortis development, so more time to obtain ultimate pH (Abdullah, Al-Beitawi, Rjoup, Qudsieh, & Ishmais, 2010). As in pigs, post-mortem pH decline in chickens strongly affects quality of meat, especially processing yield (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003). pH decline influenced changes within the muscle post-mortem on water movement from the interfilamental space into the interfibrillar fluid and from there into the extracellular space, resulting in drip loss (Honikel K. O., Kim, Hamm, & Roncales, 1968). At high ultimate pH, water holding capacity of myosin will be high (Dransfield & Sosnicki, 1999). The highest muscle pH values at 20 min and 24 hr post-mortem were associated with the highest body weights and breast meat yields (Le Bihan-Duval, Berri, Baeza, Sante, Astruc, Remignon, Le Pottier, Bentley, Beaumont, & Fernandez, 2003).

Color

Color is one of important quality attributes that influences consumer acceptance of fresh breast chicken and in many food products (Fletcher, 1999). American consumer expects breast chicken to be more yellow muscle color (Santos, Sakomura, Freitas, Fortes, & Carrilho, 2005). The major pigments responsible for color in meat are myoglobin, hemoglobin, and cytochrome C (Baeza, 2004). Skeletal muscles that have higher a* contain higher levels of iron resulting from higher levels of myoglobin because iron in myoglobin is present as heme iron (Berri, Wacrenier, Millet, & Le Bihan-Duval, 2001; Chiang, Byrem, & Strasburg, 2007). However, myoglobin in raw meat is low in broiler and turkey so it is less important in poultry (Alvarado, 2007).

There are many factors that affect meat color such as bird sex, age, strain, processing procedures, chemical exposure, etc. (Fletcher, 1999). Delayed chilling increases L*, a*, and b* of turkey breast and decreases protein extractability compared to breast meat from immediately chilled carcasses (Rathgeber, Boles, & Shand, 1999). Higher L* indicates paler meat. Variations in a* and b* in muscle will alter the consumer perception of muscle color. Lighter-than-normal meat was associated with low pH, high moisture, low emulsion capacity, and low water holding capacity (Santos, Sakomura, Freitas, Fortes, & Carrilho, 2005). The higher the ultimate pH value after rigor mortis of the breast meat, the darker the meat color and the lesser drip loss (Le Bihan-Duval, Millet, & Remignon, 1999). The b* was different between chicken strains (Santos, Sakomura, Freitas, Fortes, & Carrilho, 2005). Higher b* was found in ISA label-LSA JA 57 strain than Paraiso Pedres strain (Santos, Sakomura, Freitas, Fortes, & Carrilho, 2005). Differences in b* between slow and fast growing chicken was hypothesized to result from feed composition (Molette, Serieye, Rossignol, Babile, Fernandez, & Remignon, 2006). L* may be an effective indicator of pale, soft, exudative (PSE) in turkey meat, which results in low water holding capacity and technological yield after curing and cooking (Fernandez, Sante, Baeza, Lebihan-Duval, Berri, Remignon, Babile, Pottier, & Astruc, 2002). However, cut off value for color is highly variable in the literatures and difficult to compare due to differences in chicken genotype, color instrument, color instrument set up conditions such as light source and angle view, etc. Petracci (2009) suggested "each plant would have to determine its own lightness values for sorting PSE-like meat depending on type of birds, processing factors, and final product specifications.

Next the water-holding capacity will be reviewed. We can determine the ability of meat or meat systems to hold all or part of its own and/ or added water from water holding capacity

assessment (Honikel K.O. & Hamm, 1994). However the ability depends on the method of handling and state of the system, for examples, applying no force such as free drip, applying mechanical force such as centrifugation method or drip loss by filter paper press, and applying thermal force such as cooking losses. Selection of the method to assess water holding capacity is depending on purpose and what is required to be measured (Honikel K.O. & Hamm, 1994).

Water holding capacity (WHC)

WHC influences economic viability of poultry processors and retailers (Barbut, 1996) and affects qualitative and quantitative quality aspects of meat and meat products (Kauffman, Eikelenboom, van der Wal, Engel, & Zaar, 1986). WHC is one the protein functionalities that is important to describe how protein holds water within the protein structure (Barbut, 1996) when water is in excess and under internal force (Abdullah, Al-Beitawi, Rjoup, Qudsieh, & Ishmais, 2010). Good water holding capacity is essential in protein-based food products (Barbut, 1996; Trout, 1988), reduced weight loss during cutting and storage and improved ability of the meat to retain water during processing (Micklander, Christine Bertram, Marnø, Søvad Bak, Jørgen Andersen, Balling Engelsen, & Nørgaard, 2005).

Muscle contains approximately 75% water which exists as bound, immobilized and free water. Bound water represents less than 1% of muscle water and is associated with protein molecules with strong protein-water interaction. Immobilized or interfacial water represents approximately 10-15% of water in meat. Immobilized water is attracted to the bound water layer creating multilayers of water, each more loosely bound as the distance from the bound water layer increases. Immobilized water is usually lost with cooking (Alvarado, 2007). Free water is the remaining water in meat tissues and is associated with the extracellular space held loosely through capillary forces and easily lost during mechanical actions (Alvarado, 2007).

Water binding capacity is affected by the net charge and steric effect. Water can bind to charged amino acid side groups. The amount and type of charge on protein changes with pH. The point at which water binding is minimal on the protein is referred to isoelectric point or pI and at this point, protein-protein interactions are maximized. Myosin and actin are the predominant proteins in the muscle and most responsible for water holding capacity. Meat's water holding capacity is minimal at pI approximately 5.1 where the number of positive charges equals the number of negative charges resulting in the net zero charge or the pH (Alvarado, 2007). Therefore, part of the immobilized water may become free water and may be lost as drip (Alvarado, 2007). As pH increases, the proteins become more negatively charged. A higher net negative charge leads to an increase an repulsive force between the proteins within the myofilament, which allows the myofibril to swell and allows an increase amount of water holding capacity (Smith D. M., 2001). The steric effect or degree of contraction also effects water holding capacity. As the amount of space between the muscle protein structure decreases with contraction, less space is available for the muscle can influence the amount of interstitial space which less space within the muscle to hold water so that water is expelled into the extracellular space (Alvarado, 2007).

Salt and phosphates are commonly used ingredients to improve WHC in processed meats. Salt promotes the extraction of salt-soluble, functional proteins (Alvarado, 2007; Barbut & Findlay, 1989; Fletcher, 2004). Light meat has more extractable protein than dark meat (Khan, 1962; McCready & Cunningham, 1971). The effect of various poly phosphates and salt (NaCl) solutions on myofibril protein extraction has been studied (Alvarado, 2007; Pearson & Dutson, 1994). No significant swelling or any other changes were noted at salt concentration of 0.4 M or less. When salt concentrations were increased to 0.5 M and 0.6 M, myofibrils started to enlarge

transversely and protein extraction became significantly noticeable (Alvarado, 2007). Different methods (press method, high-speed centrifugation, low-speed centrifugation, capillary suction, optical, special instruments, etc) have been used to determine WHC (Kauffman, Eikelenboom, van der Wal, Engel, & Zaar, 1986; Trout, 1988).

Cooking yield/ cooking loss

"Cooking influences the appearance, protein, water-binding, and textural properties of meat" (Resurreccion, 1994). When meat is cooked, fat, water, and some volatiles are losses (Resurreccion, 1994).

Cooking loss percentage of commercial cross of broiler strains of chickens was found higher in male bird (29.56%) than female bird (27.95%) but there was no difference between 4 strains: Lohman, Hubbard JV, Hubbard classic, and Ross (Abdullah, Al-Beitawi, Rjoup, Qudsieh, & Ishmais, 2010). Cooking loss failed to differential between PSE and normal in post-rigor procine muscle (Honikel K.O. & Hamm, 1994; Kauffman, Eikelenboom, van der Wal, Engel, & Zaar, 1986). Cooking losses do not also depend on shortening, unlike drip loss (Honikel K.O. & Hamm, 1994).

Cooking loss depends on shape and size of the sample, temperature profile during cooking, final cooking temperature, and environment during cooking (Honikel K.O. & Hamm, 1994). The higher the final temperature and the slower the velocity of heating, the higher were the cooking loss (Honikel K.O. & Hamm, 1994).

Drip loss

Drip loss is the method to assess the water that is released by applying no force (Honikel K.O. & Hamm, 1994). The drip loss is depending on surface area and weight of the sample (Honikel K.O. & Hamm, 1994). There was the recommendation after drip loss assessment; the

same muscle then could be immediately used for cooking loss measurement (Honikel K.O. & Hamm, 1994).

Drip loss is influenced by falling pH decline causing the changes within the muscle post-mortem on water movement from the interfilamental space into the interfibrillar fluid and from there into the extracellular space (Berri, Wacrenier, Millet, & Le Bihan-Duval, 2001; Honikel K. O., Kim, Hamm, & Roncales, 1968). Drip loss was higher when bird was subjected to acute stress (Berri, 2004).

Pale, soft and exudative & dark, firm and dry meats

Pale, soft and exudative (PSE) meat is characterized by low moisture retention, soft texture, and light color. Pale fillets had significantly lower pH, greater L*, and is higher in expressible moisture, drip loss, and cook loss (Woelfel, Owens, Hirschler, Martinez-Dawson, & Sams, 2002). Smith and Northcutt (2009) suggested to use terminology "pale chicken muscle or pale poultry muscle syndrome" to describe avian breast muscle that exhibits some degree of paleness, reduced water-holding capacity, and increased drip loss. The poor water holding capacity of PSE meat results in an economic loss for processors, as well as a decrease in consumer acceptance (Alvarado, 2007; Richardson & Jones, 1987). The cause of PSE-like meat condition seem to be related to selection of the birds for growth rate and breast yield, or genetic mechanisms, as well as heat stress (Petracci, Bianchi, & Cavani, 2009). PSE-like meat can occur during hot season climate up to 40% within a flock (Petracci, Bianchi, & Cavani, 2009). Heat stress may alter the expression pattern of splice variants of ryanodine receptors which are regulators of calcium in muscle and affects postmortem calcium homeostasis (Strasburg & Chiang, 2009). High concentration of calcium ions showed abnormally rapid postmortem metabolism and associated with the incidence of PSE turkey (Strasburg & Chiang, 2009). PSE

pork muscles tend to exhibit lower protein extraction and solubility than normal meat (Barbut, Zhang, & Marcone, 2005). The lower protein extraction and solubility resulted from the rapid decline in pH occurring while muscle temperatures are still elevated (Alvarado, 2007).

Less myosin is solubilized from PSE versus normal myofibrils and phosphorylase is closely associated with myofibrils in PSE muscles (Pietrzak, Greaser, & Sosnicki, 1997). Myofibrillar and sarcroplasmic protein solubility are highly correlated with water retention measurements, such as drip loss and moisture uptake. Protein solubility affects some of the physical properties of the meat and can explain why the actual proteins extracted from PSE meat have poor functionality. Loss of functionality, due to PSE, results in lower solubility and also involves some losses of molecular functionality (Bendall & Swatland, 1988; Camou & Sebranek, 1991; Warner, Kauffman, & Greaser, 1997). Woeflfel (2002) reported that approximately 47% of the 3,554 chicken fillets were pale (L* value range >54) and these fillets potentially exhibit poor water-holding capacity. Dark, firm and dry meat is characterized when the meat has darker than normal appearance, having high pH and high water holding capacity even though the meat appears dry (Alvarado, 2007; Faustman, 1994).

Online measurement of meat composition

Rapid quality evaluation techniques for quantitative testing of meat product quality are required to meet the growing demand for bulk manufacture. These techniques require instruments that can accommodate a large sample size in a short time for routine analysis (Chizzolini, Novelli, Badiani, Rosa, & Delbono, 1993). The rigor development of chicken meat immediately after slaughter can be evaluated in-line and segregated appropriately to improve meat texture (Dransfield & Sosnicki, 1999). The Raman spectroscopic technique is a non-destructive method of online monitoring. With many advantages (Colthup, Daly, & Wiberley,

2010), Raman spectroscopy is an attractive method for use in the meat industry. It is a fast method and the spectra can be recorded in a short time. The spectrum contains information that gives both quantitative and qualitative information. Table 2.2 shows a summary of Raman spectroscopy studies on muscle.

Moisture loss or drip loss causes unattractive meat appearance and meat texture as well as influences on meat processing and contributes to the loss of sales (Forrest, Morgan, Borggaard, Rasmussen, Jespersen, & Andersen, 2000). Prediction of water holding capacity by vibrational spectroscopic methods has been investigated (Hoving-Bolink, Vedder, Merks, de Klein, Reimert, Frankhuizen, van den Broek, & Lambooij, 2005; Prieto, Roehe, Lavin, Batten, & Andres, 2009; Rosenvold, Micklander, Hansen, Burling-Claridge, Challies, Devine, & North, 2009; Samuel, Park, Sohn, & Wicker, 2011; Swatland & Barbut, 1995). Raman spectroscopy was used to investigate various sources of pork meat (research meat and commercial meat measured at slaughterhouses which differed in water holding capacity, and Raman spectroscopy predicted the quality of the muscle (Pedersen, Morel, Andersen, & Engelsen, 2003). The regions of interest for WHC were 3128-3071 and 876-951 cm^{-1} in the study of Raman spectra. There were more poor spectra considered to be outliers found from commercial meats because the speed of measure was not as fast (acquiring time 30-60 s) as the speed of the processing line. A suggestion was made to increase sample size so the Raman could reflect more representative data (Pedersen, Morel, Andersen, & Engelsen, 2003).

Changes in the Raman bands of protein chemical groups gave information of changes in secondary structure of proteins: amide conformation region, C-C stretching vibration and changes in tryptophan residues, tyrosyl doublet, aliphatic amino acids bands (Herrero, 2008b). The amide region was the most useful for observing changes in the secondary structure of

proteins (α-helix, β-sheet, turn, and random coil) which were assigned to amide I, amide II, and amide III bands (Herrero, 2008b). Amide I is usually used to quantify the secondary structure of proteins. Amide II vibration usually cannot be detected because of a small change in polarizability associated with amide II. The amide III region has some overlap between α-helix, β-sheet, turn, and random coil (Herrero, 2008b). C-C stretching vibration is also involved in the change in secondary structure of protein. The gradual loss of these structures showed the broad and weakness in intensity of this band (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008). Many Raman bands showed response to the change of tryptophan residues in proteins. Herrero (2008b) concluded from the literatures that tertiary structure of proteins can be detected from decreasing peak intensity of buried tryptophan residues in the protein. Also Herrero (2008b) concluded from previous research that the presence of a high intensity ratio of I_{1360}/I_{1340} indicated a hydrophobic environment; conversely, a low ratio indicated tryptophan was involved more in the H-bonding in a hydrophilic environment. Tyrosine is used as an indicator of the hydrogen bonding of the phenolic hydroxyl group (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008). Herrero (2008) reviewed that the decrease in intensity of aliphatic amino acids band could possibly result from hydrophobic interactions of aliphatic residues.

Other amino acids that might be useful for meat quality estimation include proline and hydroxproline, which are the amino acids of the connective tissue proteins such as collagen (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008). Collagen, the main connective tissue, contains high levels of hydroxyproline (Morse, 1933) . Collagen showed two amide bands at 1271 and 1248 cm^{-1} (Frushour & Koenig, 1975). Raman spectroscopy predicts the juiciness from the investigation of sensory evaluation of beef silverside by observing the changes in 1460-1483 cm^{-1} region corresponding to the juiciness in cooked beef (Beattie J. R., Bell,

Farmer, Moss, & Desmond, 2004). No studies have used Raman spectroscopy to observe changes in protein structure that is affected by growth rate.

Advantages of Raman

There are several types of spectroscopic instruments: circular dichroism, florescence spectroscopy, nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and Raman spectroscopy (Fourier transform Raman spectroscopy, Raman microspectroscopy) (Herrero, 2008a). Different spectroscopic methods provide information on protein structure. Raman spectroscopic technique is non-destructive online monitoring. With many advantages, Raman spectroscopy has gained more interest to be used in meat industry. It is a fast method and the spectra can be recorded in a short time. The spectrum contains information that gives both quantitative and qualitative information. Raman spectroscopy has advantages:

1) Raman spectroscopy can be used for solid samples and aqueous solution (as water has a weak Raman spectrum (Carey, 1983)), unlike circular dichroism which is not designed for measurement solid state sample
2) Raman spectroscopy provides information on the peptide backbone structure and hydrophobic side chains such as tyrosine and tryptophan, and the local conformations of disulphide bonds and methionine residue, unlike fluorescence spectroscopy where these information is limited (Herrero, 2008a).
3) Raman spectroscopy uses laser which provide convenient, intense monochromatic light source (Carey, 1983)
4) Small amount of material can be recorded using Raman spectroscopy (Carey, 1983)

5) Raman spectroscopy removed interference with Rayleigh photons which Rayleigh photons are the result of an elastic collision which have the same frequency at the incident light (Carey, 1983)

Basic principles of Raman spectroscopy

The energy of molecule consists partially of translational energy, rotational energy, vibrational energy, and electronic energy. The rotational and vibrational frequencies are most important for Raman and infared and those frequencies are related to each other and each individual has its individual strong points (Colthup, Daly, & Wiberley, 2010). The electromagnetic radiation is characterized by wavelength, frequency, and wavenumber as shown in this equation (Colthup, Daly, & Wiberley, 2010):

$$\bar{\nu} = \frac{\nu}{\left(\frac{c}{n}\right)} \qquad \bar{\nu} = \frac{1}{\lambda}$$

where: $\bar{\nu}$ is wavenumber (cm^{-1}); ν is frequency (sec^{-1} or Hertz); λ is wavelength (cm); (c/n) is the velocity of light in a medium whose refractive index is n, in which the wavenumber is measured, and c is the velocity of light in a vacuum (2.997925×10^{10} cm/sec).

According to quantum theory and principle conservation of energy, the wavenumber of the absorbed or emitted photon is equal to the changed in the molecular energy term expressed in cm^{-1} (Colthup, Daly, & Wiberley, 2010).

$$\bar{\nu} = \frac{\Delta \mathrm{Em}}{hc} = \frac{\nu}{\left(\frac{c}{n}\right)}$$

where $\Delta E_m =$ molecule of energy (gain energy if positive and loss energy if negative); h is Plank's constant; c is the velocity of light in a vacuum.

At the normal mode of vibration, all atoms in the molecule vibrate with the same frequency and all atoms pass through their equilibrium position simultaneously. Absorption

frequency depends on the molecular vibrational frequency. The absorption intensity depends on how effectively the infared photon energy can be transferred to the molecule and also depends on change in the dipole moment that occurs as a result of molecular vibration (Colthup, Daly, & Wiberley, 2010).

When the electromagnetic radiation of energy content *hv* irradiates a molecule, the energy may be transmitted, absorbed, or scattered. In Rayleigh scattering, the molecules scatter the light with no change in wavelength of the individual photons. In a Raman spectrometer, the sample is irradiated with higher radiation frequency in vibrational frequencies than electronic frequencies and normally in the visible part of the spectrum (Colthup, Daly, & Wiberley, 2010). The spectrometer analyzed the radiation scattered by the sample. Rayleigh scattering is an elastic collision between the incident photon and the molecule as frequency of the scattered photon is the same as that of the incident photon. Raman Effect is inelastic collision and the frequency of the incident photon is normally much greater than molecular frequency (Colthup, Daly, & Wiberley, 2010).

Raman and meat characteristics

Quality evaluation techniques for meat production are required to meet the demand of bulk manufacture. Instrumentation that can deal with large sample size in a short time and use in routine analysis is essential (Chizzolini, Novelli, Badiani, Rosa, & Delbono, 1993). Quality of chicken meat immediate after slaughter and deboning can be evaluated and predicted for quality attributes which are important to consumer.

Moisture loss or drip loss affects meat quality causing unattractive appearance, affect meat texture and processing of meat and sales (Forrest, Morgan, Borggaard, Rasmussen, Jespersen, & Andersen, 2000). Prediction of water holding capacity by vibrational spectroscopic

methods has been investigated. Comparing four spectroscopic instruments (fiber optical probe, visual, near infrared reflectance spectrophotometer, low field nuclear magnetic resonance) to predict water holding capacity from different stress level porcine and different type of muscle was investigated and low field magnetic resonance (LF-NMR) was a successful technique for the prediction of WHC (Brøndum, Munck, Henckel, Karlsson, Tornberg, & Engelsen, 2000). Online determination of water holding capacity at early post-mortem has been developed by using various techniques: UV fluorescence and NIR (Swatland & Barbut, 1995).

The changes in protein structure (myosin, actomyosin, collagen, etc.), intact muscle, and muscle product could be observed by Raman spectra. The myofibrillar protein conformation could be observed from the amide III region (Camou, Marchello, Thompson, Mares, & Goll, 2007). Raman spectroscopy was used to investigate the difference in water holding capacity of pork meat comparing between research meat and commercial meat and to predict quality of the muscle (Pedersen, Morel, Andersen, & Engelsen, 2003). There were several conclusions regarding to this study. First, the Raman regions of interest to WHC were 3128-3071 and 876-951 cm^{-1}. Second, there were more poor spectra considering as outliners found from commercial meats that because the speed of measure was not fast as much as the speed of the processing line. Suggestions were made to increase sample size, improve Raman to have more robust.

Peak assignment

Changes in the Raman bands of chemical groups in muscle proteins give information on changes in secondary structure of proteins: amide conformation region, C-C stretching vibration and changes in tryptophan residues, tyrosyl doublet, aliphatic amino acids bands (Herrero, 2008b). Appendix A summarizes the assignment of Raman bands that are useful in the interpretation of protein structure from the literature.

The conformational region of the amide is the most useful Raman band for the changes in secondary structure of protein (α-helix, β-sheet, turn, and random coil), which are assigned as amide I, amide II, and amide III bands (Herrero, 2008b). Amide I is usually used to quantify the secondary structure of proteins. Amide II vibration usually cannot be detected because a small change in polarizability associated with amide II. Amide III region has some overlaps between α-helix, β-sheet, turn, and random coil (Herrero, 2008b). C-C stretching vibration is also the involved in the change in secondary structure of protein. The gradual loss of these structures showed the broaden and weaken in intensity of this band (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008). Many Raman bands showed responding to the change of tryptophan residues in proteins. Herrero (2008) concluded from the literature that tertiary structure of proteins can be detected from a decrease in peak intensity when buried tryptophan residues in the protein. Also, if there is high ratio of intensity I_{1360}/I_{1340}, it indicated a hydrophobic environment, and a low ratio indicated tryptophan was involved in H-bonding of a hydrophilic environment (Herrero (2008) . Tyrosine is used an indicator of the hydrogen bonding of the phenolic hydroxyl group (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008). Herrero (2008) reported the decrease in intensity of aliphatic amino acid bands could possibly result from hydrophobic interactions of aliphatic residues. Other amino acids that might be useful for Raman assessment of meat quality include proline and hydroxproline, which are the amino acids of the connective tissue proteins such as collagen (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008).

Herrero (2008) reported that collagen, the main connective tissue, contains high levels of amino acids: proline and hydroxyproline. Collagen showed two amide bands at 1271 and 1248 cm^{-1} (Frushour & Koenig, 1975). The Raman predicted the sensory quality of beef silverside by

observing the changes in 1460-1483 cm^{-1} region, corresponding to the juiciness in cooked beef (Beattie J. R., Bell, Farmer, Moss, & Desmond, 2004).

Proteomics

Proteomics is a tool to identify proteins that might influence quality traits of meats. Proteomics is defined as "the qualitative and quantitative comparison of proteomes under different conditions to understand cellular mechanisms underlying biological processes and decipher the mechanisms of gene expression control" (Anderson & Norman, 1998). Proteomics offers a new way to identify protein in food matrix, study protein-protein interactions in raw and processed food, and study interaction between protein and other food component. Proteomics has been used in cereal science and food allergy prevention, to increase knowledge in their physiological and technological function and to identify and characterize allergenic proteins in food (Carbonaro, 2004). This technology will design better foods to enhance human health, truly understand the intrinsic and indispensable properties of foods (Han & Wang, 2008).

Two-dimension electrophoresis (2DE)

Two-dimension electrophoresis (2DE) uses electrophoretic separation based on two electrophoretic variables and can identify proteins, change in protein expression level and isoforms or post-translational modifications (Rabilloud, 2002). Mostly two-dimension electrophoresis will refer to the type of system, for example, first dimension is for isoelectric focusing which separates proteins by charge in polyacrylamide gel to generate a pH gradient followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension which separates proteins by molecular weight (Rabilloud, 2002). Most proteins are soluble in either SDS or urea or solubilization buffer. Urea is more effective than SDS for tissue

solubilization. SDS gives protein negative charge and cause protein to migrate to cathode (Adams & Gallagher, 1992).

SDS-PAGE gels are made with relatively high concentrations of acrylamide to restrict migration distance on the basis of size whereas IEF gels have lower concentrations of acrylamide so that separations strictly by differences in net charge (Bárány, Bárány, & Giometti, 1995). DS-PAGE is preferable to reverse-HPLC for separation of hydrophobic membrane proteins and peptides. Although electro-elution has largely been placed by electro-blotting, it is still applied successfully, e.g., for protein mass analysis by mass spectrometry (MALDI-MS) (Michalski & Shiell, 1999). Proteins with isoelectric points above pH 8 cannot be resolved using the classical IEF for first dimension of 2DE (Bárány, Bárány, & Giometti, 1995) because the very high electro endosmotic flow caused by charged groups on the glass walls of the gel tubes and also because of the properties of the synthetic carrier ampholytes used to generate the pH gradients. The immobilized pH gradients (IPG) have been used to overcome those problem which ampholytes have been replaced by Immobilines which can generate a pH gradient immobilised in a gel matrix. The IPG gives slope from 0.5 to 0.01 pH units/cm allowing reproducible separation of proteins that differ by 0.001 pH units in the isoelectric points(Michalski & Shiell, 1999).

Advantages of 2DE:

1) Allowing separation of proteins that would migrate as a single band using either IEF or SDS-PAGE alone (Bárány, Bárány, & Giometti, 1995) to study whole cell or tissues homogenates which contain many proteins with similar isoelectric point or molecular masses,

2) 2DE provides better resolution than either IEF or SDS-PAGE alone and appear to be a preferred method in isolating proteins for sequencing and amino acid analysis (Adams & Gallagher, 1992; Michalski & Shiell, 1999)
3) Contrast to liquid chromatography-tandem mass spectrometry based methods, which perform analysis on peptides, where molecular weight and pI information is lost, where stable isotope labeling is required for quantitative analysis (Görg, Obermaier, Boguth, Harder, Scheibe, Wildgruber, Weiss, & 2000)
4) 2DE has capability to study proteins that have undergone some form of PTM (such as phosphorylation, glycosylation or limited proteolysis) (Görg, Weiss, & Dunn, 2004)

Disadvantages of 2DE:

1) Visualization of proteins from total cell or tissue extracts lie in the high dynamic range of protein abundance, and the diversity of proteins with respect to molecular weight, pI and solubility. The presence of high-abundance proteins often masks low-abundance proteins and thus prevent their detection and identification in proteome study (Görg, Weiss, & Dunn, 2004; Hollung, Veiseth, Jia, Færgestad, & Hildrum, 2007). To avoid the problem, protein extraction or pre-fractionation is needed (Bárány, Bárány, & Giometti, 1995; Görg, Weiss, & Dunn, 2004; Rabilloud, 2002).
2) Sample preparation should be simple to increase reproducibility. Protein modifications during sample preparation must be minimized to avoid protein losses (Bárány, Bárány, & Giometti, 1995; Görg, Weiss, & Dunn, 2004; Hollung, Veiseth, Jia, Færgestad, & Hildrum, 2007).
3) There is no common protocol for choice of extraction method yet to complete proteome in one run. Very hydrophobic proteins, membrane proteins and high molecular weight

proteins are often difficult to solubilize and to analyze by 2DE (Hollung, Veiseth, Jia, Færgestad, & Hildrum, 2007)..

4) Proteolytic enzymes in the sample must be inactivated. Samples containing urea must not be heated to avoid change heterogeneitites caused by carbamylation of the proteins by isocyanate formed in the decomposition of urea (Dunbar, 1987; Görg, Weiss, & Dunn, 2004).

Major steps of the 2DE-MS include: 1) sample preparation to ensure that each spot represents an individual polypeptide and protein solubilization by cell disruption, inactivation or removal of interfering substances and solubilization of the proteins; 2) protein separation by 2DE; 3) protein detection and quantitation; 4) computer assisted analysis of 2DE pattern; 5) protein identification and characterization; 6) 2D protein database construction (Görg, Weiss, & Dunn, 2004).

Mass spectrometry (MS)

Mass spectrometry (MS) is a powerful tool that can facilitate microscale analyses of proteins and peptides (Michalski & Shiell, 1999) according to their mass to charge ratio (*m/z*). The molecule is ionized by one of several techniques, and the ion is propelled into a mass analyzer by an electric field that resolves each ion according to its *m/z* ratio. The detector passes the information to the computer for analysis. Ionization methods frequently used are matrix-assisted laser desorption/ionization (MALDI) and electro-spray ionization (ESI) (Carbonaro, 2004). The analyzer is often a time-of-flight analyzer (TOF) which the velocity of ion reaches the detector, at the constant voltage, is determined by its mass (Carbonaro, 2004).

Proteomics and meat characteristics

2DE is a useful technique for identifying differentially expressed protein that are associated with meat quality (Bendixen, 2005; Giometti & Anderson, 1982; Mullen, Stapleton, Corcoran, Hamill, & White, 2006; Rabilloud, 2002) and for indentified skeletal muscle from rat (Yan, Harry, Wait, Welson, Emery, Preedy, & Dunn, 2001). However, there are several weaknesses of this technique; difficulty in the automation of 2-D electrophoresis, pre-fractionation strategies may be needed to reach the less abundant proteins, and the problems liked with protein extraction and solubility during 2-D electrophoresis especially poorly water-soluble proteins (Rabilloud, 2002). It is advisable to keep sample preparation as simple as possible to avoid protein losses. The presence of high-abundance proteins in a tissue or cell often masks low-abundance proteins and prevent their detection and identification in proteome studies. To assist this problem, pre-fractionation is used to identify and detect low-abundance proteins. Many methods for 2DE have been published differing primarily in the apparatus used (Bárány, Bárány, & Giometti, 1995). Amino acid composition or post-translational modifications such as phosphorylation (Bárány, Bárány, & Giometti, 1995). Current proteomics studies have revealed that the majority of identified proteins are housekeeping proteins, whereas proteins such as receptor molecules which are present at much lower concentrations are usually not detected. Improved methods, such as pre-fractionation procedures, as well as more sensitive detection and quantification methods for enrichment of low-abundance proteins are required, (Görg, Obermaier, Boguth, Harder, Scheibe, Wildgruber, Weiss, & 2000).

Proteomics have been used to study the changes in pork and beef quality associated with post-mortem aging (Choi, Lee, Choe, Rhee, Lee, Joo, & Kim, 2010; Hwang, Park, Kim, Cho, & Lee, 2005; Lametsch, Kristensen, Larsen, Therkildsen, Oksbjerg, & Ertbjerg, 2006; van de Wiel

& Zhang, 2007; Zapata, Zerby, & Wick, 2009), growth development in chicken and pig (Agudo, Gómez-Esquer, Díaz-Gil, Martínez-Arribas, Delcán, Schneider, Palomar, & Linares, 2005; Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004; Hollung, Grove, Færgestad, Sidhu, & Berg, 2009; Mekchay, Teltathum, Nakasathien, & Pongpaichan, 2010; Teltathum & Mekchay, 2009), to evaluate protein changes in pork product (Luccia, Picariello, Cacace, Scaloni, Faccia, Liuzzi, Alviti, & Musso, 2005; Picariello, De Martino, Mamone, Ferranti, Addeo, Faccia, SpagnaMusso, & Di Luccia, 2006), to identify protein expression in beef post-mortem and protein mapping (Kim, Cho, Lee, Park, Lee, Cho, Choy, Yoon, Im, & Park, 2008; Muroya, Ohnishi-Kameyama, Oe, Nakajima, & Chikuni, 2007; Sawdy, Kaiser, St-Pierre, & Wick, 2004). The young birds display relatively low quantities of the glycolytic enzymes such as triosephosphate isomerase (Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004). Of the contractile proteins, actin is the most prominent in the soluble fraction of youngest birds (Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004).

The information from proteome can be used in optimizing processing method and meat technologies (Bendixen, 2005) and assist geneticist to select the marker in poultry (Anthony, 1998). Most of proteomic tools are based on protein separation in at least two dimensions, using either chromatographic methods or electrophoresis and is followed by the use of mass spectrometry.

Conclusions

Selection for rapid growth and ability to produce acceptable products to industry and consumer and decrease environmental influences are desirable. Online monitoring method is

gaining interest to be used in the production line and is benefit to further processing products. Molecular technology such proteomics approach will assist effectiveness in breeder to select the heritable growth associated with heritable traits and production yield.

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Table 2.1. Major myofibrillar proteins in skeletal muscle

Myofibriallar protein	Percent of myofibriallar protein	Function
Contractile		
Myosin	45	Muscle contraction in association with actin
Actin	20	Muscle contraction in association with myosin
Regulatory		
Troponin Complex	5	
Troponin C	1.3	Binds calcium
Troponin I	1.5	Inhibits actin/myosin interaction
Tropomyosin	5	Binds actin and troponin
Cytoskeletal		
Titin	10	Possible scaffold for sarcomere organization; myofibril elasticity
Nebulin	4	Regulates thin filament assembly and length

Adapted from Chiang (2007)

Table 2.2. Raman spectroscopy studies on muscles

Studies	Laser type	λ (nm)	Laser power (mW)	References
Raw porcine	Diode	785	30	Pedersen, Morel, Andersen, & Engelsen (2003)
	HeNe	632	5	
Lipid-prot		785	300	Meng, Chan, Rousseau, & Li-Chan (2005)
Cooked meat	Titanium:Sapphire CW	785	N/A	Beattie, Bell, Farmer, Moss, & Desmond (2004)
Meat batter	Nd:YAG	1064	300	Herrero, Carmona, Cofrades, & Jiménez-Colmenero (2008)
Porcine	Titanium:Sapphire CW	785	100	Beattie J. Renwick, Bell, Borggaard, & Moss (2008)
		633	20	
		514	10	
Pork aging, salting addition	Ar+ pumped Ti:Sapphire	785	60	Böcker, Ofstad, Wu, Bertram, Christine, Sockalingum, Manfait, Egelandsdal, & Kohler (2007)
Aging & cooked pork	Titanium:Sapphire CW	785	100	Beattie J. Renwick, Bell, Borggaard, & Moss (2008)
Surimi gel	Argon ion	488	100	Bouraoui, Nakai, & Li-Chan (1997)
Extrusion	He:Ne	785	500	Miller (2008)
Poultry	Diode	785	78	Ellis, Broadhurst, Clarke, & Goodacre (2005)
Pork postmortem	N/A	671 & 785	N/A	Jordan, Thomasius, Schröder, Wulf, Schlüter, Sumpf, Maiwald, Schmidt, Kronfeldt, Scheuer, Schwägele, & Lang (2009)
Sarcoplasmic protein, pH	He:Ne	632.8	N/A	Tadpitchayangkoon, Park, Mayer, & Yongsawatdigul (2010)

CHAPTER 3

BREAST MUSCLE ATTRIBUTES IN FAST AND SLOW GROWING CHICKENS*

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Summary

There is empirical evidence that selection for growth has affected meat attribute. Direct evidence has come from very small populations. A more reliable estimate of the relationship between growth rate and meat quality attributes is needed. Our goal was to investigate the meat physico-chemical attributes in two chicken populations with significant differences in growth rate from the same strain. We established two chicken populations from a random mating broiler control population; a slow growing sub-population (SG) with an average growth rate of 229 g/wk and a fast growing sub-population (FG) with an average growth rate of 319 g/wk. The initial and final pH was higher in the FG compared to the SG population. Whereas, there was no difference in a* between the two populations, the SG had significantly higher L* and b* compared to the FG. Water holding capacity was similar in the populations, but drip loss (DL) was higher in the SG compared to the FG. Cook yield (CY) was higher in the FG compared to the SG. There was a positive correlation between pH difference (pHDiff) and DL only in the FG. Fast growing chickens showed better technological yields than slow growth chickens. Chickens with low *P.* major weight and slow growth rate may not attain the same level of maturity as the FG chickens and should be segregated for different applications. The higher L* value and lower pH15 and pHu in the SG population coupled with higher DL and lower CY maybe an indication of pale soft exudative meat-like (PSE-like). Color parameters could be used to segregate muscle for further processing. Characteristic differences between SG and FG populations maybe related to different types and intensity of proteins found in SG and FG populations.

Keywords: Growth, poultry, meat characteristics, chicken, breast muscle

Introduction

The broiler poultry production has substantially increased, and the success of poultry production has been strongly related to improvements in growth and carcass yield. Economic viability of the poultry meat industry will depend on improving the consumer perception of poultry meat quality, such as appearance (color), eating quality (texture, flavor), and functional attributes, such as water holding capacity (WHC), drip loss, cook yield, pH, and texture (Fletcher, 2002; Forrest, Morgan, Borggaard, Rasmussen, Jespersen, & Andersen, 2000; Le Bihan-Duval, Debut, Berri, Sellier, Santé-Lhoutellier, Jégo, & Beaumont, 2008; Northcutt, 1997; Woelfel, Owens, Hirschler, Martinez-Dawson, & Sams, 2002). These quality parameters influence the profitability of poultry processors and retailers. Good WHC is essential in protein-based food products (Le Bihan-Duval, Debut, Berri, Sellier, Santé-Lhoutellier, Jégo, & Beaumont, 2008; Northcutt, 1997). Meat lighter-than-normal in color and low in pH was associated with low WHC. Variation in muscle chemical and physical composition, pH, and protein extractability directly affect WHC, emulsifying capacity, gel strength, and cook loss (Xiong, Cantor, Pescatore, Blanchard, & Straw, 1993).

Muscle pH and meat color have high correlations to meat characteristics especially at extremes low or high values. Higher muscle pH is associated with darker meat and lower muscle pH is associated with lighter meat (Fletcher, 2002). Water binding nature of the proteins is affected by pH resulting in change in physical structure of meat and light reflecting properties (Fletcher, 2002; Owens, Hirschler, McKee, Martinez-Dawson, & Sams, 2000; Woelfel, Owens, Hirschler, Martinez-Dawson, & Sams, 2002). Color measurement at 24 h post-mortem has good potential for pork meat quality grading and was used to classify meat on the basis of color intensity (C*), type of color (Hue) and exudation (L*) (Chizzolini, Novelli, Badiani, Rosa, &

Delbono, 1993). Although pH and lightness are correlated, no specification for lightness can be established that can clearly differentiate poultry meat quality (Fletcher, 2002).

A few studies have attempted to relate protein quality, type of chicken and growth rate (Berri, Elisabeth Le, Elisabeth, Pascal, Laurent, Nathalie, Maxime, Michel, & Michel Jacques, 2005; Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004; Le Bihan-Duval, Berri, Baeza, Millet, & Beaumont, 2001; Le Bihan-Duval, Debut, Berri, Sellier, Santé-Lhoutellier, Jégo, & Beaumont, 2008; Teltathum & Mekchay, 2009). The poultry industry has experience rapid growth in the past 50 year. The increase in poultry meat production is the result of intense selection for growth. Selection for growth has affected meat attributes.

Fast growing chickens are being more adapted to the further processing than slow growing in term of profitability; however, only few studies have attempted to relate growth rate in chicken to meat quality (Berri, Elisabeth Le, Elisabeth, Pascal, Laurent, Nathalie, Maxime, Michel, & Michel Jacques, 2005; Fanatico, Pillai, Emmert, & Owens, 2007; Le Bihan-Duval, Berri, Baeza, Millet, & Beaumont, 2001; Le Bihan-Duval, Debut, Berri, Sellier, Santé-Lhoutellier, Jégo, & Beaumont, 2008). The objective of this study is to investigate the variability of meat characteristics of breast muscle (*Pectoralis major*) from slow growing (SG) and fast growing (FG) chicken and ascertain the relationship of meat physico-chemical attributes in these chicken populations.

Materials and Methods

Chickens

We used the Arkansas randombred chicken population for this study. The population is a random mating broiler control line. Chicks were sexed at hatched and placed in pens (0.074 m^2/bird) with litter and fed a starter ration containing 225 g/kg protein, 52.8 g/kg fat, 25.3 g/kg fiber, 12.90 MJ ME/kg, 9.5 g/kg calcium (Ca), and 7.2 g/kg total phosphorus (P) (4.5 g/kg available P) until 18 d of age. Hereafter, they were fed a grower ration of 205 g/kg protein, 57.6 g/kg fat, 25.0 g/kg fiber, 13.20 MJ ME/kg, 9.0 g/kg Ca and 6.7 g/kg total P. (4.1 g/kg available P). The birds were kept on an 20L:4D light regimen. Body weight (BW) was measured at hatch and hereafter weekly until 6 weeks. Two sub-populations; slow growing (SG) and fast growing (FG), were created based on their growth rate from hatch until 6 wk of age. There were 572 and 574 individuals in the SG and FG populations, respectively. Growth rate (GR) was calculated as the average difference between BW at 6 wk (BW6) and BW at hatch (BW0). At 6 wk of age, the chickens were slaughtered and chilled at 4°C overnight prior to deboning. *Pectoralis* muscle weight (PMW) was measured during deboning. *Pectoralis* muscle yield (PMY) was calculated as percent PMW of BW6.

Deboned *P. major* muscle was analyzed for pH color by Minolta Chroma meter (L*, a* and b*), then packed individually in the sealed plastic bag and stored at 4 °C. Analysis for water holding capacity (WHC), drip loss (DL), and cook yield (CY) was done within 2-5 d post-mortem.

Meat Physico-Chemical Attributes

pH measurement

The pH at 15 min (pH15) and 24 h post-mortem (pHu) were measured with a portable meter (Model IQ240, IQ Scientific Instruments, San Diego, CA, USA) equipped with a Piercing Tip Micro Probe (PH56-SS). At 15 min post-mortem, the pH probe was directly inserted to the right of carcass at 1 inch deep. At 24 hr post-mortem, pH probe was directly inserted 1 inch at the side of the thickest part of right breast muscle. The difference between pHu and pH15 was denoted pHDiff.

Color measurement

Color of the left surface of thickest part of *P. major* muscle was measured in 3 replicates of each sample at 24 h post-mortem using a Minolta colorimeter (Model CR310, Minolta Corp., Osaka, Japan) with the CIE L* a* b* system (Commision International de l' Eclairage), illuminant D65, 2° observer angle, and 50 mm port diameter. L*, a*, and b* represent lightness, green to red, and blue to yellowness. Hue angle (H°) and chroma (C*) was calculated by using equation (Minolta, Co, & Ltd, 1991): $C^* = [(a^*)^2 + (b^*)^2]^{-1/2}$, $H^\circ = \tan^{-1}(b^*/a^*)$ when $a^* > 0$ and $b^* \geq 0$.

Water holding capacity (WHC)

The WHC of breast meat was measured at 2 d post-mortem modified from Barbut (Barbut, 1996). Whole breast of *P. major* muscle that ranged from 54-334 g was minced in a small chopper (Model KFC3100, Kitchen Aid, USA) for 60-90 s at ambient temperature. A 10 g aliquot of the chopped muscle was mixed with 16 mL of 0.6 mol L^{-1}NaCl and incubated for 30 min at 4 °C using a shaker waterbath (Model G76D, New Brunswick Scientific, Edison, N.J., USA) at 175 RPM. Samples were centrifuged at 7000 g at 4 ºC for 15 min (Sorvall RC-5B; rotor:

Sorvall SS-34, Du Pont Instruments, Wilmington, DE), and the excess fluid was decanted. The WHC was defined as the portion of fluid retained by the sample and expressed as a percentage of the initial weight.

Drip loss (DL)

The *P. major* muscle was weighed at 5 d post-mortem after blotting dry by paper towel. DL was calculated as a percentage relative to the weight of the muscle at 1 d post-mortem.

Cook yield (CY)

The *P. major* muscle was weighed at 5 d post-mortem, tagged, and cooked in a smokehouse at 71% relative humidity, dry bulb temperature of 87.7 °C, and wet bulb temperature of 79.4 °C until the internal product temperature reached 78 °C. Then, The *P. major* was showered with cool water for 3 min on and 1 min off, repeatedly until internal product temperature dropped to 60 °C. Cooked muscle was blotted dry by paper towel and reweighed. CY was calculated as drip weight at 5 d divided by initial weight at 1 d.

All procedures relating to the use of live animals were approved by the University of Georgia Institutional Animal Care and Use Committee.

Statistical Analyses

Data were analyzed using PROC MIXED procedure of SAS[16] and least squares means for all traits were computed. PROC CORR (SAS Institute Inc., 2002) was used to analyze linear correlations between quality parameters. A probability level of 0.05 or less was considered as significant.

Results and Discussion

The growth and meat characteristics of the two chicken populations are listed in Table 3.1. The total number of observations for each variable depended on the selection for each assay. The hatch weight, BW6, GR and PMY of the FG population were significantly higher than that of the SG population. The SG population accrued about 229 g/wk compared to almost 319 g/wk for the FG population. The initial and final pH were higher ($P<0.05$) in the FG compared to the SG population. Whereas, there was no difference in a* between the two populations, the SG population had significantly higher L* and b* compared to the FG population. Water holding capacity was similar in the populations, but DL was significant higher in the SG population compared to the FG population. On the other hand, CY was significantly higher in the FG population compared to the SG population. The Hº was significantly higher in the FG population compared to the SG population. The Pearson correlation coefficients between attribute traits were summarized in Table 3.2 and 3.3 for SG and FG populations. In both SG and FG populations, pHu was negatively correlated with L* and b*, and positively correlated with a*, however, pHDiff was positively correlated with L* and b*, but negatively correlated with a*. In both populations, CY was positively correlated with hatch weight. Hº was positively correlated with pHu and negatively correlated with pH15 and pHDiff in both chicken populations. C* was not correlated with pH15, pHu nor pHDiff, but correlated with their determinants (a* and b*) in both populations. DL was positively correlated with L* in both populations, however, the correlation coefficient was higher in SG population. DL was correlated with pHu in both populations. There were some unique differences in the relationships among meat attributes between the SG and FG populations. There was a positive correlation between pHDiff and DL in the FG population, however, there was no such correlation in the SG population. The pHDiff was

positively correlated with PMY, as well as CY and PMY only in the SG population. In the SG population, both C* and Hº were correlated with L*, however, in the FG population, Hº was correlated with L*, but not the C*.

The current studies show some differences among technological parameters in relation to growth. *P. major* muscles of fast growing chickens have relatively better meat quality attributes compared to slow growing chickens. Changes in pH tend to relate to quality of meat by modifying its DL and CY. Initial pH was higher than pHu in both SG and FG populations because immediately after slaughter, the carcass may still be warm and the presence high concentrations of ATP so post-mortem glycolysis occurred. With the breakdown of ATP and the formation of actomyosin during rigor mortis, lactic acid increased which caused a reduction in the pH, and a steric effect occurs in which there is a reduction in the space of the myofibrils. Water is forced from the intracellular spaces to the extra myofibrillar spaces where the fluid can be more easily expelled (Belitz, Grosch, & Schieberle, 2004; Huff-Lonergan & Lonergan, 2005). Differences in rates of post-mortem glycolysis may be reflected in different ultimate pH values as shown there was significant correlation between pHu and pHDiff. Even though the FG population had a higher pHu compared to the SG population, there were no difference in their WHC with very low correlation between pHu and WHC but the difference showed in DL. As in our WHC measurement, 0.6 mol L^{-1}NaCl was added, addition of salt putatively augments ionic strength promoting water-binding in meat, dissociating the actomyosin cross-bridges, and swelling of myofibrils (Barbut & Findlay, 1989). Improvement of muscle structure by adding salt solution might be a reason why we could not observe difference between WHC estimates from SG and FG population as we observed from DL. It implies that SG muscle could be used as good as FG muscle in such application i.e. marination when added additive such salt.

The selection of rapid growth and muscle development can produce meat with higher pHu and consequently lower DL and higher CY which may affect the processing quality of meat (Berri, Elisabeth Le, Elisabeth, Pascal, Laurent, Nathalie, Maxime, Michel, & Michel Jacques, 2005). In both chicken populations, there was a negative relationship between pHu and both DL and CY, however, the *P. major* from the SG population showed more prominence to unacceptable for the meat industry and consumers as they showed higher drip loss and lower cook yield. Thus birds with low hatch weight and slow growth rate may not attain the same level of maturity as the fast growing birds and should be culled prior to processing. In the current study DL was higher than literature (Berri, Elisabeth Le, Elisabeth, Pascal, Laurent, Nathalie, Maxime, Michel, & Michel Jacques, 2005; Le Bihan-Duval, Berri, Baeza, Millet, & Beaumont, 2001; Le Bihan-Duval, Debut, Berri, Sellier, Santé-Lhoutellier, Jégo, & Beaumont, 2008; Van Laack, Liu, Smith, & Loveday, 2000; Woelfel, Owens, Hirschler, Martinez-Dawson, & Sams, 2002) that probably due to how it was calculated and the difference in chicken genotypes.

The *P. major* from the SG population had higher L* and b* values compared to the FG population, but both groups had similar a* values. The higher L* value and lower pH15 and pHu in the SG population coupled with higher DL and lower CY maybe an indication of soft exudative meat-like characteristic (PSE-like). Even though the cause of PSE syndrome is not well known, rapid ante-mortem stress and glycolysis could be implicated (Sosnicki, Greaser, Pietrzak, Pospiech, & Sante, 1998). In both groups of chicken, meat lightness (L*) was positively correlated with DL. The genetic relationship between L* and DL has previously been demonstrated (Le Bihan-Duval, Berri, Baeza, Millet, & Beaumont, 2001). Therefore, the L*value could be a good selection parameter to improve DL in both fast and slow growing chicken. In both populations, the L* value was negatively correlated with the Hue value. The

relationship between the L* value and chroma values in the SG and FG populations were different. Whereas, the L* value was positively related to the chroma value in the SG population which suggests more intense color, there was not such relationship in the FG population. Obviously, the dynamics among the SG population are different from the FG population, as the Hue value is negatively correlated with DL and chroma value is positively related to CY in the SG population.

The characteristic differences between SG and FG population muscle is hypothesized by different type and intensity of protein found in SG and FG population muscles. As parts of pre-select fresh muscle for further-processed products, the results showed that L* and b* could possibly be used to predict meat characteristic, drip loss. As part of further-processed products, adding salt could be applied to improve consumer acceptance and meat texture.

Conclusions

The current study indicated differences in meat technological parameters based on growth rate. Our results support previous works that observed differences in color and pH parameters in chickens that varied in quality. Based on these results, growth rate influenced the quality of breast meat. The correlation between growth and meat attributes traits were low, suggesting that the genetic mechanisms underlying those traits could be different and further indicating that selection for growth rate or breast meat yield would not negatively alter meat attribute. It is imperative for producers to ensure that all birds in their flock are growing according the target rate in order to avoid producing meat of lesser quality for the slow growing individuals.

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Table 3.1. Descriptive statistics of meat characteristics of slow growing and fast growing chickens[1]

Parameters	Slow Growth		Fast Growth	
	N	Mean	N	Mean
pH15	407	6.45[a]	348	6.57[b]
pHu	403	5.71[a]	374	5.82[b]
pHDiff	379	0.75[a]	328	0.78[a]
L*	399	57.00[b]	372	55.77[a]
a*	399	13.75[a]	372	13.94[a]
b*	399	11.20[b]	372	10.54[a]
WHC (%)	169	27.24[a]	139	25.33[a]
DL (%)	156	8.48[b]	153	6.44[a]
CY (%)	122	85.96[a]	79	86.92[b]
Hº	399	50.4[a]	372	52.7[b]
C*	399	17.84[a]	372	17.56[a]
BW0 (g)	572	37.25[a]	574	38.92[b]
BW6 (g)	572	1413.42[a]	574	1951.13[b]
GR (g/wk)	572	229.36[a]	574	318.70[b]
PMW (g)	467	155.44[a]	467	225.81[b]
PMY (%)	467	10.92[a]	467	11.57[b]

[1]pH15 = pH at 15 min post-mortem; pHu = pH at 24 h post-mortem; pHDiff = pH difference; L* = lightness; a* = green to red, b* = blue to yellowness; WHC = water holding capacity; DL = drip loss; CY = cook yield; C* = chroma; Hº = hue; BW0 = body weight at 0 wk; BW6 = body weight at 6th wk; GR = growth rate; PMW = *P. major* weight; PMY = *P. major* yield.

[a, b] Means within a row lacking a common superscript differ ($P < 0.05$).

Table 3.2. Pearson correlation coefficients (r) between attribute traits of chicken breast meat from slow growing (SG) chicken line

SG[1]	pH15	pHu	pHDiff	L*	a*	b*	WHC	DL	CY	Hº	C*	BW0	BW6	GR	PMW
pHu	0.18*														
	(213)														
pHDiff	0.76*	-0.46*													
	(209)	(209)													
L*	-0.11*	-0.31*	0.11*												
	(211)	(209)	(207)												
a*	-0.13*	0.11*	-0.22*	-0.06											
	(211)	(209)	(207)	(219)											
b*	0.00	-0.18*	0.14*	0.52*	0.23*										
	(211)	(209)	(207)	(219)	(219)										
WHC	-0.07	-0.15	0.05	-0.03	-0.02	-0.10									
	(76)	(80)	(75)	(79)	(79)	(79)									
DL	-0.06	-0.22*	-0.01	0.47*	-0.16*	0.08	ND								
	(87)	(90)	(73)	(90)	(90)	(90)	(0)								
CY	-0.23*	-0.28*	0.04	0.06	0.10	0.26*	ND	-0.08							
	(69)	(70)	(65)	(70)	(70)	(70)	(0)	(69)							
Hº	-0.13*	0.23*	-0.31*	-0.38*	0.66*	-0.52*	0.06	-0.21*	-0.08						
	(211)	(219)	(207)	(219)	(219)	(219)	(79)	(90)	(70)						
C*	-0.10	0.00	-0.08	0.21*	0.86*	0.69*	-0.07	-0.07	0.20*	0.22*					
	(211)	(219)	(207)	(219)	(219)	(219)	(79)	(90)	(70)	(219)					
BW0	-0.05	-0.02	-0.08	-0.04	-0.06	-0.11*	0.21*	-0.02	0.20*	0.16*	-0.01				
	(223)	(221)	(209)	(219)	(219)	(219)	(98)	(91)	(71)	(219)	(219)				
BW6	0.09	0.04	0.04	-0.02	-0.12*	-0.10*	0.04	-0.11	0.08	-0.01	-0.13	0.13*			
	(223)	(221)	(209)	(219)	(219)	(219)	(98)	(91)	(71)	(219)	(219)	(347)			
GR	0.09	0.05	0.05	-0.01	-0.12*	-0.09	0.03	-0.11	0.07	-0.02	-0.13	0.10*	1.00*		
	(223)	(221)	(209)	(219)	(219)	(219)	(98)	(91)	(71)	(219)	(219)	(347)	(347)		
PMW	0.11*	0.04	0.10	-0.15*	-0.14*	0.00	0.07	-0.26*	0.28*	-0.10*	-0.10	0.06	0.68*	0.68*	
	(219)	(217)	(205)	(215)	(215)	(215)	(96)	(91)	(70)	(215)	(215)	(278)	(278)	(278)	
PMY	0.08	0.02	0.11*	-0.18*	-0.10*	0.06	0.08	-0.29*	0.32*	-0.12*	-0.05	0.00	0.24*	0.24*	0.87*
	(219)	(217)	(205)	(215)	(215)	(215)	(96)	(91)	(70)	(215)	(215)	(278)	(278)	(278)	(278)

[1]pH15 = pH at 15 min post-mortem; pHu = pH at 24 h post-mortem; pHDiff = pH difference; L* = lightness; a* = green to red, b* = blue to yellowness; WHC = water holding capacity; DL = drip loss; CY = cook yield; C* = chroma; Hº = hue; BW0 = body weight at 0 wk; BW6 = body weight at 6th wk; GR = growth rate; PMW = *P. major* weight; PMY = *P. major* yield.
ND = not detected; (n) = number of sample used to calculate correlation coefficient
* $P \leq 0.05$.

Table 3.3. Pearson correlation coefficients (r) between attribute traits of chicken breast meat from fast growing (FG) chicken line

SG[1]	pH15	pHu	pHDiff	L*	a*	b*	WHC	DL	CY	Hº	C*	BW0	BW6	GR	PMW
pHu	0.12*														
	(177)														
pHDiff	0.74*	-0.55*													
	(175)	(175)													
L*	-0.01	-0.36*	0.22*												
	(176)	(194)	(174)												
a*	-0.08	0.21*	-0.23*	-0.43*											
	(176)	(194)	(174)	(194)											
b*	0.09	-0.14*	0.16*	0.44*	-0.25*										
	(176)	(194)	(174)	(194)	(194)										
WHC	-0.20*	-0.04	-0.19	0.08*	-0.15	0.00									
	(62)	(67)	(60)	(62)	(67)	(67)									
DL	0.10	-0.26*	0.20*	0.36*	0.00	-0.07	ND								
	(61)	(71)	(59)	(69)	(69)	(69)	(0)								
CY	-0.16	-0.25*	0.08	-0.18	-0.03	-0.13	ND	0.03							
	(30)	(38)	(28)	(36)	(36)	(36)	(0)	(39)							
Hº	-0.11*	0.21*	-0.23*	-0.54*	0.67*	-0.88*	-0.07	-0.07	0.10						
	(176)	(194)	(174)	(194)	(194)	(194)	(67)	(69)	(36)						
C*	0.01	0.06	-0.05	-0.00	0.62*	0.59*	-0.13	0.04	-0.12	-0.16*					
	(176)	(194)	(174)	(194)	(194)	(194)	(67)	(69)	(36)	(194)					
BW0	-0.08	0.05	-0.05	-0.01	0.06	-0.06	0.08	-0.14	0.23*	0.08	0.00				
	(187)	(196)	(175)	(194)	(194)	(194)	(88)	(73)	(40)	(194)	(194)				
BW6	0.08	0.17*	0.04	-0.05	0.01	-0.07	-0.16	-0.13	-0.04	0.06	-0.04	0.19*			
	(187)	(196)	(175)	(194)	(194)	(194)	(88)	(73)	(40)	(194)	(194)	(386)			
GR	0.09	0.17*	0.04	-0.05	0.01	-0.07	-0.16*	-0.13	-0.04	0.05	-0.04	0.16*	1.00*		
	(187)	(196)	(175)	(194)	(194)	(194)	(88)	(73)	(40)	(194)	(194)	(386)	(386)		
PMW	0.17*	0.25*	0.00	-0.12*	-0.10	0.08	-0.13	-0.13	0.18	-0.10*	-0.01	0.13*	0.51*	0.51*	
	(184)	(193)	(172)	(191)	(191)	(191)	(87)	(72)	(39)	(191)	(191)	(283)	(283)	(283)	
PMY	0.15*	0.19*	0.00	-0.13*	-0.12*	0.13*	-0.08	-0.09	0.22	-0.15*	0.01	0.06	0.06	0.06	0.89*
	(184)	(193)	(172)	(191)	(191)	(191)	(87)	(72)	(39)	(191)	(191)	(283)	(283)	(283)	(283)

[1]pH15 = pH at 15 min post-mortem; pHu = pH at 24 h post-mortem; pHDiff = pH difference; L* = lightness; a* = green to red, b* = blue to yellowness; WHC = water holding capacity; DL = drip loss; CY = cook yield; C* = chroma; Hº = hue; BW0 = body weight at 0 wk; BW6 = body weight at 6th wk; GR = growth rate; PMW = *P. major* weight; PMY = *P. major* yield.
ND = not detected; (n) = number of sample used to calculate correlation coefficient
* $P \leq 0.05$.

CHAPTER 4

PROTEOMIC ANALYSIS OF CHICKEN BREAST MUSCLE: DIFFERENTIAL PROTEIN EXPRESSION WITH VARYING GROWTH RATE AND WATER HOLDING CAPACITY

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Summary

Growth rate (GR) and water holding capacity (WHC) are important meat quality parameters. The objective of this study was to identify protein markers associated with slow- and fast-GR and low- and high-WHC from water soluble protein (WSP) and non-water soluble protein (non-WSP) extracts of breast chicken muscles by using 2-dimensional electrophoresis and MALDI-TOF mass spectrophotometry. A total of 22 selected protein spots were excised and analyzed by in-gel tryptic digestion and MALDI-TOF mass spectrometry. Expressed proteins in extracts from slow- and fast-GR and low- and high-WHC included: metabolic enzymes, such as creatine kinase, pyruvate kinase, triosephosphate isomerase, ubiqitin; housekeeping proteins, such as heat shock protein; contractile proteins, such as myosin heavy chain, actin and also MHC isoforms and actin isoforms. The mass spectra of 20 protein spots significantly matched (protein score > 83; $p<0.05$) online database. In non-WSP, there were unique proteins that were present only in fast-GR population: gi|118099530; gi|20664362; gi|71895043; gi|114794125; gi|297343122; gi|71895043. This information identified protein markers associated with growth rate and water holding capacity. Some of those protein markers could be added to chicken database.

Keywords: 2DE, growth rate, poultry, muscle proteome, proteomics, MALDI

Introduction

Proteomic methods are useful for the identification of protein markers associated with meat quality traits in animal species. These methods have been used to study the changes in pork and beef quality associated with post-mortem aging (Choi, Lee, Choe, Rhee, Lee, Joo, & Kim, 2010; Hwang, Park, Kim, Cho, & Lee, 2005; Lametsch R., Kristensen, Larsen, Therkildsen, Oksbjerg, & Ertbjerg, 2006; van de Wiel & Zhang, 2007; Zapata, Zerby, & Wick, 2009), growth development in chicken and pig (Agudo, Gómez-Esquer, Díaz-Gil, Martínez-Arribas, Delcán, Schneider, Palomar, & Linares, 2005; Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004; Hollung, Grove, Færgestad, Sidhu, & Berg, 2009; Mekchay, Teltathum, Nakasathien, & Pongpaichan, 2010; Teltathum & Mekchay, 2009), to evaluate protein changes in pork product (Luccia, Picariello, Cacace, Scaloni, Faccia, Liuzzi, Alviti, & Musso, 2005; Picariello, De Martino, Mamone, Ferranti, Addeo, Faccia, SpagnaMusso, & Di Luccia, 2006), to identify protein expression in beef post-mortem and protein mapping (Kim, Cho, Lee, Park, Lee, Cho, Choy, Yoon, Im, & Park, 2008; Muroya, Ohnishi-Kameyama, Oe, Nakajima, & Chikuni, 2007; Sawdy, Kaiser, St-Pierre, & Wick, 2004). The information from proteome can be used in optimizing processing method and meat technologies (Bendixen, 2005) and assist geneticist to select the marker in poultry (Anthony, 1998). Most proteomic techniques are based on protein separation in at least two dimensions, dependent on differing protein characteristics, using either chromatographic methods or electrophoresis, followed by mass spectrometry of selected chromatographic peaks or gel spots.

Growth rate, muscle yield and water holding capacity (WHC) are important quality attributes in the poultry industry. Body weight and breast meat yield of six-week-old chickens were considered for improvements of bird growth performance and muscle development (Berri,

2004). Curing-cooking yield was higher in fast growth chicken breast meat than slow growth chicken breast meat, and was attributed to a less rapid pH decline (Debut, Berri, Baéza, Sellier, Arnould, Guemené, Jehl, Boutten, Jego, Beaumont, & Le Bihan-Duval, 2003). Growth rate influenced some quality attributes of chicken breast meat, and the correlation coefficients between growth and meat quality traits were low (Phongpa-ngan, Mulligan, Aggrey, & Wicker, 2010). This relationship suggests that there are different genetic mechanisms underlying various quality traits; consequently selecting for growth rate or breast meat yield would not negatively affect meat quality.

Pale, soft, and exudative (PSE) pork meat is correlated with a defect in the expression of the halothane gene (Barbut, Sosnicki, Lonergan, Knapp, Ciobanu, Gatcliffe, Huff-Lonergan, & Wilson, 2008). Expression of halothane gene in muscle results in a defective Ca2+ release channel associated with the sarcoplasmic reticulum at twice the rate of release in normal pigs, hence it caused an augmented rate of muscle metabolism and lactic acid accumulation (Bowker, Grant, Forrest, & Gerrard, 2000). High concentration of calcium ions showed abnormally rapid postmortem metabolism and associated with the incidence of PSE-like in turkey (Strasburg & Chiang, 2009). PSE pork muscles tend to exhibit lower protein extraction and solubility than normal meat (Barbut, Zhang, & Marcone, 2005) and losses of molecular functionality (Bendall & Swatland, 1988; Camou & Sebranek, 1991) as well as poor water holding capacity (Alvarado, 2007; Richardson & Jones, 1987). The causes of PSE-like meat condition seemed to be related to selection of the birds for growth rate (Phongpa-ngan, Mulligan, Aggrey, & Wicker, 2010) and breast yield, or genetic mechanisms, as well as heat stress (Petracci, Bianchi, & Cavani, 2009).

The economic value of fast growth rate cannot be realized if quality attributes are compromised. The objectives of this study were to identify water soluble protein and non-water

soluble protein of chicken breast muscle that were segregated by growth rate and water holding capacity, and to use 2-dimensional electrophoresis and MALDI-TOF mass spectrophotometry to identify protein markers associated with growth rate and water holding capacity.

Material and Methods

P. major muscle was obtained and meat quality traits were measured as described in Phongpa-ngan (2010). From the previous results, slow-GR was defined as GR that was lower than or equal to 250 g/wk and fast-GR was defined as GR that was higher than or equal to 299 g/wk. Low-WHC was defined as WHC that was lower than or equal to 4.08% and high-WHC was defined as WHC that was higher than or equal to 89.26%. All muscles in this study were 2 d post-mortem, previously frozen at -20°C and thawed to 4 °C before extracting protein. A total of 6 breast muscles from 6 chickens of extreme GR; 3 slow- and 3 fast-GR, were used for protein extraction. There were a total of 6 breast muscles from 6 chickens of extreme WHC; 3 low- and 3 high-WHC for protein extraction.

Protein extraction

Water soluble protein and non-water soluble protein were extracted from selected samples according to the following procedures. The protein extraction was done in duplicate experiment.

Water soluble protein (WSP)

Extraction procedures were followed according to Barbut (2005). WSP was extracted from 5 g of minced meat sample using 50 mL of 0.025 M sodium phosphate buffer, pH 7.2. The sample was mixed using magnetic bar and magnetic stirrer (Model R015S1, IKA®_WERKE, Staufen, Germany) on ice to minimize protein denaturation for 1 h at setting speed 3.

Homogenates were centrifuged in a Sorvall RC-5B refrigerated super-speed centrifuge (Du Pont Instruments, Wilmington, DE, USA) at 7000 g for 15 min at 4°C. The supernatants were collected and labeled as WSP. The pellets were then processed further for extraction of non-WSP.

Non-water soluble protein (Non-WSP)

The pellets were mixed with buffer, containing 20 mM Tris (Catalog# 161-0719, Bio-Rad Laboratories, Hercules, CA, USA), 8M urea (Catalog# BP169-212, Fisher Scientific, Fair Lawn, New Jersey, USA), 1% SDS (Catalog# 161-0302, Bio-Rad Laboratories, Hercules, CA, USA). Homogenates were stirred setting speed 3 for 1 h on ice and centrifuged at 7000 g for 15 min at 4°C. The supernatants were collected as non-water soluble proteins.

Protein determination

Protein solubility

The concentration of proteins in the supernatants (non-WSP and WSP) was determined in duplicate experiment and duplicate assay using the test tube procedure for BCA protein assay with BSA as standard protein (Pierce, Rockford, IL) and the absorbance was read by UV-visible spectrophotometer (UV-pharmaspec-1700, Shimadzu, Japan) at 565 nm.

2DE

WSP and non-WSP were separated by isoelectric focusing (IEF) (Protean IEF cell, Bio-Rad Laboratories, Hercules, CA, USA) in the first dimension and the SDS-PAGE (15% gel) in the second dimension. A concentration of 300 µg protein of WSP samples was denatured before mixing with rehydration buffer by adding 25 µl of 5 mM tributylphosphine (TBP, Sigma-Aldrich Co., St. Louis, MO, USA) per 1 ml of protein and 30 µl of 15 mM iodoacetamide (Catalog# 163-2109, Bio-Rad Laboratories, Hercules, CA, USA) per 1 ml of protein solution. Then mixture was

centrifuged (Eppendorf 5415, Brinkman, Burtonsville, MD, USA) at 14,000 × g at room temperature for 5 min to precipitate insoluble material. An aliquot of 400 µl of cold acetone was added to the pellet and equilibrated for 20 min at -20ºC and non-protein contaminants were removed by centrifugation (Sorvall Legend Micro21R, Thermo Scientific, Germany) at 10,000 × g at 4ºC for 30 min.

Acetone precipitated WSP pellet was mixed with 125 µL of rehydration buffer. The rehydration buffer consisted of 1g of protein solubilization powder; PSB powder, 1.1 mL PSB diluents (Chemicals in ReadyPrep™ Protein Extraction Kit, catalog# 163-2087, Bio-Rad Laboratories, Inc., Hercules, CA, USA), 20 µl of 200 mM (in N-methyl-2-pyrrolidinone) tributylphosphine (TBP) (Catalog# T7567, Sigma-Aldrich Co., St. Louis, MO, USA), 20 µl Bio-Lyte 3/10 buffer (100x, Catalog# 163-2094, Bio-Rad Laboratories, Inc., Hercules, CA, USA), 5 µl of 0.002% (w/v) bromophonol blue (Catalog# 80-0082, ESA Inc., Chemlmsford, MA, USA). The protein mixture was centrifuged (Eppendorf (Model 5415, Brinkman, Burtonsville, MD, USA) at 14,000 × g at room temperature for 10 min to precipitate undissolved particles. For non-water soluble samples, a concentration of 300 µg protein of non-WSP was directly mixed with rehydration buffer to a total volume of 125 µL per IPG gel strip (Catalog# 163-2000, length 7 cm, pH range 3-10, Bio-Rad Laboratories, Hercules, CA, USA).

Each loaded sample IPG gel strip was overlaid with mineral oil (Catalog# 163-2169, Bio-Rad Laboratories, Hercules, CA, USA) to prevent evaporation during rehydration process and rehydrated for 12-16 hours at 20ºC at 50 µA/IPG strip. After rehydration was complete, a wet paper wick (Catalog# 165-4071, Bio-Rad Laboratories, Hercules, CA, USA) was placed at each end of the channels as receptacles for salts and other non-amphoteric constituents of the samples.

Rapid ramping protocol was used to focus WSP. The voltage was raised from 250 V to 4,000 V in 1h 30 min then maintained at 4,000 V for 20,000 V-hr. For non-WSP, a membrane method was selected to focus, and the voltage was raised from 250 V to 500 V in 1 h with rapid ramping, then from 500 V to 4,000 V in 1 h with slow ramping, then with slow ramping for 2 hours to increase the voltage. As in the previous step, if the voltage had not reached 4000 V, then the final step was maintained at 4,000 V for 20,000 V-hr with rapid ramping to ensure that final voltage was reached to focus samples.

At the completion of focusing, the IPG strips were equilibrated in SDS-PAGE equilibration buffer containing 6 M urea (Catalog# 80-0070, ESA Inc., Chelmsford, MA, USA), 20% glycerol (Catalog# G5516, Sigma-Aldrich Co., St. Louis, MO, USA), 2% SDS (Catalog# 161-0416, 10% (w/v) SDS solution, Bio-Rad Laboratories, Hercules, CA, USA), 0.375 M Tris-HCl, pH 8.8 (Catalog# 161-0798, 1.5M resolving gel buffer, Bio-Rad Laboratories, Hercules, CA, USA), 2 mM TBP (Catalog# 126-73-8, Sigma-Aldrich Co., St. Louis, MO, USA), for 20 min at room temperature before application onto vertical sodium dodecyl sulphate (SDS)-polyacrylamide gels.

The gel strip was loaded onto large format (22 cm × 22 cm × 1 mm) 15% Tris-HCl acrylamide slab gels that were prepared according to the manufacturer's instructions (Protein electrophoresis application guide, Hoefer Scientific Instruments, SF, CA, USA) for the second dimension. The tank buffer contained 25 mM Tris, 192 mM glycine (Catalog# G-7126, Sigma-Aldrich Co., St. Louis, MO, USA) and 0.1% SDS. The separation was performed by protein electrophoresis apparatus connected with power supply (Model EV 265, Hoefer Scientific, SF, CA, USA) and with refrigerated circulation bath (RCB-20 Hoefer, Hoefer Scientific, SF, CA,

USA) to run gel at 30 mA/gel at 16ºC until the dye front reached within 8 cm from the top of the gel.

Fixing and Staining

After running the second dimension, the gels were fixed for 30 min with fixing solution containing 40% methanol (Catalog# 9093-03, J. T. Baker, Inc., Phillipsburg, New Jersey, USA), 10% acetic acid (J. T. Baker, Inc., Phillipsburg, New Jersey, USA), and 50% Type I water and stained overnight in stain solution containing 0.1% Coomassie Brilliant Blue G-250 (Bio-Rad, Laboratories, Richmond, CA, USA) in 40% methanol, 7% acetic acid, and 53% Type I water. They were washed with Type I water several times until background was removed. The gels were scanned using scanner (Model C9850A, Hewlett-Packard, China), and temporarily kept in closed plastic bag with a small amount of Type I water and stored at 4 ºC.

Proteomics

A reference gel was selected as the gel among the replications which had more counts of protein spots. Manual editing was performed to remove artifacts and mismatched spots. The landmark spots on reference gel were selected from most spots presented in all gels. Protein spots on the 2DE gel were matched to the reference gel by aligning the gel with the landmark spot on the reference gel. Relative density ratio for each spot was calculated from combination of spot area and spot density. The spots that were statistically different expressed between slow- and fast-GR and between low- and high-WHC greater than 1.5 fold in relative density ratio were excised for proteomic analysis.

Proteomic analysis was completed at the University of Georgia Proteomics and Mass Spectrometry Facility (University of Georgia, Athens, GA, USA). The target was analyzed using a Bruker Daultonics Autoflex MALDI-TOF mass spectrometer to identify peptide mass

fingerprints. The data was internally calibrated using trypsin auto-digestion peaks. Mascot, a powerful search engine that uses mass spectrometry data to identify proteins from primary sequence databases at www.matrixscience.com (Perkins, Pappin, Creasy, & Cottrell, 1999) was used. The nominal mass and calculated pI value were obtained from the National Centre for Biotechnology Information (NCBI) BLAST search against nr, as a set of possible amino acid sequence pattern. Carbamidomethyl modification of cysteine residues (C) was selected as a fixed modification and oxidation of methionine (M) as a variable modification. The peptide tolerance was set at 0.1 Da and the number of missed cleavages ranged from 1-3.

Statistical Analysis

PROC student's *t*-test procedure of SAS (SAS Institute Inc., 2002) was used to compare statistical difference between slow- and fast-GR relative density ratio as well as between low- and high-WHC and differences were considered significant at $P<0.05$.

Results and Discussion

Selected muscle characteristics of the two groups, slow- and fast-GR and low- and high-WHC, are shown in Table 4.1. The data summarized the physico-chemical characteristics of the specific muscle samples tested in this proteomic study. The averages of pHu, L*, a*, *P. major* weight, growth rate, and water holding capacity were different between slow- and fast-GR chicken breast muscles. The averages of pHu of slow- and fast-GR chicken breast muscles were 5.43 and 6.01. The averages of L* of slow- and fast-GR chicken breast muscles were 70.84 and 55.77. The averages of a* of slow- and fast-GR chicken breast muscles were 9.77 and 13.58. The averages of *P. major* weight of slow- and fast-GR chicken breast muscles were 60 and 305 g.

The averages of growth rate of slow- and fast-GR chicken breast muscles were 138.91 and 356.6 g/wk. The averages of WHC of slow- and fast-GR chicken breast muscles were 15.92 and 17.61. Protein solubility of WSP and non-WSP of GR and WHC populations evaluated in this study are presented in Table 4.2. Protein solubility of WSP was higher in muscle from fast-GR samples than from slow-GR samples. No differences were observed in protein solubility of WSP based on WHC or from non-WSP based on GR. Analysis of 2DE gels of chicken muscle revealed differences in WSP and non-WSP. Figures 4.1 shows the proteome profile for WSP and non-WSP, based on GR; while Figures 4.2 shows the proteome profile for WSP and non-WSP, based on WHC. In WSP, 4 peptides based on GR (Fig. 4.1-A) and 4 peptides based on WHC (Fig. 4.2-A) were unique. In non-WSP, 13 unique peptides based on GR (Fig. 4.1-B) and 1 peptide based on WHC (Fig. 4.2-B) were noted. These 22 protein spots were excised and identified by MALDI-TOF.

Differentially expressed proteins in slow- and fast-GR of chicken breast muscle are summarized in Table 4.3 for WSP and Table 4.4 for non-WSP with information on average spot density, protein identity, accession, peptides matched, protein score, sequence coverage rate, pI, and molecular weight. A negative value under column average protein density denoted that protein was over-expressed in slow-GR. Notably, spot# 1, 8, and 14 potentially identified as serum albumin precursor, creatine kinase M-type, and protein DJ-1 were over-expressed in slow-GR, and spot# 43, ubiquitin, is under-expressed in slow-GR (Table 4.3). In non-WSP protein, 17 protein spots were significantly ($P<0.05$) up- or down-regulated in which 13 protein spots had increased expression levels in fast-GR and 4 protein spots had decreased expression levels in fast-GR group of chicken muscles (Table 4.4, Fig. 4.1-B). However, there were some spots that had the same protein identities as shown in Table 4.4. Two protein spot (spot# 167 and spot#

182) were identified as chain A, spvb ADP-ribosylated actin: hexagonal crystal form. Other protein spots that had the same protein identity were spot# 54, 174 and 192 which were identified as Chain A, structures of actin-bound wh2 domains of spire and the implifilament nucleation. The different migration patterns of these spots with identical identities may indicate that these proteins underwent different post-translational modifications. Out of 17 protein spots from GR population, only one spot (spot# 26; aspartate aminotransferase) was not significantly matched to the protein database (protein score < 83; $p>0.05$).

Tables 4.5 and 4.6 summarized differentially expressed WSP (Table 4.5) and non-WSP (Table 4.6) in low- and high-WHC groups of chicken breast muscles. A negative value under column average protein density showed when the protein was over-expressed in slow-WHC. There were 5 protein spots which were significantly up and down-regulated in which 4 protein spots had increased expression levels in high-WHC and 1 protein spot had increased expression levels in low-WHC group. Out of 5 protein spots from WHC population, one spot (spot# 26; triosephosphate isomerase) was not significantly matched (protein score < 83; $p>0.05$) to the protein database. There were 2 of 5 proteins spots that had the same protein identity but differentially expressed in low- and high-WHC.

Matches between peptides identified in these samples were made to proteins extracted from non-chicken source such as human, rabbit, monkey unless chicken source was not available. Expressed proteins in extracts from low- and high-WHC and slow- and fast-GR included metabolic enzymes, such as creatine kinase, pyruvate kinase, triosephosphate isomerase, ubiqitin and housekeeping proteins, such as heat shock protein.

Triosephosphate isomerase, creatine kinase and pyruvate kinase were reported as the major soluble proteins found in the growth development study (Doherty, McLean, R. Hayter, M.

Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004). Triosephosphate isomerase (TPI) is an essential enzyme in all living cells, and plays an important role in glycolysis and is essential for energy production. TPI catalyses the conversion of dihydroxyacetone phosphate to glyceraldehyde-3-phosphate (Lawrie, 1988). Decreased TPI was observed in pork muscle after extended aging which was associated with lower shear force (r=0.46), but not significantly related to Hunter L-value and drip loss (Hwang, Park, Kim, Cho, & Lee, 2005). In an investigation of muscle proteome, TPI had increased expression in grass-fed compared to green-fed cattle (Shibata, Matsumoto, Oe, Ohnishi-Kameyama, Ojima, Nakajima, Muroya, & Chikuni, 2009). In our study, TPI was identified in two locations on the gel. Whereas one spot (spot# 6) had increased expression level in low-WHC and the other spot (spot# 21) had increased expression level in high-WHC (Table 4.5). The different spot location might be because post-translational protein modification or isoforms of the protein (Remignon, Molette, Babile, & Fernandez, 2006).

Pyruvate kinase muscle isozyme (PKM) is a glycolytic enzyme that catalyzes the transfer of phosphoryl group from phosphoenolpyruvate to ADP and generating ATP (Lawrie, 1988). PKM in this study had increased expression level in fast-GR muscle (Table 4.5). TPI and PKM were also expressed proteins from Thai native chicken and commercial broiler chicken muscles that differed at high-shear force (Mekchay, Teltathum, Nakasathien, & Pongpaichan, 2010). TPI and PKM were present in the proteome of male layer chickens during growth between 1-27 d but in lower quantities (Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004).

Heat shock protein (HSC) is reported to be involved with cell thermo tolerance when exposed to elevated temperatures or other stress (De Maio, 1999). HSC protein in this study had

increased expression level in high-WHC chicken muscle (Table5.1). Heat shock protein was found in this study which has a molecular weight around 70 kDa. In a proteome study on pigs, HSC70 was affected by compensatory growth and increased protein turnover (Lametsch R., Kristensen, Larsen, Therkildsen, Oksbjerg, & Ertbjerg, 2006). In the study to identify specific oxidatively modified proteins in chicken muscles, HSC70 showed a very faint carbonyl, but strong 3-nitrotyrosine (3-NT) reactivity (Stagsted, Bendixen, & Andersen, 2004). 3-NT is a marker of oxidative stress and potentially results in oxidative differences from the diets whether it is a low-antioxidant diet or diet supplemented with antioxidant-rich fruits/vegetables (Stagsted, Bendixen, & Andersen, 2004).

Creatine kinase M-type (M-CK), one of four forms of CK, had increased expression in slow-GR chicken muscle in this study (Table 4.3). M-CK is a constitutive protein of the M-line of muscle. The M-line of muscle ties to thick filaments of the A-band and ensures that these myosin filaments act in concert during contraction. M-CK is a globular protein that has both structural and enzymatic properties. CK was reported as a potential candidate marker protein for WHC as identified from low- and high-drip loss of pork muscles (van de Wiel & Zhang, 2007) and is known for enzymatic conversion of creatine phosphate into creatine and ATP (van de Wiel & Zhang, 2007), as a phophoprotein plays central role in energy transduction in muscle with large fluctuating energy demands (Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004). CK is identified as oxidation-sensitive (Stagsted, Bendixen, & Andersen, 2004) and maybe an in-vivo substrate for m-calpain, and m-calpain may regulate the degradation of CK and possibly its activity (Purintrapiban, Wang, & Forsberg, 2001).

Serum albumin precursor is the main protein in plasma and has a good binding capacity for water Ca^{2+}, Na^{+}, K^{+}, fatty acid, hormones, etc. Serum albumin precursor had increased expression level in slow-GR chicken muscle in this study. Serum albumin precursor was also found in the proteome of male layer chickens during growth between 1-27 d and a decline of serum albumin precursor with time in the soluble fraction was reported (Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004).

Ubiquitin had increased expression in fast-GR chicken muscle. Ubiquitin exists either covalently attached to another protein or free (unanchored). When covalently bound, it is conjugated to target proteins via an isopeptide bond either as a monomer (monoubiquitin), a polymer linked via different Lys residues of the ubiquitin (polyubiquitin chains). Ubiquitin was found in chicken embryo fibroblasts and denoted as a heat shock protein in chicken (Bond & Schlesinger, 1985). Increase expression of ubiquitin is associated with an increase in proteolysis in skeletal muscle during the damage of cells (Jia, Ekman, Grove, Færgestad, Aass, Hildrum, & Hollung, 2007).

There were contractile proteins, such as myosin heavy chain, actin, keratin, and found in slow- and fast-GR of chicken muscles, but mostly increased their expression in fast-GR chicken muscle. Observation of either post-translational modification or breakdown fragmentation of parent molecules into different fragments of actin and myosin occurred (Remignon, Molette, Babile, & Fernandez, 2006). This post-translational modification might because muscle in this study was aged 24 h before they were measured for ultimate pH and stored for 5d at 4ºC before it was frozen for proteomic study. During aging, some proteins might degrade (Sawdy, Kaiser, St-Pierre, & Wick, 2004). Protein identification for aged muscle (i.e. at 3 d) proved extremely complicated (van de Wiel & Zhang, 2007). However actin and myosin fragments were also

reported at 48 h post-mortem in pork and related to meat tenderness (Choi, Lee, Choe, Rhee, Lee, Joo, & Kim, 2010; Lametsch R. , Karlsson, Rosenvold, Andersen, Roepstorff, & Bendixen, 2003).

Myosin heavy chain (MHC) is a large polypeptide chain of the myosin molecule. MHC had increased expression in low-GR chicken muscle. Myosin expression in muscle has been related to glycolysis post-mortem and protein solubility. At 24 h post-mortem, MHC was lower in muscle which underwent rapid glycolysis rate in turkey breast muscle (Eadmusik, Molette, Rémignon, & Fernandez, 2009). In PSE pork muscle, MHC showed lower protein extractability (Lovell & Harrington, 1981). The lower extractability in PSE pork suggested a result from a tighter association of actin and MHC than the normal pork muscle (Lovell & Harrington, 1981).

Actin has increased expression in fast-GR chicken muscle (Table 4.4). In rapidly glycolysing turkey breast muscle, actin showed a lower amount at 24 post-mortem than in normal glycolysing muscle (Eadmusik, Molette, Rémignon, & Fernandez, 2009). Alpha actin was found in the proteome of male layer chicken during growth between 1-27 d (Doherty, McLean, R. Hayter, M. Pratt, H. L. Robertson, El-Shafei, J. Gaskell, & J. Beynon, 2004). In meat aging or ripening, actin fragments derived from myofibrillar protein hydrolysis, could be considered molecular markers for proteolysis (Luccia, Picariello, Cacace, Scaloni, Faccia, Liuzzi, Alviti, & Musso, 2005). Keratin, Type 1 cytoskeletal 9 (CK-9 or K-9) plays a role in keratin filament assembly. Keratin Type 1 is one of two types of cytoskeletal and microfibrillar keratin, I (acidic) and II (neutral to basic) with 40-55 and 56-70 kDa (UniProt, 2010). Tropomyosin alpha-1 had a high expression in fast-GR breast muscle. The Tropomyosin alpha-chain is one of the two polypeptides of tropomyosin (Asghar, Samejima, & Yasui, 1985).

Conclusion

This present study showed proteomic approach could be used to identify protein markers that are associated with growth rate and water holding capacity. These findings should be of great advantage for breeding programs in the selection of genes for the improvement of chicken production and meat quality. Gene improvement helps to provide high quality meat characteristics for consumers, and optimize the conversion of muscle to meat, and development of protein array.

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Table 4.1. Averages and standard deviations of selected muscle physico-chemical characteristics[1]

Sample	pH15	pHu	L*	a*	b*	Hue	Chroma	Pmajwt	GR	WHC
GR[2]										
Slow	6.74 ± 0.26	5.43 ± 0.20	70.84 ± 0.63	9.77 ± 2.13	8.98 ± 0.12	42.98 ± 6.30	13.33 ± 0.11	60 ± 6	138.91 ± 15.52	15.92 ± 20.95
Fast	6.69 ± ND	6.01 ± 0.04	55.77 ± 0.74	13.58 ± 0.81	9.44 ± 0.23	34.95 ± 2.29	16.54 ± 0.53	305 ± 29	356.60 ± 32.20	17.61 ± 13.14
WHC[3]										
Low	6.30 ± 0.20	5.74 ± 0.04	55.16 ± 3.15	14.96 ± 2.72	10.95 ± 2.80	53.29 ± 11.46	18.79 ± 1.01	143 ± 45	208.17 ± 42.50	3.50 ± 0.95
High	6.17 ± 0.67	5.59 ± 0.14	58.40 ± 5.02	12.90 ± 2.78	10.94 ± 1.70	49.28 ± 10.34	17.10 ± 1.17	192 ± 35	267.63 ± 17.90	93.97 ± 4.12

[1]Adapted from Phongpa-ngan (2010); pH15 = pH 15 min post-mortem; pHu = pH at 24 h post-mortem; L* = lightness; a* = green to red, b* = blue to yellowness; Hue = hue angle; Pmajwt = *P. major* weight; GR = growth rate; WHC = water holding capacity; ND = not detected

[2] N=6, slow-GR is defined as GR that is ≤ 250 g/wk; fast-GR is defined as GR that is ≥ 299 g/wk.

[3] N=6, low-WHC is defined as WHC that is ≤ 12.04 %; high-WHC is defined as WHC that is ≥ 33.30 %.

Table 4.2. Protein solubility (mg/mL) of water soluble protein and non-water soluble protein in slow- and fast-GR chicken breast muscle and low- and high-WHC breast muscle

Proteins*	GR[1]				WHC[2]			
	ID	Slow	ID	Fast	ID	Low	ID	High
WSP	2810	10.84	2724	15.78	3224	18.14	3439	12.96
	3124	11.16	4276	15.76	3256	13.89	3653	16.11
	4423	12.20	4533	16.60	3792	17.71	3819	18.34
	Ave	*11.40*	*Ave*	*16.05*	*Ave*	*16.58*	*Ave*	*15.80*
	SD	*0.71*	*SD*	*0.48*	*SD*	*2.34*	*SD*	*2.70*
Non-WSP	2810	19.48	2724	19.73	3224	17.47	3439	15.64
	3124	19.88	4276	18.80	3256	17.47	3653	13.38
	4423	21.47	4533	18.58	3792	15.98	3819	15.87
	Ave	*20.28*	*Ave*	*19.04*	*Ave*	*16.97*	*Ave*	*14.96*
	SD	*1.05*	*SD*	*0.61*	*SD*	*0.86*	*SD*	*1.38*

* WSP = water soluble protein, Non-WSP = non-water soluble protein
[1] GR = growth rate, slow-GR ≤ 250 g/wk; fast-GR ≥ 299 g/wk
[2] WHC = water holding capacity, low-WHC ≤4.08%; high-WHC ≥89.26%
[3] Ave = Average
[4] SD = Standard deviation

Table 4.3. List of spot number (No.) consensus water soluble protein marker for slow- and fast-growth rate, accession, peptides matched, protein score (PS), sequence coverage rate (SC, %), isolectric point (pI) and molecular weight (MW, Da) on 2DE gel using peptide mass fingerprint of MALDI-TOF spectra

No.	Av Spot density[1]	Consensus protein identity	Accession	Type	Matched	PS[2]	SC	pI/MW
1	-2.06	Serum albumin precursor [Gallus gallus]	gi|45383974	chicken	21	118	39	5.51/71868
8	-1.35	Creatine kinase M-type [Gallus gallus]	gi|45382875	chicken	39	262	74	6.50/43529
14	-0.70	Protein DJ-1 [Gallus gallus]	gi|45383015	chicken	10	81	51	6.32/20159
43	1.14	Ubiquitin	gi|78099807	earth worm	9	154	96	5.24/7199

[1]Av spot density is the average spot density. A negative was showed when the protein was over-expressed in slow-GR.
[2]Protein score is -10*Log(P), where P is the probability that the observed match is a random event. Protein scores, greater than 83, are significant ($p<0.05$).

Table 4.4. List of spot number (No.) consensus non-water soluble protein marker for slow- and fast-growth rate, accession, peptides matched, protein score (PS), sequence coverage rate (SC, %), isolectric point (pI) and molecular weight (MW, Da) on 2DE gel using peptide mass fingerprint of MALDI-TOF spectra

No.	Av Spot density[1]	Consensus protein identity	Accession	Type	Matched	PS[2]	SC	pI/MW
26	0.56	Aspartate aminotransferase, mitochondrial-like [Pongo abelii]	gi|297698888	orangutan	3	67	43	7.82/9659
54	0.13	Chain A, structures of actin-bound wh2 domains of spire and the impli filament nucleation	gi|297343122	rabbit	7	97	21	5.10/40304
67	-1.58	Myosin heavy chain, skeletal muscle, adult	gi|13432175	chicken	35	154	16	5.63/223976
68	0.97	Similar to myosin, heavy polypeptide 1, skeletal muscle, adult [Gallus gallus]	gi|118099530	chicken	24	136	26	5.39/116569
89	0.39	Actin, alpha cardiac muscle 1 isoform 2 [Callithrix jacchus]	gi|296214263	common marmoset	15	133	36	5.16/40342
90	0.41	Chain A, polylysine induces an antiparallel actin dimer that nucleates filament assembly: crystal structure at 3.5 A resolution	gi|20664362	rabbit	12	134	33	5.46/41558
91	0.77	Keratin, type I cytoskeletal 9	gi|55956899	human	33	153	41	5.14/62255
155	0.45	Tropomyosin alpha-1 chain OS=danio rerio GN=tpma PE=2 SV=2	TPM1 CHICK	chicken	14	112	41	4.70/32823
165	0.82	Actin, aortic smooth muscle [Gallus gallus]	gi|71895043	chicken	15	147	39	5.23/42367
167	1.28	Chain A, Spvb ADP-ribosylated actin: hexagonal crystal form	gi|114794125	rabbit	21	195	43	5.48/41561
174	1.38	Chain A, structures of actin-bound wh2 domains of spire and the impli filament nucleation	gi|297343122	rabbit	16	163	40	5.10/40304
182	0.87	Chain A, Spvb ADP-ribosylated actin: hexagonal crystal form	gi|114794125	rabbit	13	170	33	5.48/41561
192	0.54	Chain A, structures of actin-bound wh2 domains of spire and the impli filament nucleation	gi|297343122	rabbit	9	101	28	5.10/40304

[1]Av spot density is the average spot density. A negative was showed when the protein was over-expressed in slow-GR.
[2]Protein score is -10*Log(P), where P is the probability that the observed match is a random event. Protein scores, greater than 83, are significant ($p<0.05$).

Table 4.5. List of spot number (No.) consensus water soluble protein marker for low- and high-WHC, accession, peptides matched, protein score (PS), sequence coverage rate (SC, %), isolectric point (pI) and molecular weight (MW, Da) on 2DE gel using peptide mass fingerprint of MALDI-TOF spectra

No.	Av Spot density[1]	Consensus protein identity	Accession	Type	Matched	PS[2]	SC	pI/MW
5	2.39	Heat shock protein [Numida meleagris]	gi|45544523	bird	25	200	36	5.37/71055
6	-4.51	Triosephosphate isomerase [Gallus gallus]	gi|45382061	chicken	21	238	72	6.71/26832
10	1.11	Pyruvate kinase muscle isozyme [Gallus gallus]	gi|45382651	chicken	27	245	51	7.29/58434
21	4.57	Triosephosphate isomerase [Gallus gallus]	gi|45382061	chicken	31	367	96	6.71/26832

[1]Av spot density is the average spot density. A negative was showed when the protein was over-expressed in low-WHC.
[2]Protein score is -10*Log(P), where P is the probability that the observed match is a random event. Protein scores, greater than 83, are significant ($p<0.05$).

Table 4.6. List of spot number (No.) consensus non-water soluble protein marker for low- and high-WHC, accession, peptides matched, protein score (PS), sequence coverage rate (SC, %), isolectric point (pI) and molecular weight (MW, Da) on 2DE gel using peptide mass fingerprint of MALDI-TOF spectra

No.	Av Spot density[1]	Consensus protein identity	Accession	Type	Matched	PS[2]	SC	pI/MW
26	1.19	Triosephosphate isomerase [Gallus gallus]	gi|45382061	chicken	26	297	76	6.71/26832

[1]Av spot density is the average spot density. A negative was showed when the protein was over-expressed in low-WHC.
[1]Protein score is -10*Log(P), where P is the probability that the observed match is a random event. Protein scores, greater than 83, are significant ($p<0.05$).

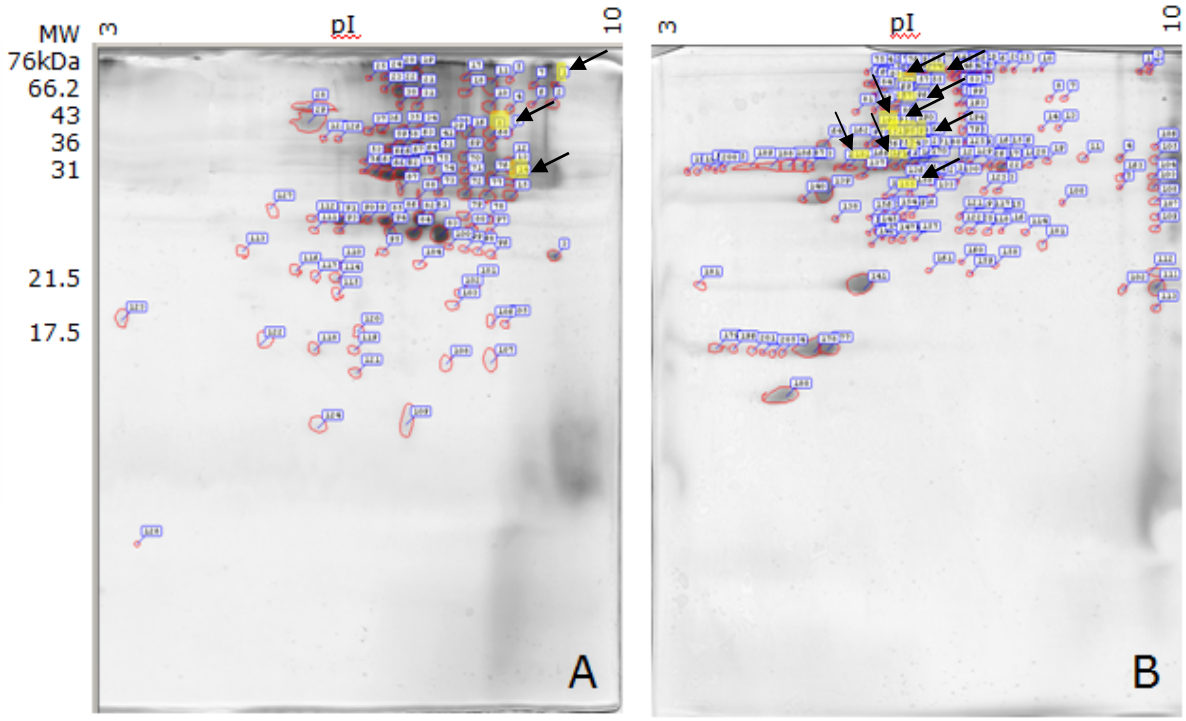

Fig. 4.1. Representation 2DE image of growth rate (A) WSP extract and (B) non-WSP extract. The protein loading was 300 µg and the gels were stained with coomassie brilliant blue G-250. The arrows indicate the identified protein positions that were differentially expressed between slow- and fast-GR.

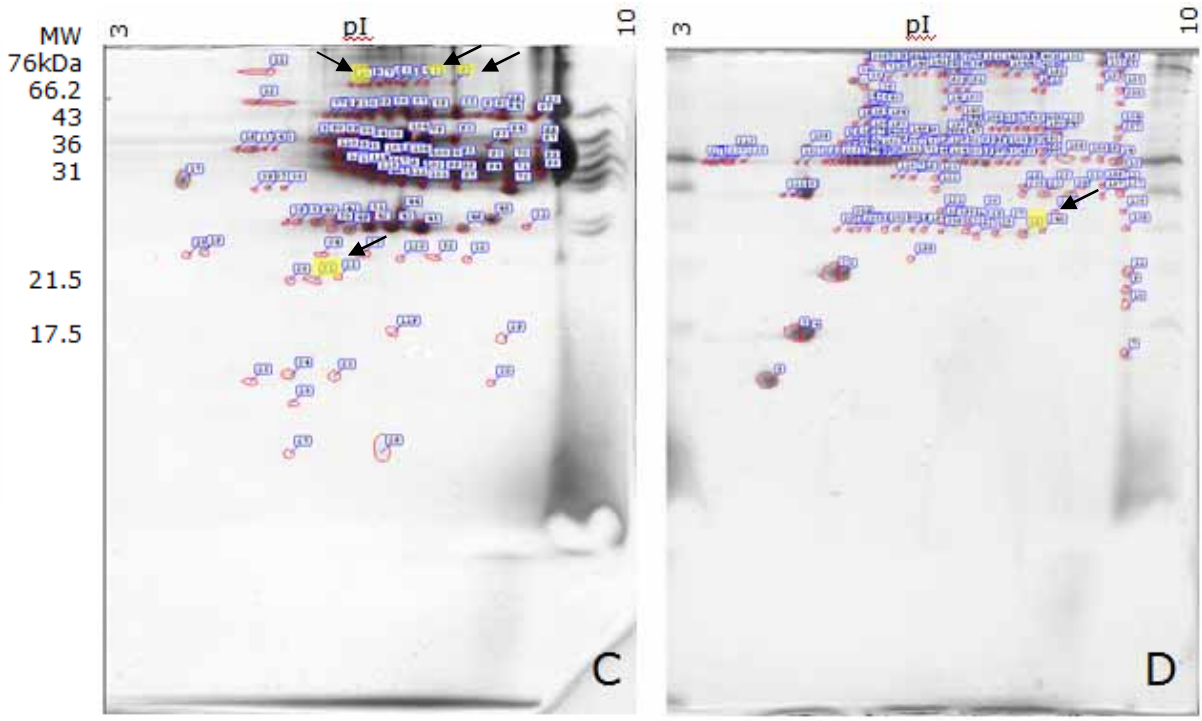

Fig. 4.2. Representation 2DE image of WHC (C) WSP extract and (D) non-WSP extract. The protein loading was 300 µg and the gels were stained with coomassie brilliant blue G-250. The arrows indicate the identified protein positions that were differentially expressed between low- and high-WHC.

CHAPTER 5

RAMAN SPECTROSCOPY AS A MONITORING TOOL FOR MEAT CHARACTERISTICS IN FAST AND SLOW GROWING CHICKENS

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Summary

Growth rate has affected the characteristics of chicken breast muscle. Breast muscle from slow growing bird exhibited pale, soft, and exudative (PSE) characteristics. PSE meat has poor water holding capacity (WHC) which results in an economic loss for processors, as well as a decrease in consumer acceptance. Rapid and non-destructive method dealing with large product quantity within short time period at very early stages of production is attractive for meat production. The objective of this study was to observe the change in protein structure through Raman spectra with regard to growth rate and WHC. The slow- and fast-growing chicken muscle and the low- and high-WHC were scanned by Raman Spectroscopy at 785 nm excitation, 500 mW laser power, 10 s total integration time. There were 8 significant bands (P<0.05) from growth population spectrum: 538, 582, 682, 691, 1367, 1625, 1704, 1743 cm^{-1}, and 5 significant bands (P<0.05) from WHC population spectrum: 1270, 1277, 1354, 1653, 1737 cm^{-1}, that potentially could be used to identify muscle attribute variation.

Keywords: Raman spectroscopy, growth rate, water holding capacity, meat attribute, poultry, chicken

Introduction

Rapid quality evaluation techniques for quantitative testing of meat product industries are required to meet the growing demand of bulk manufacture. These techniques require instruments that can accommodate a large sample size in a short time for routine analysis (Chizzolini, Novelli, Badiani, Rosa, & Delbono, 1993). The quality of chicken meat immediately after slaughter and deboning can be evaluated and predicted for quality attributes which are important to the consumer. The Raman spectroscopic technique is a non-destructive method of online monitoring. With many advantages (Colthup, Daly, & Wiberley, 2010), Raman spectroscopy is becoming an attractive method for use in the meat industry. It is a fast method and the spectra can be recorded in a short time. The spectrum contains information that gives both quantitative and qualitative information. Table 3.1 shows a summary of Raman spectroscopy studies on muscle.

Moisture loss or drip loss causes unattractive meat appearance and meat texture as well as influences on meat processing and contributes to the loss of sales (Forrest, Morgan, Borggaard, Rasmussen, Jespersen, & Andersen, 2000). Prediction of water holding capacity by vibrational spectroscopic methods has been investigated by various studies (Hoving-Bolink, Vedder, Merks, de Klein, Reimert, Frankhuizen, van den Broek, & Lambooij, 2005; Prieto, Roehe, Lavin, Batten, & Andres, 2009; Rosenvold, Micklander, Hansen, Burling-Claridge, Challies, Devine, & North, 2009; Samuel, Park, Sohn, & Wicker, 2011; Swatland & Barbut, 1995). Raman spectroscopy was used to investigate various sources of pork meat (research meat and commercial meat measured at slaughterhouses which differed in water holding capacity, and Raman spectroscopy predicted the quality of the muscle (Pedersen, Morel, Andersen, & Engelsen, 2003). The regions of interest for WHC were 3128-3071 and 876-951 cm^{-1} in the

study of Raman spectra. There were more poor spectra considered to be outliers found from commercial meats because the speed of measure was not as fast (acquiring time 30-60 s) as the speed of the processing line. A suggestion was made to increase sample size so the Raman could reflect more representative data (Pedersen, Morel, Andersen, & Engelsen, 2003).

Changes in the Raman bands of protein chemical groups gave information of changes in secondary structure of proteins: amide conformation region, C-C stretching vibration and changes in tryptophan residues, tyrosil doublet, aliphatic aminoacids bands (Herrero, 2008). The conformation region of amide was the most useful Raman bands for observing changes in the secondary structure of proteins (α-helix, β-sheet, turn, and random coil) which were assigned to amide I, amide II, and amide III bands (Herrero, 2008). Amide I is usually used to quantify the secondary structure of proteins. Amide II vibration usually cannot be detected because of a small change in polarizability associated with amide II. The amide III region has some overlaps between α-helix, β-sheet, turn, and random coil (Herrero, 2008). C-C stretching vibration is also the involved in the change in secondary structure of protein. The gradual loss of these structures showed the broad and weakness in intensity of this band (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008). Many Raman bands showed response to the change of Tryptophan residues in proteins. Herrero (2008) concluded from the literatures that tertiary structure of proteins can be detected from decreasing peak intensity of buried tryptophan residues in the protein. Also Herrero (2008) concluded from previous research that the presence of a high intensity ratio of I_{1360}/I_{1340} indicated a hydrophobic environment; conversely, a low ratio indicated tryptophan was involved more in the H-bonding of a hydrophilic environment. Tyrosine is used as an indicator of the hydrogen bonding of the phenolic hydroxyl group (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008). Herrero (2008) reviewed that the

decrease in intensity of aliphatic amino acids band could possibly result from hydrophobic interactions of aliphatic residues. Other amino acids that might be useful for meat that strong band of Raman can be observed such as proline and hydroxproline which are the amino acids of the connective tissue proteins such as collagen (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008).

The collagen, the main connective tissue, contains high levels of hydroxyproline amino acid (Morse, 1933) . Collagen showed two amide bands at 1271 and 1248 cm^{-1} (Frushour & Koenig, 1975). Raman spectroscopy could predict the juiciness from the investigation of sensory evaluation of beef silverside by observing the changes in 1460-1483 cm^{-1} region corresponding to the juiciness in cooked beef (Beattie, Bell, Farmer, Moss, & Desmond, 2004). No studies have used Raman spectroscopy to observe changes in protein structure that is affected by growth rate.

The objective of this study was to study the change that could be observed through Raman spectra with regard to growth rate and WHC from different level of growth and WHC chicken breast muscle.

Material and Methods

Chicken muscle

P. major muscle was obtained and meat attribute traits were measured as described in Phongpa-ngan (2010). From the previous result, slow-growing (SG) was defined as growing with a growth rate lower than or equal 250 g/wk; and fast-growing (FG) was defined as growing with the growth rate higher than or equal 299 g/wk. Low-WHC was defined as WHC that was lower than or equal 4.08%, and high-WHC was defined as WHC that was higher than or equal 89.26%.

There were total of 181 chicken breast muscles from individual chickens for a growth rate population. Two sub-populations were created based on their extreme growth rate; 45 slow- and 45 fast-growing. There were total of 78 breast muscles from individual chickens for WHC population. Two sub-populations were created based on their extremes: WHC; 19 low- and 19 high-WHC.

Raman spectroscopy measurement

Chicken breast muscles at 2 d post-mortem were scanned for Raman spectra. A HRC-10HT Bruker Optics Sentinel Raman Spectrometer (Bruker Optics, Billerica, MA) was used for the measurement of Raman spectra of the 2 d post-mortem *P. major* chicken breast muscle. A 785 nm helium-neon (He-Ne) laser with 500 mW of power was the excitation source. A background was collected for 10 seconds followed by the spectra for another 10 seconds in order to achieve an acceptable signal to noise ratio (S/N); a charge coupled device (CCD) was in place to decrease background noise. The Raman background and Raman scatter were corrected. The spectral that was measured ranged between 220 cm^{-1} and 2250 cm^{-1} with baseline correction and normalization taking place after collection using Sure_Cal. Raman spectra were taken in triplicate for every sample at different spot on the chicken breast. The average of Raman spectra for each sample was processed using Opus Spectroscopic Software V.6 (Bruker, Karlsruhe, Germany). The peak selection command was used to identify peaks in spectra with defining frequency range from 500 cm^{-1} to 1900 cm^{-1} and setting sensitivity at 1%. The relative peak intensity for each spectrum and selected Raman band were exported into a .txt file and opened in Microsoft Excel to calculate peak ratios manually. Raman band 1849 cm^{-1} was selected as an

internal standard peak. The peak ratio was calculated by dividing relative peak intensity at each selected Raman band with relative peak intensity at Raman band 1849 cm^{-1} for each spectrum.

Statistics

PROC TTEST (SAS Institute Inc., 2002) was used to compare statistical difference between SG and FG relative peak intensity ratio as well as between low- and high-WHC. PROC CORR (SAS Institute Inc., 2002) was used to analyze linear correlations between studied parameters and relative peak intensity ratio. A probability level of 0.05 or less was considered to be significant.

Results and Discussion

The characteristics of selected muscles are shown in Table 3.2. Muscles from the SG population showed significantly lower pH level measured at 15 min post-mortem (pH15); pH measured at 24 h post-mortem (pHu); hatch weight (BW0); *P. major* yield (PMY), and hue angle (Hº) than muscle from the FG population. Muscles from FG population showed significantly lower color value than muscle from SG population both in lightness (L*) and blue to yellowness (b*). There was no statistical difference between the SG and FG populations in the difference between pH15 and pHu (pHDiff), green to red (a*), chroma (C*), drip loss (DL), and cook yield (CY). There was no statistical difference between the low- and high-WHC in all muscle characteristic.

After peak selection, considering good representation of the data, only Raman bands that had at least 9 replications of either slow- or fast-growing and at least 4 replications of either low- or high-WHC were included for further analysis. Relative peak intensity ratios at each Raman

band were calculated. There were a total of 156 Raman bands for the growth population and 85 Raman bands for the WHC population and the comparisons for each Raman band were made in between group.

Of 156 selected Raman bands, there were a total of 21 Raman bands displayed statistical significant difference in relative peak intensity ratio between slow- and fast-growing populations as shown in Table 3.3. These 21 Raman bands occurred in several regions: 501-510 cm^{-1} (cys); 963-987 cm^{-1} (C-C stretching); 1079-1155 cm^{-1} (C-O streching); 1270-1354 cm^{-1}; (amide III, α), 1535 cm^{-1} (amide II); 1652-1653 (amide I, α); 1718 (acid C=O), and 1737 (COOH, amide II). Out of 85 selected Raman bands, there were a total of 8 Raman bands displayed statistical significant difference in relative peak intensity ratio between low- and high-WHC populations as shown in Table 3.4. These 8 Raman bands were in several regions: 538-583 cm^{-1} (Amide NH_2 and NH group), 682-691 cm^{-1} (Met), 1367 cm^{-1} (Trp), 1625 cm^{-1}, (amide III, α), and 1704-1743 cm^{-1} (COOH, amide II).

Out of 21 significant selected Raman bands in slow- and fast-growing populations, all relative peak intensity ratios were significantly higher in FG population than in the SG population. Out of 8 significant selected Raman bands in low- and high-WHC populations, 5 relative peak intensity ratios were significantly higher in high-WHC than in low-WHC populations and 3 relative peak intensity ratios were significantly lower in high-WHC than in low-WHC populations.

The Pearson correlation coefficients of between ratios of peak intensities and growth rate are shown in Table 3.5, and ratios of peak intensities and WHC are shown in Table 3.6.

Growth rate played an important role all in Raman bands. Relationships between ratios of peak intensities (I_{1277}/I_{1849}, $I_{1653}/I_{1849,}$ I_{1277}/I_{1849}, and I_{1737}/I_{1849}) and growth rate were all positively

statistically significant (P<0.05) with correlation coefficients (r) = 0.44, 0.55, 0.50, 0.47, and 0.55. There were significant correlations between relative peak intensity ratios between I_{1277}/I_{1849} and I_{1653}/I_{1849} and between I_{1277}/I_{1849} and I_{1737}/I_{1849} with correlation coefficient of 0.63 and 0.64. These correlation coefficients indicated positive relationship between amide III and amide I and between Amide III and amide II. For non significant correlation coefficients, the correlation coefficients were positive, except the correlation coefficient between I_{1270}/I_{1849} and I_{1277}/I_{1849} was negative.

WHC played an important role in all Raman bands. However, there were 5 out of 8 that showed significant correlations (P<0.05). Relationships between ratios of peak intensities (I_{538}/I_{1849}, $I_{691}/I_{184,}$ I_{1367}/I_{1849}, I_{1625}/I_{1849}, I_{1704}/I_{1849}) and WHC were statistically significant (P<0.05) with correlation coefficients (r) = -0.92, 0.92, 0.74, 0.94, and 0.83. These relationships showed the shift position of COOH and explosion of tryptophan in the excess water condition. Although statistically significant relationships were not shown, the correlation coefficients of these ratios of peak intensities ($I_{582}/I_{1849,}$ I_{682}/I_{1849}, and I_{1743}/I_{1849}) and WHC were high with correlation coefficients of (r) = -0.34, 0.49, and -0.44. The correlation coefficient was significant between I_{538}/I_{1849} with the following peak ratios: I_{682}/I_{1849} (r = -1.00), I_{1704}/I_{1849} (r = -0.95), and I_{1743}/I_{1849} (r=0.90). There was significantly correlation coefficient between I_{582}/I_{1849} and I_{1743}/I_{1849} (r = 0.84); between I_{682}/I_{1849} and I_{1704}/I_{1849} (r = 0.95); and between I_{691}/I_{1849} and I_{1367}/I_{1849} (r = 0.92).

Conclusion

These results show promise for the purpose of finding an online method for monitoring and classify chicken breast muscle, and this study showed that Raman spectroscopy provides good predictive information in wavenumbers according to growth rate and WHC of the chicken

breast muscle. This information could be used to improve the conditions of handling, processing, and storage of chicken breast muscle.

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Table 5.1. Raman spectroscopy studies on muscles

Studies	Laser	λ (nm)	Laser power (mW)	References
Raw porcine	Diode	785	30	(Pedersen, et al., 2003)
	HeNe	632	5	
Lipid-prot		785	300	(Pedersen, et al., 2003)
Cooked meat	Titanium:Sapphire CW	785	N/A	(Beattie, et al., 2004b)
Meat batter	Nd:YAG	1064	300	(Herrero, Carmona, Lopez-Lopez, et al., 2008c)
Porcine	Titanium:Sapphire CW	785	100	(Beattie, Brockbank, McGarvey, & Curry, 2005c)
		633	20	
		514	10	
Pork aging, salting addition	Ar+ pumped Ti:Sapphire	785	60	(Böcker et al., 2007)
Aging & cooked pork	Titanium:Sapphire CW	785	100	(Beattie, Bell, Borggaard, & Moss, 2008b)
Surimi gel	Argon ion	488	100	(Bouraoui, Nakai, & Li-Chan, 1997)
Extrusion	He:Ne	785	500	(Miller, 2008)
Poultry	Diode	785	78	(Ellis, Broadhurst, Clarke, & Goodacre, 2005)
Pork postmortem	N/A	671, 785	N/A	(Jordan, Thomasius, Schröder, Wulf, Schlüter, Sumpf, Maiwald, Schmidt, Kronfeldt, Scheuer, Schwägele, & Lang, 2009)
Sarcoplasmic protein, pH	He:Ne	632.8	N/A	(Tadpitchayangkoon, Park, Mayer, & Yongsawatdigul, 2010)

Table 5.2. Descriptive statistics of selected muscle characteristics[1]

Sample[2]	pH15	pHu	pHDiff	DL	CY	BW0	BW6	PMY	GR	WHC
Growth rate[3]										
Slow	6.43[a] ± 0.29	5.85[a] ± 0.16	0.64 ± 0.26	6.85 ± 2.88	85.04 ± 2.86	37.01[a] ± 4.06	1358.20[a] ± 101.90	10.64[a] ± 12.5	220.20[a] ± 17.02	25.48 ± 28.14
Fast	6.70[b] ± 0.22	5.99[b] ± 0.16	0.75 ± 0.24	6.05 ± 2.45	86.39 ± 1.66	39.93[b] ± 3.45	2070[a] ± 86.80	11.61[b] ±1.21	338.31[b] ±14.33	18.12 ± 10.07
WHC[4]										
Low	6.66 ± 0.21	5.93 ± 0.17	0.77 ± 0.25	ND	ND	36.99 ± 4.29	1792.80 ± 299.88	11.48 ± 1.03	292.63 ± 49.90	9.69[a] ± 1.13
High	6.63 ± 0.17	5.92 ± 0.22	0.76 ± 0.23	ND	ND	39.42 ± 4.04	1816.50 ± 317.47	11.65 ± 1.17	296.18 ± 52.53	41.06[b] ± 19.68

[1]Adapted from (Phongpa-ngan, et al., 2010)

[2]pH15 = pH 15 min post-mortem; pHu = pH at 24 h post-mortem; pHDiff = pH difference; DL = drip loss; CY = cook yield, BW0 = body weight at 0 wk; BW6 = body weight at 6th wk; PMY = *P. Major* yield; GR = growth rate; WHC = water holding capacity; L* = lightness; a* = green to red; b* = blue to yellowness; H^{o} = hue angle; C* = chroma; ND = not detected

[3] Slow growth rate is defined as growth rate that is ≤ 250 g/wk; fast growth rate is defined as growth rate that is ≥ 299 g/wk.

[4] Low water holding capacity is defined as water holding capacity that is ≤ 12.04 %; high water holding capacity is defined as water holding capacity that is ≥ 33.30 %.

[a,b] Means within a row lacking a common superscript differ ($P < 0.05$)

Table 5.2. Cont.

Sample[2]	L*	a*	b*	Hº	C*
Growth rate[3]					
Slow	54.64[b] ± 2.96	14.32 ± 1.82	11.19[b] ± 1.85	43.0[a] ± 5.73	18.27 ± 1.85
Fast	55.48[a] ± 2.45	14.31 ± 1.24	10.19[a] ± 1.36	35.0[a] ± 4.58	17.62 ± 1.13
WHC[4]					
Low	56.08 ± 3.06	14.31 ± 1.18	10.81 ± 1.91	53.3 ± 5.16	18.00 ± 1.52
High	55.34 ± 3.07	14.98 ± 1.06	9.83 ± 2.34	56.72 ± 6.88	18.05 ± 1.28

[1]Adapted from (Phongpa-ngan, et al., 2010)

[2]pH15 = pH 15 min post-mortem; pHu = pH at 24 h post-mortem; pHDiff = pH difference; DL = drip loss; CY = cook yield, BW0 = body weight at 0 wk; BW6 = body weight at 6th wk; PMY = *P. Major* yield; GR = growth rate; WHC = water holding capacity; L* = lightness; a* = green to red; b* = blue to yellowness; H^{o} = hue angle; C* = chroma; ND = not detected

[3] Slow growth rate is defined as growth rate that is ≤ 250 g/wk; fast growth rate is defined as growth rate that is ≥ 299 g/wk.

[4] Low water holding capacity is defined as water holding capacity that is ≤ 12.04 %; high water holding capacity is defined as water holding capacity that is ≥ 33.30 %.

[a,b] Means within a row lacking a common superscript differ ($P < 0.05$)

Table 5.3. Significant Raman bands difference ($P < 0.05$) in ratio of peak intensities between slow- and fast-growing chicken muscles

Raman band (cm^{-1})	Assignment	N	Peak ratio* Slow growing	N	Peak ratio Fast growing
501	Cys	4	$0.023^{b} \pm 0.012$	9	$0.180^{a} \pm 0.184$
502	Cys	5	$0.070^{b} \pm 0.017$	9	$0.137^{a} \pm 0.165$
510	Cys	12	$0.054^{b} \pm 0.025$	4	$0.166^{a} \pm 0.107$
963	C-C stretching	2	$0.062^{b} \pm 0.026$	11	$0.138^{a} \pm 0.066$
987	C-C stretching	7	$0.044^{b} \pm 0.028$	10	$0.102^{a} \pm 0.067$
1079	C-O stretching	11	$0.068^{b} \pm 0.042$	5	$0.129^{a} \pm 0.047$
1086	C-O stretching	2	$0.045^{b} \pm 0.009$	12	$0.095^{a} \pm 0.066$
1127	C-O stretching	10	$0.273^{b} \pm 0.160$	6	$0.529^{a} \pm 0.151$
1133	C-O stretching	5	$0.043^{b} \pm 0.039$	13	$0.145^{a} \pm 0.147$
1155	C-O stretching	5	$0.064^{b} \pm 0.029$	12	$0.122^{a} \pm 0.074$
1270	Amide III-α	15	$0.132^{b} \pm 0.067$	13	$0.242^{a} \pm 0.153$
1277	Amide III-α	11	$0.103^{b} \pm 0.092$	9	$0.240^{a} \pm 0.128$
1354	Amide III-α	9	$0.057^{b} \pm 0.019$	9	$0.120^{a} \pm 0.080$
1535	Amide II-NH	11	$0.181^{b} \pm 0.106$	7	$0.333^{a} \pm 0.138$
1652	Amide I-α	9	$0.114^{b} \pm 0.061$	8	$0.258^{a} \pm 0.161$
1653	Amide I-α	10	$0.159^{b} \pm 0.137$	9	$0.269^{a} \pm 0.055$
1718	C=O	5	$0.041^{b} \pm 0.024$	10	$0.046^{a} \pm 0.097$
1737	Amide II-COOH	15	$0.088^{b} \pm 0.052$	10	$0.183^{a} \pm 0.099$
1807	Anhydrides	4	$0.088^{b} \pm 0.042$	12	$0.199^{a} \pm 0.072$
1863	Anhydrides	9	$0.110^{b} \pm 0.046$	8	$0.204^{a} \pm 0.091$
1876	Anhydrides	3	$0.085^{b} \pm 0.026$	11	$0.181^{a} \pm 0.069$

* Peak ratio ± standard deviation

[a,b] Means within a row lacking a common superscript differ ($P < 0.05$)

Table 5.4. Significant Raman bands difference ($P < 0.05$) in ratios of peak intensities between low- and high-water holding capacity of chicken breast muscles

Raman band (cm^{-1})	Assignment	N	Peak ratio* Low-WHC	N	Peak ratio High-WHC
538	Amide II-NH	2	$0.286^{a} \pm 0.042$	4	$0.117^{b} \pm 0.056$
582	Amide II-NH	9	$0.163^{a} \pm 0.099$	2	$0.051^{b} \pm 0.002$
682	Met	5	$0.071^{b} \pm 0.061$	2	$0.154^{a} \pm 0.007$
691	Met	4	$0.049^{b} \pm 0.024$	2	$0.146^{a} \pm 0.016$
1367	Trp	6	$0.466^{b} \pm 0.142$	4	$10.562^{a} \pm 6.753$
1625	Amide III-α	2	$0.105^{b} \pm 0.080$	5	$0.361^{a} \pm 0.062$
1704	Amide II-NH	3	$0.071^{b} \pm 0.054$	5	$0.184^{a} \pm 0.047$
1743	Amide II-NH	4	$0.143^{a} \pm 0.062$	4	$0.056^{b} \pm 0.029$

* Peak ratio ± standard deviation

[a,b] Means within a row that lacks a common superscript difference ($P < 0.05$)

Table 5.5. Pearson correlation coefficients (r) between ratios from each relative selected peak intensities to the relative peak intensity at Raman band 1849 cm^{-1} from Raman spectra scanned at 785 nm excitation from 2 d post-mortem breast muscle from slow- and fast-growing chicken muscles

Variables	Growth rate	I_{1270}/I_{1849}	I_{1277}/I_{1849}	I_{1354}/I_{1849}	I_{1653}/I_{1849}
I_{1270}/I_{1849}	0.44*				
I_{1277}/I_{1849}	0.55*	-0.21			
I_{1354}/I_{1849}	0.50*	0.39	0.38		
I_{1653}/I_{1849}	0.47*	0.11	0.63*	0.32	
I_{1737}/I_{1849}	0.55*	0.07	0.64*	0.12	0.36

* $P < 0.05$.
n=32

Table 5.6. Pearson correlation coefficients (r) between ratios from each relative selected peak intensities to the relative peak intensity at Raman band 1849 cm^{-1} from Raman spectra scanned at 785 nm excitation from 2 d post-mortem breast muscle from low- and high-water holding capacity (WHC) chicken breast muscles

Variables	WHC	I_{538}/I_{1849}	I_{582}/I_{1849}	I_{682}/I_{1849}	I_{691}/I_{1849}	I_{1367}/I_{1849}	I_{1625}/I_{1849}	I_{1704}/I_{1849}
I_{538}/I_{1849}	-0.92*							
I_{582}/I_{1849}	-0.34	-0.67						
I_{682}/I_{1849}	0.49	-1.00*	0.50					
I_{691}/I_{1849}	0.92*	-0.81	-0.12	0.60				
I_{1367}/I_{1849}	0.74*	-0.78	-0.26	0.50	0.92*			
I_{1625}/I_{1849}	0.94*	-0.79	0.02	0.78	0.96	0.53		
I_{1704}/I_{1849}	0.83*	-0.95*	-0.56	0.96*	0.40	0.71	0.64	
I_{1743}/I_{1849}	-0.44	0.90*	0.84*	0.58	-0.63	-0.33	-0.66	-0.67

* $P < 0.05$.
n=15

CHAPTER 6

CONCLUSIONS

Fast growing chickens showed better technological yields than slow growth chickens as seen in higher cook yield and lower drip loss. Color parameters could be used to segregate muscle for further processing because lightness (L*) and blue to yellowness (b*) showed difference between different growth rate chicken. L* was also correlated with drip loss. Drip loss is an important attribute that causes unattractive appearance, affect to meat texture and processing of meat and the loss of sales. Monitor changes in chicken breast muscles from different growth rate levels and different water holding capacity levels by using non-destructive online monitoring method, Raman spectroscopy, revealed the prominent Raman bands that could be attributed to changes of protein structure resulting in different muscle characteristics. The Raman spectroscopy method which is rapid, easy to use, non-destructive, could be a potential tool for authentication and quality control of poultry products with further development. Fractionation and evaluation of protein quality and quantity difference in chicken breast muscle from different growth rate levels and different water holding capacity levels by using proteomics approaches (2-dimensional electrophoresis and MALDI-TOF mass spectrophotometry) showed different protein expressions in different levels of studied parameters. This finding should be of great advantage for breeding program in selection of gene for improvement of chicken production and meat quality traits to provide good quality of meat for consumer, optimization the conversion of muscle to meat, and development of protein array.

APPENDICE A

SUMMARY OF WAVENUMBER ASSIGNMENTS FROM LITERATURES

Chapter 5. Summary of wavenumber assignments from the literature*

Frequency (cm^{-1})	Structure	Products	Characteristics
160			Associated with conformational transitions of muscle proteins, to changes in the structure of muscle water, and/or alterations in protein water interactions during frozen
180	Water		Motion of water molecules involved in hydrogen bond interactions which bind other molecules such as protein
510 w	*v*SS (cystine)		
525 w	*v*SS		
544	Trp		
545 w	*v*SS		
577	Trp		
600-750	*v*sMet, *v*sCys		
620-640 w	Phe		
630-670 w	*v*CS		
644 w	Tyr		
655-724	Methionine in the trans form		
700-745 w	*v*CS		

Chapter 5. Cont.

Frequency (cm^{-1})	Structure	Products	Characteristics
759	Tryptophan residues		Decrease of peak intensity at 759, modification in the tertiary structure of protein
760	Trp		
760 m	Trp		
830 w	Tyr *v*-ring		
850 w	Tyr *v*-ring		
855	Proline		Proline is amino acid of the connective tissue, show strong band due to aromatic or saturated shide chain rings
876-951			water holding capacity
879	Hydroxyproline		
880 w	Trp *v*-ring, hydroxyl proline residue		Proline is amino acid of the connective tissue, show strong band due to aromatic or saturated shide chain rings
890-945	α-helix		
890-1060	C-C stretching		
900	*vs*C-C		
921	Proline		Proline is amino acid of the connective tissue, show strong band due to aromatic or saturated shide chain rings
933-944	*vs*C-C, amide III		
940	*vs*C-C		Loss of a-helix structure
940 m	*v*CC (*α*-helix)		
1003-1006	Phe v-ring		Insenstitive to conformation or microenvironment, suggested to be used for the normalization of the protein Raman spectra
1006 m	Phe *v*-ring		

Chapter 5. Cont.

Frequency (cm^{-1})	Structure	Products	Characteristics
1014	Trp		
1020-1060	β-sheets		
1200-1300	Amide III		C-N stretching and N-H in plane bending vibration of the peptide bond and contribution from Cα-C stretching and C=o in-plane bending
1230	Amide III		
1244	Amide III (*β*-sheets, random coil)		Fibrous helical conformation of the myosin tail region
1240-1250 sh	Amide III (*β*-sheets, random coil)		
1260-1300	Amide III (*α*-helix)		
1265	Amide III (*α*-sheets, random coil)		Globular myosin head
1273 m	Amide III (*α*-helix)		
1304	Amide III (*α*-sheets, random coil)		
1309 sh	amide III (*α*-helix)		
1309 sh	Amide III (*α*-helix)		
1321 m	*δ*CH		
1340	Trp		
1340	Amide III		
1341 m	*δ*CH		
1360 w	Trp*ν*-ring		
1363	Trp		
1400-1430 w	*νs*COO− (Asp, Glu)		
1409 W	His		
1410	His		In D2O solution of His, used to monitor the ionization state of aminoacid residues of the protein
1425 sh	Asp, Glu, Lys		

Chapter 5. Cont.

Frequency (cm^{-1})	Structure	Products	Characteristics
1450 s	$\delta_{as}CH_3$, δCH_2, δCH		
1490-1500	Histidine		
1510-1560	Amide II		N-H in-plane bending and C-N stretching of the trans peptide group
1553	Trp		
1554 w	Trp*v*-ring		
1582	Trp		
1606 sh	Trp, Phe, Tyr *v*-ring		
1618 sh	Trp, Phe, Tyr *v*-ring		
1645-1685 vs	Amide I		
1650-1680	Amide I, *vs*CC		Actomyosin structure
1650-1657	Amide I		C=O stretching vibrations, C-N stretching, Cα-C-N bending and N-H in-plane bending of peptide group
1656	Amide I	Meat batter	C-N stretching and N-H in-plane bending vibrations of the peptide bond
1660-1665	Amide I	Fresh fish muscle	Random coil or disorder structure
1665-1680	β sheet	Fresh fish muscle	
1700-1720	COOH		
2550-2580 w	*vs*SH (cysteinyl residues)		
2800-3000	Aliphatic C-H stretching		C-H stretching vibrational bands
2800-3000 vs	*v*CH		
2860	v_sCH_2		
2935	v_sCH_2, y v_sCH_3	Surimi gel	Protein unfolding leading to solvent exposure of methyl and methylene groups produce this wavenumber

Chapter 5. Cont.

Frequency (cm^{-1})	Structure	Products	Characteristics
2940	Aliphatic residue		C-H stretching, involvement of hydrophobic interactions
2970	$\nu_{as}CH_3$		
3128-3071			Water holding capacity
3140	NH stretching of primimary amides		

*Adapted from Herrero (2008), Bouraoui (1997), Pederson (2003).

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