
รายงานวิจัยฉบับสมบูรณ์

# โครงการโพรไฟล์ทรานสคริปโตมที่เกี่ยวข้องกับการเกิดตำหนิแบบ white striping ในไก่เนื้อส่วนอก

# Muscle transcriptome profiles associated with development of white striping defect in broiler breast meat

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## Executive summary

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# บทคัดย่อ

การเกิดตำหนิแบบแถบลายสีขาว (white striping; WS) บนเนื้อส่วนอกไก่เป็นปัญหาสำคัญของอุตสาหกรรมไก่เนื้อ งานวิจัยนี้มีวัตถุประสงค์เพื่อบ่งชี้กลไกชีวภาพที่เกี่ยวข้องกับการเกิดตำหนิ WS บนเนื้อส่วนอกของไก่เนื้อโดยใช้เทคนิคไมโครอะเรย์ในการวิเคราะห์โพรไฟล์ทรานสคริปโตมของตัวอย่าง งานวิจัยนี้แบ่งออกเป็น 2 ส่วน โดยส่วนแรกเป็นการศึกษาอุบัติการณ์ของตำหนิแบบ WS ในไก่ที่เก็บจากโรงเชือดอุตสาหกรรม รวมทั้งวิเคราะห์ผลกระทบของการเกิดตำหนิต่อคุณภาพของเนื้ออกไก่ ผลการศึกษาตัวอย่างไก่เนื้อตัวผู้สายพันธุ์ Ross 308 (n=184) ที่มีอายุระหว่าง 39 วัน ถึง 49 วัน พบว่า มีเนื้อไก่ 4 ชิ้น (คิดเป็น 2.2%) ที่ไม่พบตำหนิดังกล่าว แต่พบว่ามีตัวอย่างเนื้อไก่จำนวน 102 ชิ้น (55%) 71 ชิ้น (39%) และ 7 ชิ้น (3.8%) จัดเป็น WS ระดับเล็กน้อย ปานกลาง และรุนแรงมาก ตามลำดับ ตัวอย่างเนื้อไก่ที่เป็น WS ระดับรุนแรงมากประกอบด้วยไขมันมากขึ้น แต่มีโปรตีนและเถ้าลดลง ($p<0.05$) ลักษณะภาพตัดขวางเส้นใยกล้ามเนื้อเมื่อส่องภายใต้กล้องจุลทรรศน์อิเล็กตรอน พบว่า ตัวอย่างที่มีตำหนิ WS แบบรุนแรงมากมีพื้นที่หน้าตัดใหญ่กว่าระดับรุนแรงเล็กน้อยและตัวอย่างที่ไม่ผิดปกติ ตัวอย่างเนื้อไก่ที่มีตำหนิมีค่า pH สูงแต่ปริมาณกรดแลคติกต่ำกว่าตัวอย่างที่ไม่มีตำหนิอย่างมีนัยสำคัญ ตัวอย่างที่มีตำหนิระดับรุนแรงมากเมื่อทำให้สุกมีค่า hardness springiness และ chewiness สูง แต่มีค่าแรงและพลังงานที่ใช้เฉือนผ่านตัวอย่างต่ำกว่าตัวอย่างที่ไม่มีตำหนิอย่างมีนัยสำคัญ จากการวิเคราะห์ ordinal logistic regression model (OLR) พบว่าหากเพิ่มอายุของไก่ 1 วัน หรือเพิ่มสัดส่วนเนื้ออกขึ้น 1 % จะทำให้มีโอกาสเกิด WS ระดับรุนแรงขึ้น 11.8% และ 51.5% ตามลำดับ ในงานวิจัยส่วนที่สองเป็นการเปรียบเทียบการแสดงออกของยีนในกล้ามเนื้อไก่ *pectoralis major* ในกลุ่มตัวอย่างที่มีตำหนิ WS ระดับต่างๆ โดยใช้ total RNA จากกล้ามเนื้อของตัวอย่างที่มีตำหนิระดับเล็กน้อย (n=6) ระดับปานกลาง (n=6) และระดับรุนแรงมาก (n=4) สำหรับไฮบริไดซ์บน Agilent SurePrint G3 Custom GE 8×60K chicken gene expression ไมโครอะเรย์ พบว่ามียีนที่แสดงออกแตกต่างกัน (DEGs) เมื่อเปรียบเทียบตัวอย่างระหว่างระดับรุนแรงต่อระดับเล็กน้อย (WS1) จำนวน 2,517 ยีน เปรียบเทียบระหว่างระดับรุนแรงต่อระดับปานกลาง (WS2) จำนวน 1,615 ยีน และเทียบระหว่างตัวอย่างปานกลางต่อระดับเล็กน้อย (WS3) จำนวน 2,483 ยีน นอกจากนี้ ยีนประมาณ 80% ของ DEGs ทั้งหมดมีค่า foldchange ระหว่าง 1.0 ถึง 1.5 นอกจากนี้ ยืนยันข้อมูลไมโครอะเรย์ด้วยเทคนิค quantitative real-time polymerase chain reaction (qPCR) จาก pathway analysis พบ metabolic pathway necroptosis และ vascular smooth muscle contraction เป็นกลไกทางชีวภาพสามอันดับแรก ที่เกี่ยวข้องกับการเกิดตำหนิแบบ WS ที่รุนแรงต่างกัน นอกจากนี้ การเกิด WS ยังเกี่ยวข้องกับกลไกทางชีวภาพที่ทำหน้าที่เมื่อเซลล์ได้รับความเครียดจากภาวะออกซิเจนจำกัด และเกิดการอักเสบด้วย ในส่วนของ metabolic processes พบว่าการแสดงออกของยีน lactate dehydrogenase A และ phosphorylase kinase regulatory subunit beta ลดลงอย่างมีนัยสำคัญในตัวอย่างที่มี WS ระดับรุนแรงมาก สัมพันธ์กับปริมาณไกลโคเจนและกรดแลคติกที่ลดลง รวมถึงค่า pH ที่สูงขึ้นในเนื้อสัตว์ ผลงานวิจัยนี้แสดงให้เห็นว่าการเกิดตำหนิแบบ WS ในเนื้ออกไก่เกี่ยวข้องกับการเปลี่ยนแปลงกลไกทางชีวภาพ โดยเฉพาะความผิดปกติของระบบเมตาบอลิสมกลุ่มคาร์โบไฮเดรต และการซ่อมแซมของกล้ามเนื้อภายใต้สภาวะออกซิเจนจำกัดในอกไก่ที่มีขนาดใหญ่กว่าปกติอีกด้วย

**คำสำคัญ**: ตำหนิแบบแถบลายสีขาว, ไก่เนื้อทางการค้า, เนื้ออกไก่, การถดถอยโลจิสติค (ordinal logistic regression) ไมโครอะเรย์ โพรไฟล์ทรานสคริปโตม

# ABSTRACT

Development of white striping (WS) defect has been in great concern of poultry industry. The overall goal of this study was to obtain better understanding regarding biological pathways associated with development of WS in the breast muscle. The current investigation was divided into two parts. The first part was aimed at estimating incidence of WS defect in commercial broilers. The second part was to compare gene expression patterns in chicken skeletal muscle associated with WS defects. In the first part, commercial broilers (n=184, male Ross 308) slaughtered at the ages varying from 39d to 49d were collected. Impact of WS development on meat quality indices was also evaluated. The meat samples were classified as non-defective, mild WS, moderate WS and severe WS based upon numbers and thickness of white striation appeared on the surface of the meat. Of 184, only 4 (2.2%) samples were grouped as non-defective whereas 102 (55%), 71 (39%) and 7 (3.8%) were mild, moderate and severe, respectively. Severe WS samples showed elevated fat content but reduced protein and ash content ($p<0.05$). Muscle fiber cross-sectional area of moderate and severe WS, as observed under scanning electron microscope, was larger than those of mild and non-defective samples. The meat classified as WS samples exhibited a higher ultimate pH but lower lactic acid content compared to those of non-defective meat. Increases in hardness, springiness, and chewiness, but decreases in shear force and shear energy were observed in cooked severe WS samples ($p<0.05$). Based upon an ordinal logistic regression, an increase in slaughter age and percentage of breast weight by one unit elevated the likelihoods of increasing WS severity by 11.8% and 51.5%, respectively. In the second part, gene expression patterns of *pectoralis major* muscle associated with WS severity were profiled using Agilent SurePrint G3 Custom GE 8×60K chicken gene expression microarray. Total RNA was isolated from the muscle samples of 49d broiler breast classified as mild (n=6), moderate (n=6) and severe WS (n=4). The transcriptome profiling revealed that 2,517, 1,615 and 2,483 transcripts were differentially expressed (DEGs) in comparisons between severe WS and mild WS (WS 1), between severe WS and moderate WS (WS 2), and between moderate WS and mild WS (WS 3), respectively. Approximately 80% of the DEGs in all comparisons showed absolute foldchange ranging between 1.0 and 1.5. Microarray data was confirmed using quantitative real-time polymerase chain reaction (qPCR). Pathway analysis highlighted metabolic pathway, necroptosis and vascular smooth muscle contraction as the top pathways associated with WS severity. Upstream and downstream of responsive pathways for stress under hypoxic condition and inflammations were revealed. Declined expressions of lactate dehydrogenase A and phosphorylase kinase regulatory subunit beta were identified in severe WS compared with mild WS corresponding with reduced muscular glycogen and lactic acid content as well as increased ultimate meat pH in the severe WS. Overall, the current findings supported altered biological mechanistic pathways, particularly carbohydrate metabolism, occurred under limited oxygen supply within the enlarged breast muscle of the broilers exhibiting severe WS defect.

**Keywords:** white striping, commercial broilers, breast meat, meat defect, ordinal logistic regression, microarray, transcriptome profile

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# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND

The demand for poultry meat has been steadily grown as reflected by an increase in global poultry meat consumption from 11.1 kg (per person in the year of 2000) to 13.6 kg (per person in 2009, USDA, 2015). Among all poultry meat, the commercial meat-type chicken, known as broiler, is the most consumed poultry meat throughout regions as it is an inexpensive, good source of protein. In Thailand alone, 1.57 million tons of broilers were produced in 2013 (USDA, 2015), and the country earned 6.3 billions THB by exporting 0.5 million tons of chicken meat (USDA, 2015). An increase in export volume is anticipated as EU and Japan markets for raw and frozen chicken commodities have recently been re-opened.

To meet an increasing demand for chicken meat, broilers have been intensively selected for rapid growth rate and heavy carcass yield. In Thailand, however, broiler producers generally purchase the commercial meat-type chicken breeds from abroad and applied their own production systems in order to enhance growth efficiency of the birds. Today, commercial broilers reach market weight of 2.0 kg approximately within 40 days. Those birds are sold as fresh or frozen cuts, or as further-processed products. In addition to the normal commercial broilers, some Thai poultry producers obtain a group of heavy broilers with live weight of 3.2 to 3.5 kg by expanding slaughter age with or without modifying feed formula.

A success in improvement of poultry fast growth rate and heavy body mass coincides with an increasing meat quality problem (Dransfield and Sosnicki, 1999; Duclos et al. 2007; Petracci et al., 2013b; Lorenzi et al., 2014; Mudalal et al., 2015; Petracci et al., 2015). One of the concerned quality defects is white striping. The emerging defect is characterized by the appearance of white striation parallel to muscle fiber on the surface of breast fillets (Petracci et al., 2013b). The white-striped meat received a significant decrease in consumer's acceptance because the white striation on the meat was a sign of high fat content, meat toughness and a spoilage condition to consumers (Kuttappan et al., 2012b).

Additionally, chemical composition, protein quality and quantity, as well as protein functionality of the white-striped chicken meat were altered (Mudalal et al., 2014). Petracci et al. (2013b) reported an occurrence of white-striped breast meat in poultry commercial plant in Italy was approximately 10% in medium-size broilers (2.7 kg live weight). Later, Lorenzi et al. (2014) found an increased incidence (48%) and severity of the defect in the population of heavy chickens (average live weight of 3.9 kg). Based on this information, a group of heavy broilers produced by Thai producers using the modified production scheme is more likely to yield the white-striped breast meat. Additional to an undesirable white-striping appearance, the white-striped meat adversely diluted final quality of processed meat products due to its inferior protein functionality. Such problems potentially cause economic loss in Thai poultry industry. However, the incidence of white striping defect in Thailand poultry industry has not been assessed. The cause of white striping in chicken meat is still unclear. The main objectives of this study was to define

molecular pathways associated with development of white striping defect in skeletal muscle obtained from commercial broilers.

**1.2 OBJECTIVES**

- To estimate occurrence and severity of white striping defect among commercial broilers collected from commercial slaughterhouse in Thailand.
- To compare gene expression patterns in skeletal muscle associated with WS defect using microarray technique
- To define biological pathways associated with WS defect in broiler breast muscle

# CHAPTER 2

# INCIDENCE OF WHITE STRIPING DEFECTS IN BROILERS COLLECTED FROM COMMERCIAL SLAUGHTERHOUSE

## 2.1 ABSTRACT

Development of white striping (WS) defect has been in great concern of poultry industry. The objectives of this study were to estimate incidence of WS defect in breast meat collected from commercial broilers (n=184, male Ross 308) slaughtered at the ages varying from 39d to 49d. Impact of WS development on meat quality indices was also evaluated. The meat samples were classified as non-defective, mild WS, moderate WS and severe WS based upon numbers and thickness of white striation appeared on the surface of the meat. Of 184, only 4 (2.2%) samples were grouped as non-defective whereas 102 (55%), 71 (39%) and 7 (3.8%) were mild, moderate and severe WS, respectively. As slaughter age increased to 45d and 49d, moderate WS samples were predominant. Carcasses and breast meat of non-defective samples were lighter in weight than those of the severe and moderate WS groups regardless of age ($p<0.05$). Severe WS samples showed elevated fat content but reduced protein and ash content ($p<0.05$). Muscle fiber cross-sectional area of moderate and severe WS, as observed under scanning electron microscope, was larger than those of mild and non-defective samples. In 20-min postmortem skeletal muscle samples, no significant differences in pH20, R-value and lactic acid content were found ($p\geq0.05$). On the other hands, the 24-h-postmortem meat classified as WS samples exhibited a higher pHu but lower lactic content compared to those of non-defective meat. Increases in hardness, springiness, and chewiness, but decreases in shear force and shear energy were observed in cooked severe WS samples ($p<0.05$), suggesting altered texture of defective samples. Based upon an ordinal logistic regression, an increase in slaughter age and percentage of breast weight was associated with an increased severity of WS defect in commercial broiler breast meat.

## 2.2 INTRODUCTION

An advance in poultry production has allowed the manufacturers to produce fast-growing chickens with enlarged muscle mass, particularly breast muscle, the most valuable chicken part in broiler industry. The success in chicken production, however, has coincided with an increased incidence of quality abnormalities in chicken breast (Dransfield and Sosnicki, 1999; Duclos et al. 2007; Petracci et al., 2013a; Lorenzi et al., 2014; Mudalal et al., 2015). The emerging white striping (WS), characterized by visual appearance of white lines parallel to muscle fiber on the surface of chicken breast meat, are of great concern.

The WS occurrences have been extensively reported in European countries (Petracci et al., 2013a; Sihvo et al., 2014; Tasoniero et al., 2016), in the US (Kuttappan et al., 2012a) and in Brazil (Ferreira et al.,

2014) over the past decade. In the early studies, the prevalence of WS chicken breast was reported at approximately 10% depending on live weight of the chickens (Petracci et al., 2013a). Within a few years, the incidence of WS has drastically increased, varying from 20% to as high as 96% (Kuttappan et al., 2013a; Lorenzi et al., 2014; Trocino et al., 2015; Russo et al., 2015; Tijare et al., 2016). The increased WS prevalence was reported in the birds experimentally fed with high-energy diets in comparison with low-energy diets (Kuttappan et al., 2013a; Kindlein et al., 2017). The defective breasts showed negative changes in chemical composition, technological properties as well as consumer acceptance (Kuttappan et al., 2012b; 2013b; Petracci et al., 2013b; Petracci et al, 2014; Mudalal et al., 2015; Tasoniero et al., 2016; Tijare et al., 2016).

Occurrence of WS defect in commercial broilers around Eastern and Southeast Asia has not been widely discussed. One explanation could be the fact that most commercial broilers distributed in this region are generally slaughtered at the age of 35 days to 42 days and sold with skin covering, if exists, any undesirable appearances. Only small percentage of broilers are reared longer, up to 49 days with an initial attempt to produce heavier broilers to be sold locally or exported to the western countries in the forms of premium-grade whole carcasses or fresh cuts. To date, however, the growing numbers of the heavy broilers have been attainable among the birds slaughtered within the normal period. Based on the previous evidences regarding WS, those rapidly grown birds were prone to WS which could adversely affect quality of the breast meat. The main objective of this study was to monitor WS cases among commercial broilers randomly collected at commercial slaughterhouse with the age varying from 39 to 49 days. Additional information regarding quality indices influenced by WS defect was subsequently examined. The chance of increasing WS severity within the broiler breast meat associated with slaughter age and breast yield was also determined using ordinal logistic regression.

## 2.2 SPECIFIC AIMS

- To monitor WS cases among commercial broilers collected at commercial slaughterhouse with the age varying from 39 to 49 days.
- To determine influence of WS defect on meat quality indices of the chicken breast
- To identify association between WS severity and slaughter age and breast yield

## 2.3 EXPERIMENTAL DESIGN AND METHODOLOGY

### 2.3.1 Animals and samples collection

A total of 184 breast samples were collected from male ROSS 308 broilers at the age varying from 39d to 49d. The birds were reared by five farms located in Saraburi, Lopburi, and Nakhon Ratchasima (Thailand) under the uniform standard practices of Sun Food International Company, Ltd (Thailand). The collection process was conducted at the industrial slaughterhouse (Saraburi, Thailand). Slaughtering process was operated by the company's trained staff according to the Halal standard practice. On the day of slaughter, the birds were fasted for 12 h and transferred to the industrial slaughtering facility. The birds

were manually hung on shackles, electrically stunned using water bath, and slaughtered by manual neck cut. The birds were then bled for 3 min, scalded at 70°C for 2 min, and subsequently put in a rotary drum picker for 30 s. Immediately after de-feathering, a random chicken carcass was collected from the conveying belt, weighed and immediately advanced to evisceration. One side of chicken breast was dissected from the eviscerated carcass, sliced into small cubes, and snap-frozen in liquid nitrogen. The snap-frozen samples, referred to as 20-min postmortem skeletal muscle samples, were kept in liquid nitrogen while transporting back to Food Biotechnology Laboratory, BIOTEC. Upon arrival, the muscle samples were immediately stored at -80°C until needed for determination of pH at 20 min postmortem (pH20), lactic acid content and ATP status. The other side of the breast was dissected, placed in a plastic bag and kept on ice while being transferred back to the lab. This side of the meat was referred as 24-h postmortem meat samples and was utilized for WS defect classification and determination of breast weight, microscopic images, drip loss, cook loss, physicochemical as well as textural properties. Based on its carcass weight, the birds were graded into “medium” or “heavy” groups using the cut-off value of 2.5 kg (medium; weight ≤ 2.5 kg, heavy; weight > 2.5 kg) according to the local poultry industry criteria.

Carcass weight and dress weight of the samples referred to as the weight of the whole carcass prior and after evisceration, respectively. Breast weight was the weight of the breast meat determined at 24 h postmortem. Percentage of breast weight was expressed as the percentage of the breast meat weight relative to the whole carcass weight.

**2.3.2 Classification of white striping defect**

After 24 h postmortem, white striping (WS) defect and its severity were subsequently inspected using the criteria as previously described (Kuttappan et al., 2012b) with modifications. Four categories were classified based on appearance and thickness of the white striation on the surface of the breast (Figure 2.1).

- Non-defective: no white striation was found on the meat surface
- Mild: 1 to 40 white lines with the thickness of ≤ 0.5 mm
- Moderate: more than 40 white lines or 1 to 5 line(s) with the thickness of 1- to 1.9-mm
- Severe: more than 5 lines with the thickness of 1- to 1.9-mm thickness or at least 1 white line with thickness > 2-mm

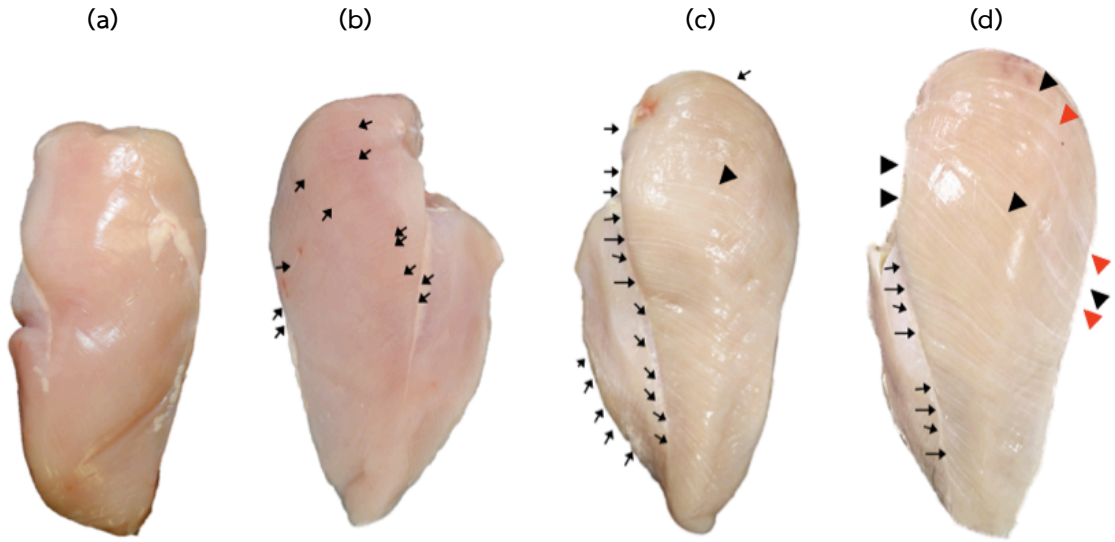


**Figure 2.1** White striping (WS) defect at four degrees of severity, (a) normal, (b) mild, (c) moderate and (d) severe. Black arrows, black triangle, and red triangle indicate white lines with thickness < 1.0 mm, 1.0-1.9 mm and ≥2.0 mm, respectively.

### 2.3.3 Determination of meat quality indices

Following the WS inspection, ultimate pH (pHu) and surface color of the meat were determined immediately. Top and caudal parts of the meat were then removed, thoroughly chopped and used for evaluation of chemical compositions and physicochemical properties. The trimmed sample was subjected to drip loss and cook loss analyses.

***Chemical composition***. Moisture, protein and ash contents of the samples were determined following the recommended methods of AOAC (2000). Total fat content was measured using a modification of the chloroform: methanol procedure described by Bligh and Dyer (1959). Briefly, 10 g of chopped raw meat were homogenized for 2 min, on ice, with 80 mL of a solvent mixture consisting of 1 volume of chloroform, 2 volumes of methanol and 1 volume of distilled water. Twenty milliliters of chloroform and 10 mL of distilled water were added into the homogenate. Each solvent addition was followed by homogenization for 1 min and 30 s, respectively. After centrifugation at 3,000×g, 4°C for 15 min, the supernatant was transferred into a separating flask, and let stand for 1 h. The organic phase was filtered through a filter paper containing 2 g of anhydrous sodium sulfate into a pre-weighed dry round-bottom flask. The solvent was removed by evaporating at 65°C using a rotary evaporator. The crude fat was dried at 70°C for 4 h to remove any residual solvent and water and reweighed afterwards. The fat to protein quantity ratio was expressed in percentage.

Total collagen content was determined after acid hydrolysis as described by Bergman and Loxley (1963) with a modification. Briefly, for total collagen, 0.5 g of chopped raw and cooked samples was hydrolyzed with 5 mL of 6 N HCl at 110°C for 24 h. The neutralized hydrolysate (pH 7.0 ± 0.05) was

filtered and diluted with distilled water to a final volume of 50 mL. Ten microliters of the hydrolysate or hydroxyproline standard was reacted with 10 µL of oxidizing reagent (one volume of 7% w/v chloramin T in aqueous solution and four volumes of acetate/citrate buffer, pH 6.0), 20 µL of isopropanol and 139 µL of Ehrlich's reagent. After incubation at 60°C for 25 min, absorbance at 558 nm of the mixture was measured. Concentration of hydroxyproline was converted to collagen content using the factor of 7.25 and expressed as milligrams of collagen per gram meat sample.

To assess lactic acid content in the muscle and meat samples, 750 mg of the samples were homogenized with 10 mL of 1 M perchloric acid for 2 min. The pH of the homogenate was adjusted to pH 8.0 using KOH followed by adjusting final volume to 25 mL using distilled water. The solution was then incubated on ice for 20 min to precipitate potassium perchlorate and centrifuged at 13,000×g for 10 min. Lactic acid content in the supernatant was determined using L-Lactic Acid Assay kit (Megazyme, Ireland).

Mineral profiles of breast samples were determined using an Orbis PC Micro-x-ray fluorescence (XRF) analyzer (EDAX, AMETEK, Inc.). Samples were prepared by pressed between two layers of 4-µm thick polycarbonate film and placed in an EDXRF sample holder. Elemental profile was accomplished under a vacuum condition using the x-ray beamline at 30KV and 1000 µA. The acquisition time was 60s for single point analysis on the prepared pellets with the x-ray tube radius of 1 mm and 1mm penetration depth. The data was recognized against the available library provided by the Orbis Vision software (EDAX). The signal intensity of each element was reported using the Orbis Vision software (EDAX).

***Physicochemical properties.*** Surface color in CIE L*a* b* system of the meat was measured using a Minolta CR300 Chroma Meter (Minolta, Japan) and estimated after the meat was bloomed at 4°C for 30 min. The values of pH20 and pHu of each sample were determined in triplicates according to Eadmusik et al. (2011) and Petracci and Baéza (2009), respectively. In brief, for pH20, 1 g of the 20-min postmortem muscle samples was ground thoroughly in liquid nitrogen and subsequently homogenized, on ice, with 10 mL of iced-cold iodoacetate buffer (5mM sodium iodoacetate, 150 mM potassium chloride, pH 7) for 30 s. The pH value of the homogenate was immediately measured using a pH meter. For 24-h postmortem meat sample, the pH was directly determined by inserting a spear-shape glass pH-probe (Mettler-Toledo Seven Easy, Mettler-Toledo, Inc., Switzerland) into three assigned positions (top, middle, and caudal) of each meat sample.

The ATP status in 20-min postmortem specimen was assessed as R-value (Ryu and Kim 2006). Briefly, 750 mg samples were homogenized, on ice, with 5 mL of 1 M perchloric acid for 40 s. After centrifugation at 5,000×g, 4°C for 10 min, 100 µL of supernatant was mixed with 4 mL of 0.1 M phosphate buffer (pH 7). Absorbance at 250 nm and 260 nm of the mixture was subsequently measured using a UV-Vis spectrophotometer (Model Helios omega, Thermo Scientific, Inc.). R-value, evaluated in duplicates, was expressed as a ratio of absorbance at wavelength 250 nm to 260 nm.

Lipid oxidation of the meat was determined using Thiobarbituric acid-reactive substances (TBARS) assay. Determination of TBARS, used as an index of lipid oxidation, was performed in duplicates according to the method described by Buege and Aust (1978). The results were expressed as mg malonaldehyde per kilogram meat sample.

To assess degree of myofibril degradation in the 24-h postmortem breast meat, Myofibril fragmentation index (MFI) was measured in triplicates following the protocol of Hopkins et al. (2000). In brief, 0.5 g of chopped raw meat sample was homogenized, on ice, with 30 mL of ice-cold MFI buffer (25 mM potassium phosphate buffer, pH 7.0 containing 0.1 M KCl, 1 mM EDTA and 1 mM sodium azide) at 13,500 rpm for 2 min (30 sec on followed by 30 sec rest, 2 cycles). The homogenate was filtered through two layers of gauzes and rinsed with 10 ml cold MFI buffer. The filtrate was subsequently centrifuged at 1,000×g for 10 min at 2°C. The resulting pellets of myofibrils were re-suspended in 10 ml of cold MFI buffer. The extraction was repeated twice and the pellet was finally re-suspended in 10 ml of cold MFI buffer. The protein concentration of the suspensions was determined using the Pierce Bicinchoninic acid (BCA) Protein Assay kit (Thermo Scientific, Rockford, Illinois, USA) following manufacturer's recommendation. Absorbance of the myofibril suspensions, diluted in MFI buffer to a final protein concentration of 0.5 mg/mL with a total volume of 2 mL, was determined at 540 nm using MFI buffer as blank. MFI was calculated by multiplying the average absorbance with 200.

***Water holding capacity and texture analyses.*** Water holding capacity of the meat, in terms of drip loss and cook loss was determined as previously described (Chiang et al., 2008). Drip loss, cook loss and cooked meat texture were determined consecutively within each breast sample. Following the trimming of the top and caudal parts, the meat was weighed and individually packed in a plastic bag and hung at 4°C. Once reached 24 h, the meat was re-weighed. Drip loss was expressed as percentage of the weight loss due to gravitational force during 24-h hanging relative to the initial weight. Afterwards, the meat was vacuum-packed in a polyethylene plastic bag and cooked at 95°C by water immersion. Internal temperature of the meat thickest portion was monitored throughout the cooking using a thermocouple. When the core temperature reached 80°C, the meat was then cooled in an iced water bath until its temperature declined to below 15°C. The cooked meat was rested at 4°C for at least 2 h before re-weighed. Cook loss represented percentage of the weight loss owing to cooking. Texture of the cooked samples was determined using Warner-Bratzler Shear (WBS) and texture profile analysis (TPA) using TA-XTi texture analyzer (Stable Micro System, Godalming, UK) following protocol described by U-chupaj et al. (2017). Each breast was cut into four rectangular cuboids (10 × 20 × 10 mm) for the WBS test and three cubes (10 × 10 × 10 mm) for TPA. All meat samples were cut parallel to muscle fiber alignment to minimize the variation from muscle fiber direction. The testing conditions were set as follows.

- **WBS:** The texture analyzer was equipped with a Warner-Bratzler shear blade with a 25-kg loading cell. The parameters were 1 mm/s test speed, 25 to 30 mm working distance, and 0.2 N trigger force. The actual cross-sectional area at the shearing point indicates WBS force (N), while work of shear (N

mm) represents the area under the force deformation curve. Cooked meat strips were sheared perpendicularly to the muscle fiber direction

- **TPA:** Cooked meat cubes were double-compressed to 40% of their initial height using a 25-kg loading cell connected to a 50-mm cylinder aluminum probe. Test conditions were: 1 mm/s probe velocity, 1 s holding time, and 0.1 N trigger force. The TPA parameters hardness (N), springiness (ratio), cohesiveness (ratio), gumminess (N), and chewiness (N×mm) were calculated from the force-time curves recorded for each sample using Texture Expert software version 1.0 (Stable Micro System).

All textural parameters were automatically calculated and reported by the Exponent software (Stable Micro Systems).

**2.3.4 Microscopic images**

Cross-sectional microscopic view of the meat samples (n=3) was depicted under a SU5000 field emission-scanning electron microscope (FE-SEM, HITACHI Ltd., Tokyo, Japan) according to the method of Wattanachant et al. (2005) with modifications. The meat (5 × 5 × 5 mm) was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 for 2 h at ambient temperature. After washing with 0.1 M phosphate buffer, pH 7.3, the meat was dehydrated in ethanol solutions with a serial concentration of 50%, 70%, 80%, 90% and 100%. The dehydration was done twice and three times, 30 min each, in 50% to 90%, and 100% ethanol, respectively. The specimen was cut in liquid nitrogen, mounted on an aluminum stub using carbon tape and splutter-coated with gold for 15 sec. Cross-sectional images (300× magnification) of the sample were displayed under FE-SEM using an acceleration voltage of 10 kV and subsequently analyzed using WinROOF software (Mitani Corporation, Japan). The microscopic images were processed using ImageJ 1.46r software (Rasband, 2012). The fiber density was estimated by counting total fiber number (TFN) in nine fields per specimen (modified from Alves et al., 2012). The average fiber diameter was calculated from the mean cross-sectional area of the fiber, which was obtained by dividing total cross-sectional fiber area with TFN within each field.

Size distribution of muscle fiber was estimated and expressed as percentage of relative to total fibers within each WS severity (Petracci et al., 2013a). Fibers were grouped into four subdivisions; group I (< 1,000 $\mu m^2$), group II (≥ 1,000 to < 2,000 $\mu m^2$), group III (≥ 2,000 to < 3,000 $\mu m^2$) and group IV (≥ 3,000 $\mu m^2$) based on individual cross-sectional area.

**2.3.5 Statistical analysis**

The statistical analysis was performed using the R package version 3.2.1. The dataset was firstly subjected to normality and variance equality testing using Shapiro-Wilk Normality Test and Bartlett Test, respectively. Mean difference among the treatments was analyzed using analysis of variance (ANOVA). The Duncan's new multiple range test was used for post hoc multiple comparisons. In case normality and

homogeneity of variance were violated, prior ANOVA and multiple range test, function *varIdent* from library nlme of R package was pursued to account for the difference in variance. The significant level for all statistical analyses was set as $\alpha$=0.05. Ordinal logistic regression model (OLR) was constructed to determine the effects of slaughter age and percentage of breast weight on WS severity in broiler breast (model 1). Severity of WS defect was defined as the ordinal response with non-defective = 1, mild = 2, moderate = 3 and severe = 4.

The OLR model is as follow.

$$\text{logit}\,[P(Y \le j \mid X_1, X_2, .., X_k)] = \alpha_j + \beta_{j1}X_1 + \beta_{j2}X_2 + \ldots + \beta_{jk}X_k$$

where $P(Y \le j \mid X_1, X_2, .., X_k)$ is the probability of being at or below category j, given a set of X independent variables, $\alpha_j$ is an intercept, $X_1, X_2, .., X_k$ denote the independent variables and $\beta_{jk}X_k$ is the logistic coefficient for the $j^{th}$ category and $k^{th}$ independent variable. Independent variables were age and percent of breast weight. With R, a function *polr* from MASS package was used for the analysis. The results from the OLR analysis were reported as the odds ratio. The odds ratio >1 indicates an increased chance whereas <1 denotes a decreased chance of a dependent category as a result of an increase in the continuous independent variable by one unit.

## 2.4 RESULTS AND DISCUSSION

### 2.4.1 Prevalence of WS defects in the commercial broilers

According to the current observation, whole carcass weight of the 184 collected broilers, with the slaughter ages from 39d to 49d, was in the range of 2.03 kg to 3.94 kg (Figure 2.2a). Among the collected samples, 4 (2.2% of total broilers) samples were classified as non-defective meat whereas the other 180 (97.8%) samples exhibited WS defect (Figure 2.2b). It must be denoted that no statistical effect of rearing location was detected in this study ($p \ge 0.05$).

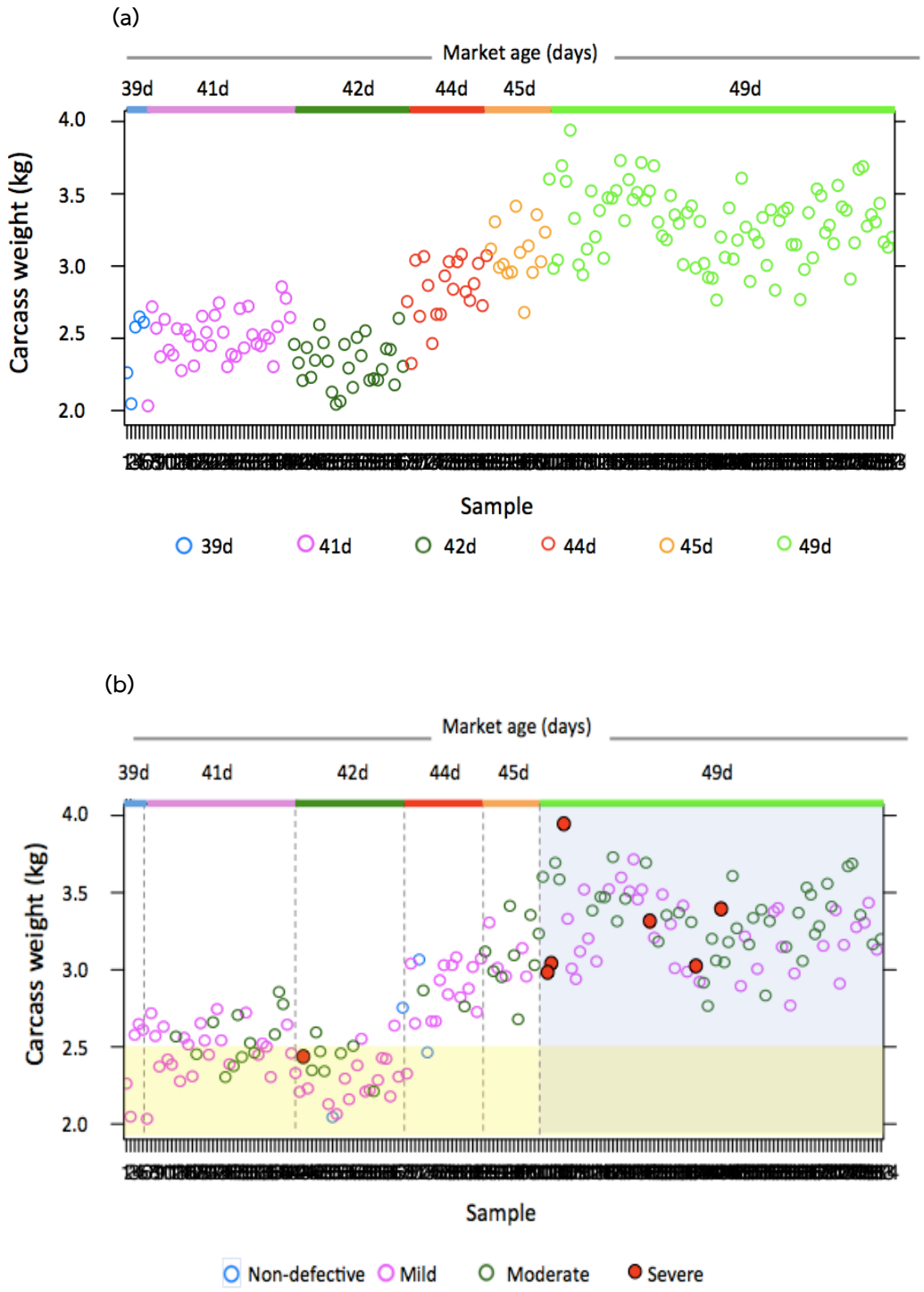


**Figure 2.2.** Scatter plot illustrates carcass weight of each commercial broiler. (a) A total of 184 male broilers were grouped based on market age into 6 groups; 39d (blue), 41d (pink), 42d (dark green), 44d (red), 45d (orange) and 49d (light green). (b) The birds were grouped based on white striping severity, i.e. non-defective (blue), mild (pink), moderate (green) and severe (red)

The WS degree has been classified into four levels, non-defective, mild, moderate and severe based upon numbers and thickness of those white lines appeared on surface of the meat. The current criteria have been modified from the study of Kuttappan et al. (2012b) in which WS severity was categorized based solely on thickness of the white lines (thickness ≤ 1 mm = moderate, thickness > 1 mm = severe). Because some breasts collected in this study were almost entirely covered with several white fine lines on the surface but none of those lines was thicker than 1 mm (Figure 1b), total number of the lines was incorporated in the current WS classification. As shown in Table 2.1, the numbers of mild, moderate and severe WS samples observed in this study were 102 (55%), 71 (39%) and 7 (3.8%), respectively. The majority of the samples from broilers with the age of 39d to 44d were classified as mild WS whereas, within the groups of 45d and 49d birds, moderate WS was predominant. Six out of seven severe WS were detected in 49d broilers. Incidence of WS defect reported herein was more pronounced than those earlier observed by Lorenzi et al. (2014) and Alnahhas et al. (2016).

Regardless of age, carcass weight of non-defective group was lighter than those of the moderate and severe WS samples but was not different from mild WS (Table 2.2). Breast weight and percentage of breast weight of the non-defective was also lower than those samples exhibiting WS defect ($p<0.05$).

**Table 2.1** Numbers of broilers yielding breast meat white striping defects

| WS degree | Slaughter age (days) | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | **39** | **41** | **42** | **44** | **45** | **49** | |
| Non-defective | 0 | 0 | 1 | 3 | 0 | 0 | 4 |
| Mild WS | 5 | 23 | 18 | 15 | 5 | 36 | 102 |
| Moderate WS | 0 | 12 | 7 | 2 | 9 | 41 | 71 |
| Severe WS | 0 | 0 | 1 | 0 | 0 | 6 | 7 |
| **Total** | 5 | 35 | 27 | 20 | 14 | 83 | 184 |

**Table 2.2** Carcass and breast weights of non-defective or white striping breast meat[1, 2]

| Quality | Degree of white striping | | | |
|---|---|---|---|---|
| | **Non-defective (n=4)** | **Mild (n=102)** | **Moderate (n=71)** | **Severe (n=7)** |
| Carcass weight (kg) | 2.58 $^{b}$ ± 0.43 | 2.80 $^{ab}$ ± 0.43 | 3.07 $^{a}$ ± 0.42 | 3.16 $^{a}$ ± 0.46 |
| Dress weight (kg) | 2.21 $^{b}$ ± 0.32 | 2.46 $^{ab}$ ± 0.38 | 2.72 $^{a}$ ± 0.37 | 2.80 $^{a}$ ± 0.41 |
| Breast weight (g) | 188.36 $^{c}$ ± 32.73 | 240.07 $^{b}$ ± 48.64 | 278.48 $^{ab}$ ± 47.07 | 303.08 $^{a}$ ± 50.30 |
| Percentage of breast weight (%) | 7.35 $^{c}$ ± 1.04 | 8.54 $^{b}$ ± 0.85 | 9.06 $^{ab}$ ± 0.80 | 9.58 $^{a}$ ±0.66 |

[1]Data are presented as mean ± standard deviation.

[2]Different superscripts in the same row indicate statistical difference.

### 2.4.2 Impact of WS development on quality of broiler breast meat

#### 2.4.2.1 Chemical compositions

Chemical compositions of the non-defective and WS breast samples are shown in Table 2.3. The severe WS samples showed the greatest fat content but the lowest protein and ash content ($p<0.05$). No significant differences in those compositions were found among non-defective, mild and moderate samples ($p\geq0.05$). Changes in chemical composition in WS breast agreed well with previous observation (Kuttappan et al., 2012a; Mudalal et al., 2014; Petracci et al., 2014; Alnahhas et al., 2016; Soglia, et al., 2016). Total collagen analyzed in this study was not significantly different among the breast samples ($p\geq0.05$). Petracci et al. (2014) previously addressed greater collagen content in WS breasts, plausibly due to fibrosis in the defective samples (Kuttappan et al., 2013b; Trocino et al., 2015**;** Soglia et al., 2016). The different breeds of the broilers may explain dissimilar results between the previous and the current studies. Nonetheless, the findings herein suggested that the lines on the surface of the defective meat could be a complex deposition of fat, collagen and other fibrous connective tissues (Mann et al., 2011). The current findings affirmed the reduced nutritional quality in term of high fat, low protein content of the severe WS breasts.

Considering lactic acid content in 20min postmortem muscle, no significant difference was observed among WS levels. As postmortem duration prolonged, lactic acid was generated to the greater extent through anaerobic glycolysis to produce ATP as the energy source for cellular activities. At 24h postmortem when the chicken skeletal muscle was considered to be at the complete rigor state, the non-defective samples contained the greater lactic acid in comparison with the WS samples ($p<0.05$).

**Table 2.3** Chemical compositions of non-defective or white striping breast meat[1, 2]

| Property | Degree of white striping | | | |
|---|---|---|---|---|
| | Non-defective (n=4) | Mild (n=102) | Moderate (n=71) | Severe (n=7) |
| Moisture (%) | 75.01 ± 0.68 | 75.14 ± 0.98 | 75.33 ± 1.03 | 75.76 ± 1.95 |
| Total fat (%) | 1.34 $^{b}$ ± 0.19 | 1.54 $^{ab}$ ± 0.32 | 1.63 $^{ab}$ ± 0.40 | 1.88 $^{a}$ ± 0.98 |
| Total protein (%) | 23.02 $^{a}$ ± 1.04 | 22.07 $^{ab}$ ± 1.08 | 21.77 $^{b}$ ± 1.20 | 21.07 $^{b}$ ± 1.50 |
| Total ash (%) | 1.22 $^{a}$ ± 0.04 | 1.17 $^{ab}$ ± 0.09 | 1.16 $^{ab}$ ± 0.07 | 1.12 $^{b}$ ± 0.08 |
| Total collagen (mg/g sample) | 3.01 ± 1.02 | 2.87 ± 1.35 | 3.00 ± 0.90 | 3.92 ± 1.03 |
| Lactic acid (mg/g sample) | | | | |
| at 20 min | 2.61 ± 0.18 | 2.20 ± 0.68 | 2.63 ± 0.77 | 2.40 ± 0.32 |
| at 24 h | 9.11$^{a}$ ± 1.34 | 7.55$^{b}$ ± 1.58 | 7.10$^{b}$ ± 0.85 | 6.87$^{b}$ ± 0.84 |

[1]Data are presented as mean ± standard deviation.

[2]Different superscripts in the same row indicate statistical difference.

**2.4.2.2 Mineral profiles**

Minerals and trace elements consisted in the chicken breast samples are shown in Table 2.4. The severe WS samples showed the greater amounts of calcium, iron and zinc than those of the others ($p<0.05$). Concentration of sulfur in such samples was also higher than that of non-defective meat ($p<0.05$). On the other hand, as the WS severity elevated, phosphorous concentration gradually decreased ($p<0.05$). Similar changes in mineral profile in accordance with WS development were addressed in the study of Tasoniero et al. (2016) in which iron and sodium decreased whereas phosphorous and potassium increased ($p<0.05$). The current findings supported the overloaded of $Ca^{2+}$ within the myopathic muscle potentially due to muscle tissue degeneration (Mutryn et al., 2015; Petracci et al., 2015; Tasoniero et al., 2016). As [$Ca^{2+}$] plays crucial role in various cellular signaling pathways, an imbalanced [$Ca^{2+}$] could trigger downstream cellular dysregulation. The declined phosphorous concentration in the severe WS muscle could also be attributed by muscle damage (Fuller et al., 1976). Interestingly, aluminum was and selenium were detected in moderate WS samples. In case of selenium, the element was also found in the non-defective meat.

**Table 2.4** Elements and trace elements detected in non-defective or white striping breast meat[1, 2]

| Mineral | Percentage of total elements | | | |
|---|---|---|---|---|
| | Non-defective (n=4) | Mild (n=102) | Moderate (n=71) | Severe (n=7) |
| Aluminum (Al) | ND | ND | 5.82 ± 0.37 | ND |
| Calcium (Ca) | 4.82 $^{bc}$ ± 1.36 | 5.56 $^{b}$ ± 0.63 | 4.23 $^{c}$ ± 0.67 | 6.85 $^{a}$ ± 1.77 |
| Chromium (Cr) | 0.49 ± 0.06 | 0.50 ± 0.05 | 0.50 ± 0.05 | 0.49 ± 0.05 |
| Copper (Cu) | 0.31 ± 0.37 | 0.19 ± 0.03 | 0.18 ± 0.12 | 0.21 ± 0.04 |
| Iron (Fe) | 0.74 $^{b}$ ± 0.09 | 0.77 $^{b}$ ± 0.05 | 0.73 $^{b}$ ± 0.07 | 0.91 $^{a}$ ± 0.15 |
| Magnesium (Mg) | 30.28 ± 6.89 | 28.59 ± 3.10 | 29.21 ± 1.98 | 29.22 ± 3.76 |
| Phosphorous (P) | 382.62 $^{a}$ ± 7.80 | 373.33 $^{bc}$ ± 8.26 | 378.09 $^{ab}$ ± 9.26 | 367.04 $^{c}$ ± 11.08 |
| Potassium (K) | 543.78 ± 11.42 | 549.93 ± 13.39 | 540.54 ± 9.81 | 545.61 ± 16.36 |
| Rubidium (Rb) | 0.96 ± 0.55 | 1.24 ± 0.37 | 0.99 ± 0.34 | 0.96 ± 0.30 |
| Selenium | 0.03 ± 0.10 | ND | 0.025 ± 0.05 | ND |
| Sodium (Na) | 33.80 ± 7.01 | 34.84 ± 6.22 | 34.69 ± 4.36 | 40.65 ± 11.74 |
| Sulfur (S) | 0.96 $^{b}$ ± 1.12 | 3.75 $^{ab}$ ± 3.46 | 3.78 $^{ab}$ ± 1.99 | 6.57 $^{a}$ ± 5.01 |
| Zinc (Zn) | 1.23 $^{b}$ ± 0.20 | 1.28 $^{b}$ ± 0.08 | 1.24 $^{b}$ ± 0.18 | 1.51 $^{a}$ ± 0.31 |

[1]Data are presented as mean ± standard deviation.

[2]Different superscripts in the same row indicates statistical difference.

ND = not detected

**2.4.2.3 Physicochemical properties**

Influences of WS defects on physicochemical properties of the breast meat are shown in Table 2.5. No significant differences in lightness and redness/greenness, as indicated by L*-value and a*-value, respectively, were observed among the WS severity levels ($p \geq 0.05$). However, b*-values of the moderate and severe WS samples were significantly higher than that of non-defective ones ($p < 0.05$). An increase in yellowness in WS breast has been addressed in previous studies (Kuttppan et al., 2013b; Petracci et al., 2013b; Trocino et al., 2015; Tasoniero et al., 2016) and was hypothesized to be corresponded with high accumulation of fat content within the meat (Kuttappan et al., 2013b).

**Table 2.5** Physicochemical properties of non-defective or white striping breast meat[1, 2]

| Property | Degree of white striping | | | |
|---|---|---|---|---|
| | Non-defective (n=4) | Mild (n=102) | Moderate (n=71) | Severe (n=7) |
| L*-value | 51.53 ± 2.07 | 50.46 ± 2.22 | 51.34 ± 2.59 | 52.26 ± 3.15 |
| a*-value | 3.12 ± 0.52 | 3.00 ± 0.80 | 1.02 ± 0.74 | 3.16 ± 0.56 |
| b*-value | -0.98 $^{b}$ ± 1.01 | 0.22 $^{ab}$ ± 1.58 | 1.05 $^{a}$ ± 1.52 | 0.35 $^{a}$ ± 1.43 |
| pH20 | 6.79 ± 0.09 | 6.90 ± 0.11 | 6.87 ± 0.10 | 6.86 ± 0.08 |
| pHu | 5.70 $^{b}$ ± 0.16 | 5.99 $^{a}$ ± 0.20 | 5.97 $^{a}$ ± 0.12 | 6.00 $^{a}$ ± 0.12 |
| R-value | 0.81 ± 0.02 | 0.77 ± 0.05 | 0.76 ± 0.05 | 0.80 ± 0.01 |
| TBARS (mg MDA/kg sample) | 0.95 ± 0.13 | 0.94 ± 0.31 | 0.99 ± 0.31 | 1.24 ± 0.38 |
| MFI | 235.79 $^{a}$ ± 21.70 | 195.58 $^{ab}$ ± 53.15 | 146.09 $^{b}$ ± 18.43 | 169.22 $^{b}$ ± 3.00 |

[1]Data are presented as mean ± standard deviation.

[2] Different superscript indicates statistical difference.

pH20 = pH at 20 min postmortem, pHu = ultimate pH, R-value = ratio of absorbance at 250 nm to 260 nm, TBARS = Thiobarbituric acid-reactive substances, TBARS = Thiobarbuturic acid reactive substances, MDA = malonaldehyde, MFI = Myofibril fragmentation index

No significant differences in pH20 and R-value in the 20-min postmortem muscle samples were observed among different WS degree ($p \geq 0.05$). In the 24-h postmortem meat, pHu values of the WS samples at all severity levels were higher than that of the non-defective ones, corresponding with the lower lactic acid in 24-h postmortem meat ($p < 0.05$). Consistent results were addressed (Kuttappan et al., 2013b; Mudalal et al., 2015; Trocino et al., 2015; Tasoniero et al., 2016; Zambonelli et al., 2016). Production of ATP postmortem via anaerobic metabolism for maintenance of cellular activities leads to accumulation of lactic acid, resulting in pH decline from neutral pH (~7.0) in the muscle to the range of pH 5.3-5.8 in the meat (Smulders et al., 1992). The pHu varies among animals based upon species, muscle type, antemortem conditions of animals as well as surrounding environment. Either reduction of pHu to the greater extent or the rapid pH drop, or combination of both induces muscle protein denaturation, lowering water holding capacity, impairing texture and increasing lightness, in term of L*-value, of the meat. On the other hand,

an inadequate decline of pHu results in dark meat with dry and tough texture (Barbut et al., 2005). The correlation between pHu and meat L*-value, however, was more pronounced in red meat, e.g. pork and beef, compared to white meat, e.g. chicken breast meat (Barbut et al., 2008). This could explain why no difference in L*-value was observed despite of a greater degree of pHu in WS samples. Kuttappan et al. (2013b) reported that, based on multinomial regression, development of WS in broilers with the age of 59d, 61d and 63d was not associated with neither of pHu, L* or a* ($p > 0.05$); thus, WS defect might not cause metabolic changes in the muscle tissue. The current findings partially support an above argument as no difference in the R-value suggested similar early postmortem metabolic rate among the present sample groups. However, the greater pHu and lower content of lactic acid in the WS meat samples implied an insufficient glycogen stored inside the muscle cells (Nadaf et al., 2007) due potentially to histological degeneration of muscle fibers (Shivo et al, 2014; Mudalal et al., 2015). Besides, an altered metabolic event regarding conversion of glycogen into lactic acid to generate ATP postmortem could be anticipated. The current speculation was supported by protein profiles of severe WS relative to normal skeletal muscle collected from 52-d-old broilers (Kuttappan et al., 2017b). Decreased abundances of enzymes involving in carbohydrate metabolism may alter glycolytic potential in severe samples, eventually altering pHu.

Lipid oxidation in chicken breast meat was estimated using TBARS assay. In this study, no statistical difference in TBARS values of raw breast meat was found among the samples with different WS degree ($p \geq 0.05$). The observation corresponds with the previous studies (Alnahhas et al., 2016; Soglia et al., 2016).

The value of Myofibril fragmentation index (MFI) has been applied as an indicator of muscle fiber degradation (Yu et al., 2005; Wilhelm et al., 2010). In this study, moderate and severe WS samples exhibited lower MFI than the non-defective samples ($p<0.05$). No significant difference was observed between non-defective and mild samples ($p \geq 0.05$). In general, MFI indicates the extent of proteolysis caused by the rupture of the I-band of the sarcomere and breakage of intermyofibril linkages (Taylor et al., 1995). The current results suggested the effect of WS development, particularly at severe level, on degree of muscle fiber degradation postmortem. Degradation of myofibrillar proteins might be suppressed in the moderate and severe WS defective samples, possibly affecting texture of the samples.

**2.4.2.4 Water holding capacity and cooked meat texture**

As shown in Table 2.6, severe WS samples showed significantly greater degree of cook loss compared to those with mild and moderate degree ($p<0.05$). Cook loss of the non-defective breast, however, was not different from those of the defective groups ($p \geq 0.05$). Inconsistent impact of WS development on water holding capacity in terms of drip loss and cook loss were shown in previous studies. Increased cook loss due to WS defect ($p<0.05$) was previously reported by Petracci et al. (2013b), Mudalal et al. (2014; 2015), Tijare et al. (2016) and Zambonelli et al. (2016). Different scenario was observed by Kuttappan et al. (2013b), Trocino et al. (2015), Bowker and Zhuang (2016) as well as Tasoniero et al. (2016). Those authors stated no significant difference in cook loss between normal and WS samples ($p \geq 0.05$). The

discrepancy could be owing to varying broiler lines, slaughter ages, positions of tested samples and different cooking methods.

**Table 2.6** Water holding capacity and cooked meat texture of non-defective or white striping breast meat[1,2]

| Property | Degree of white striping | | | |
|---|---|---|---|---|
| | Non-defective (n=4) | Mild (n=102) | Moderate (n=71) | Severe (n=7) |
| Drip loss (%) | 1.02 ± 0.30 | 0.82 ± 0.43 | 0.86 ± 0.50 | 0.82 ± 0.29 |
| Cook loss (%) | 18.81 $^{ab}$ ± 2.24 | 17.86 $^{b}$ ± 4.33 | 17.33 $^{b}$ ± 5.29 | 22.77 $^{a}$ ± 7.06 |
| Shear force (N) | 54.40 $^{a}$ ± 17.69 | 43.09 $^{ab}$ ± 14.62 | 39.20 $^{b}$ ± 12.34 | 34.23 $^{b}$ ± 14.13 |
| Shear energy(N×mm) | 459.18 $^{a}$ ± 118.44 | 352.73 $^{b}$ ± 123.50 | 314.13 $^{b}$ ± 104.06 | 270.51 $^{b}$ ± 120.45 |
| Hardness (N) | 23.10 $^{b}$ ± 2.80 | 26.03 $^{b}$ ± 5.17 | 26.99 $^{b}$ ± 5.29 | 34.92 $^{a}$ ± 8.16 |
| Springiness (ratio) | 0.61 $^{b}$ ± 0.03 | 0.64 $^{ab}$ ± 0.04 | 0.63 $^{ab}$ ± 0.04 | 0.67 $^{a}$ ± 0.04 |
| Cohesiveness | 0.41 ± 0.01 | 0.42 ± 0.06 | 0.43 ± 0.07 | 0.39 ± 0.04 |
| Gumminess (N) | 9.50 $^{b}$ ± 1.02 | 10.23 $^{b}$ ± 3.31 | 11.55 $^{ab}$ ± 2.70 | 13.83 $^{a}$ ± 4.33 |
| Chewiness (N×mm) | 5.88 $^{b}$ ± 0.88 | 6.63 $^{b}$ ± 2.16 | 7.33 $^{b}$ ± 1.75 | 9.37 $^{a}$ ± 3.53 |

[1]Data are presented as mean ± standard deviation.

[2] Different superscript indicates statistical difference.

Considering texture of cooked meat, WBS shear force and shear energy of the defective samples was significantly lower than those of non-defective samples ($p<0.05$), which was consistent with previous report of Petracci et al. (2013b) in which the low shear force of WS meat was associated with myopathic lesion causing the fibers to separate (poor cohesion). The link between high shear force and muscle integrity was previously discussed by Lonergan et al. (2003), supporting that arrangement of muscle fibers was disrupted in the severe WS breast reducing muscle integrity, hence declined shear force. Different scenario was revealed by Zambonelli et al. (2016) showing a greater shear force required to cut through cooked WS samples compared to normal meat. No difference in shear force between WS and normal cooked breast meat was also observed (Kuttappan et al., 2013b; Trocino et al., 2015; Alnahhas et al., 2016; Tasoniero et al., 2016). Previous investigation generally applied either shear or compression test for determining texture of WS meat. Apart from WBS test, the study herein utilized TPA, a double compression test imitating mastication, to complement the results from the shear test. The severe WS breasts exhibited the greatest degree in hardness and chewiness ($p<0.05$). Although springiness and gumminess of the severe WS samples did not significantly differed from those of mild and moderate WS samples ($p\geq0.05$), the values of those textural parameters of the severe samples were greater than those of non-defective meat ($p<0.05$). Cohesiveness, however, was not different among the samples ($p\geq0.05$). The current TPA datasets was in agreement with the findings of Soglia et al. (2016). Due to no significant difference in cohesiveness, the low degree of shear force and shear energy in cooked severe WS samples could be attributed by large muscle fiber cross-sectional area (Dransfield and Sosnicki, 1999; Ryu and Kim, 2005). Tough texture, as indicated by great hardness and chewiness, of severe WS samples was consistent with the lower degree

of MFI (Obanor, 2002; Taylor et al., 1995 and Moller et al., 1973). Overall, the textural parameters imply that texture of the cooked severe WS breast meat was tough but easily deformed under shear force.

### 2.4.3 Microscopic images of chicken skeletal muscle

Structure of pectoralis major muscle fibers excised from the non-defective and defective samples were observed under an SEM (Figure 2.3a). The average fiber cross-sectional area of non-defective samples was smaller than those of moderate and severe WS but did not significantly differ from those of the mild sample (Table 2.7). The fibers of non-defective meat mainly composed of fibers with cross-sectional area between ≥ 1,000 to < 2,000 μm2 (Figure 2.3b). None of the fibers found in non-defective meat were larger than 3,000 μm2. As the WS severity increased, the size of muscle fiber tends to be larger. In the severe WS meat, fiber with area < 1,000 μm2 was not found. The greater muscle fiber size in the defective meat could partly be associated with the age of the birds. In this study, most of the severe WS breasts were detected in the group of 49-day-old birds. It is worth noting that WS defect at mild and moderate degree were developed in the broilers at all slaughter ages. Thus, the large muscle fiber size in mild and moderate WS samples could also be the effect of WS defect.

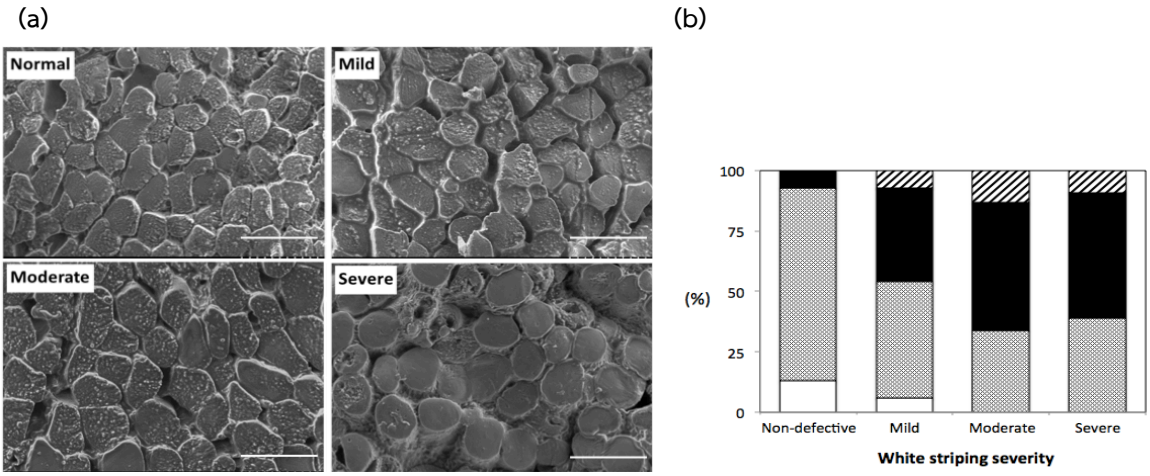


**Figure 2.3** Muscle fibers of breast meat collected from commercial broilers as affected by development of white striping defect. (a) Microscopic images of cross-sectional *pectoralis major* muscle fibers (300× magnification) displayed under field-emission scanning electron microscope using an acceleration voltage of 10 kV. Bar represents 100 μm. (b) Size distribution (%) of muscle fiber regarding cross-sectional area within each WS degree. The muscle fibers were divided into the group with cross-sectional area of <1,000 $\mu m^2$ (□), ≥1,000 to <2,000 $\mu m^2$ (▩), ≥2,000 to <3,000 $\mu m^2$ (■) and ≥3,000 $\mu m^2$ (▨).

**Table 2.7** Characteristics of muscle fibers as affected by development of white striping[1, 2]

| Property | Degree of white striping | | | |
|---|---|---|---|---|
| | Non-defective (n=4) | Mild (n=102) | Moderate (n=71) | Severe (n=7) |
| Average muscle fiber cross-sectional area (×1000 $\mu m^2$) | $1.71^{c} \pm 0.21$ | $2.22^{bc} \pm 0.38$ | $3.02^{a} \pm 0.68$ | $2.77^{ab} \pm 0.94$ |
| Muscle fiber density (fiber/$mm^2$) | $500^{a} \pm 97$ | $402^{b} \pm 73$ | $331^{b} \pm 88$ | $256^{c} \pm 92$ |

[1]Data are presented as mean ± standard deviation.

[2]Different superscript indicates statistical difference.

### 2.4.4 Association between white striping defect and slaughter age and breast yield

Influences of slaughter age and percentage of breast weight on WS severity were determined using OLR (Table 2.8). The OLR regression model where response variable, e.g. WS severity, is categorical and ordered (i.e. non-defective, mild, moderate and severe). The resulting OLR showed that development of WS in commercial broilers was associated with slaughter age and percentage of breast weight ($p<0.001$) with the odds ratios of 1.118 and 1.515, respectively. The odds ratios suggested that when the age increased one unit while percentage of breast weight was kept constant, there was 11.8% likelihood that the WS severity increased. Likewise, there was 51.5% that the birds would exhibit a greater WS severity as percentage of breast weight increased by one unit. In other words, the OLR implies that, between two broilers yielding the same percentage of breast weight, the older one was prone to greater severity degree of WS. If the birds were slaughtered at the same age, the one with greater breast yield was more likely to develop such abnormality. It must be pointed out that an initial intention was to assess effects of slaughter age, carcass weight and breast weight on WS severity using the OLR. However, collinearity was identified between carcass weight and slaughter age. In this case, carcass weight was excluded from the model to avoid risk of false-positive results (Tu et al., 2005). Percentage of breast was, thereafter, incorporated in the model as this parameter reflected an increased proportion of breast size relative to the carcass weight. Previously, relation pattern between development of WS in broilers of 4 strains (59d to 63d) and some factors was characterized by Kuttappan et al. (2013b) using multinomial logistic regression. Their findings demonstrated an increased WS degree with respect to a large cranial fillet thickness and heavy deboned carcass ($p<0.05$). Later, Alnahhas et al. (2016) estimated genetic parameters of WS defect in broilers (42d) and reported a significant genetic correlation between WS defect with body weight and breast meat. The currently acquired OLR model emphasized an increased risk of WS development and its severity in the commercial broilers, at the age of 39d to 49d, that have been undergone breeding selection for intensified breast muscle mass.

**Table 2.8** Coefficients, standard error and odds ratio for the variables included in the logistic model

| Predictor | Estimated coefficient | Standard error | Odds ratio (95% CI) | Residual deviance | AIC[1] |
|---|---|---|---|---|---|
| **Effects of age and percent of breast weight** | | | | 299.702 | 309.702 |
| Slaughter age | 0.112 | 0.044 | 1.118 *** (1.024,1.221) | | |
| Percent of breast weight | 0.415 | 0.098 | 1.515*** (1.249, 1.836) | | |

AIC = Akaike information criterion, ***p<0.001

The causative factors of both WS remain unclear. So far, several studies have suggested that such myopathies are likely the undesirable inherent side effect of the modern breeding selection (Kuttappan et al., 2013a; 2013b; Petracci et al., 2013a; Lorenzi et al., 2014; Sihvo et al., 2014; Bailey et al., 2015; Mazzoni et al., 2015; Mutryn et al., 2015; Alnahhas et al., 2016; Clark and Velleman, 2017; Griffin et al., 2018). As the breeding scheme favored maximum growth rate and breast mass, the hypertrophied muscle fibers occupied areas originally maintained by connective tissues layers limiting vascularization of muscle tissue (Sosnicki and Wilson, 1991; Velleman, 2015; Kindlein et al., 2017). Inadequate oxygen supply and waste removal induced oxidative stress, necrosis and ultimately muscle damage (Mutryn et al., 2015; Clark and Velleman, 2017). On the other hand, Bailey et al. (2015) estimated heritability between the meat defects and production traits and identified a low to moderate level for WS. Their previous results suggested the potential contribution of systematic environmental and management factors in development of WS rather than the consequence of breeding selection. Nonetheless, Bailey et al. (2015) supported the possibilities of limited capillaries and altered muscle growth and development to be associated with the myopathy traits. Corresponding with previous reports, the findings herein support the association between development of WS defect and the intensified breast muscle mass, potentially through the modern breeding selection scheme.

As addressed by Petracci et al. (2014), chicken breast exhibiting severe WS (white lines thickness > 1mm) is usually downgraded and used in manufacturing meat processed products because of its blemish leading to decreased consumer acceptance in Italy. Only moderate WS breasts (white lines thickness < 1mm) are marketed in fresh retails. To our knowledge, majority of either processors or consumers in Asian region lacked awareness of WS defect. In Thailand alone, numbers of consumers could not distinguish between non-defective and WS samples. Among those noticing the lines, they perceived the striation as normal appearance of the chicken breast. Thus, reduced visual attraction due to the WS defect may not be the major concern. However, because WS defect has been neglected by the processors, the abnormality could silently be responsible for technological challenges, such as excessive water loss and inconsistent quality of further processed products. Additionally, nutritional quality of the moderate and severe WS breast may differ from consumer expectation.

## 2.5 SUMMARY

- The WS defect in commercial broilers was evidently found associated among the birds with ages of 39d to 49d.
- Of 184, 4 breasts exhibited non-defective characteristics whereas 102 (55%), 71 (39%) and 7 (3.8%), developed mild, moderate and severe WS, respectively.
- The WS samples showed increases in fat, iron, calcium, sulfur and zinc but decreases in protein, ash and phosphorous in comparison to non-defect meat. The alteration was more pronounced as WS severity elevated.
- The inferior water holding capacity and tough texture of cooked WS severe meat were observed.
- Based on microscopic images, as the WS severity increased, the average muscle fiber cross-sectional area tended to be larger. However, reduced MFI suggested low degree of muscle fiber integrity in the moderate and severe WS.
- By increasing breast yield 1%, the chance that the broilers would develop WS with increasing severity was 51.5%. Delaying slaughter ages also increased the likelihood by 11.8%.
- The findings were congruent with those of previous studies that the causal of WS defect might be linked to the intensive broiler breeding selection for massive breast yield.
- The next chapter describes transcriptional profiles of *pectroralis major* associated with increasing WS severity with an attempt to define key molecular mechanisms underlying development of WS defect in commercial broilers.

# CHAPTER 3

# TRANSCRIPTOME PROFILING OF CHICKEN SKELETAL MUSCLE ASSOCIATED WITH WHITE STRIPING SEVERITY

## 3.1 ABSTRACT

In response to high consumer demand, chickens have been intensively selected for rapid growth rate, and breast muscle mass and conformation. The success in breeding selection has coincided with an increasing incidence of white striping (WS) meat defect. especially in response to heat stress. The objective of this study was to determine differential gene expression profiles of *pectoralis major* muscle associated with WS severity using Agilent SurePrint G3 Custom GE 8×60K chicken gene expression microarray based on one-color hybridization technique. Total RNA was isolated from the muscle samples of 49d broiler breast classified as mild (n=6), moderate (n=6) and severe WS (n=4). Microarray data was confirmed using quantitative real-time polymerase chain reaction (qPCR). The transcriptome profiling revealed that 2,517, 1,615 and 2,483 transcripts were differentially expressed (DE) in comparisons between severe WS and mild WS (WS 1), between severe WS and moderate WS (WS 2), and between moderate WS and mild WS (WS 3), respectively. Approximately 80% of the DE transcripts in all comparisons showed absolute foldchange ranging between 1.0 and 1.5. Pathway analysis highlighted metabolic pathway, necroptosis and vascular smooth muscle contraction as the top pathways associated with differential gene expression due to development of WS defect. Declined expressions of lactate dehydrogenase A and phosphorylase kinase regulatory subunit beta were identified in severe WS compared with mild WS corresponding with reduced muscular glycogen and lactic acid content, hence dysregulated glycolysis/gluconeogenesis in the severely defected breasts.

## 3.2 INTRODUCTION

White striping (WS) is a recently recognised chicken meat quality defect, characterized by an appearance of white striations parallel to the direction of muscle fibres, on broiler breast or thighs. Prevalence and degree of severity of such defects are associated with growth rate and size of the broilers. In the study of Kuttappan et al. (2012a), occurrence of WS associated with broiler growth rate was estimated. Broilers fed with high-energy diets exhibited heavier live weight and greater breast yield, as well as an increased prevalence and severity of WS. Association between degree of severity and nutritional compositions was reported (Kuttappan et al., 2013a; Petracci et al., 2014). As the degree of WS defect was more severe, muscle fat and energy content increased while protein level decreased. Upon histological examination, a great degree of fat cells and connective tissue was observed in the WS samples (Kuttappan et al., 2013a; Ferreira et al., 2014). Histopathology indicated degenerative myopathy, fibrosis and lipidosis in the moderate and severe samples (Kuttappan et al., 2013b; Ferreira et al., 2014). The lesions were more pronounced in the breast muscle compared with thigh or drumsticks, suggesting that the defect is dependent on myofiber type and distribution. Kuttappan et al. (2013b) also suggested that an impaired

cation regulation combined with inflammatory response during myofiber degeneration might initiate myopathic changes in white-striped meat. To repair the damaged myofibers, signals from inflammatory cells activate satellite cells to enter regenerative process. However, under intense damages, regenerative process was ineffective. The satellite cells differentiate into fibroblasts or adipocytes despite of myoblasts, ultimately resulting in fibrosis and lipidosis of the WS meat.

The WS defect can be accompanied by another type of muscle abnormality known as wooden breast, characterised by bulging and pale area in the caudal part of chicken breast meat (Sihvo et al., 2014). Both abnormalities exhibited similar histological changes consisting of moderate-to-severe myo-degeneration, as well as variable amounts of interstitial connective tissue accumulation or fibrosis (Sihvo et al., 2014). Mutryn et al. (2015) compared transcriptome profiles between normal and wooden broiler breast meat using RNA-sequencing technique. They identified localized hypoxia, oxidative stress, increased intracellular $Ca^{2+}$ concentration, and muscle type switching, as key biological pathways associated with development of wooden meat. The findings support that the defective wooden meat is associated with the modulation of muscle fiber as well as disruption of cation regulation through changes at transcript level.

Recent transcriptional (Zambonelli et al., 2016) and proteomic (Kuttappan et al., 2017b) analyses of the breasts exhibiting hardened areas and superficial white striation, relevant to wooden breast overlapped with WS defect revealed that altered expressions of multiple genes and proteins involved in muscle degeneration and regeneration, oxidative stress response and metabolic processes. However, transcriptome profile of broiler skeletal muscle affected with WS alone has not been elucidated. Besides, the skeletal muscle used in the study of Zambonelli et al. (2016) was excised and collected at 2 h postmortem. Information of some genes playing critical roles in WS development could be lost as the genes could be degraded during the prolonged postmortem duration (Malila et al., 2015). This chapter describes transcriotome profiling in *pectoralis major* muscle associated with increasing WS severity and pathway analysis with the aim of defining molecular mechanisms associated with WS severity in commercial broilers. The present findings could augment and complement the currently documented information regarding development WS defect in commercial broilers.

**3.2 SPECIFIC AIMS**

- To compare gene expression patterns in skeletal muscle obtained from 49d broilers exhibited different WS severity levels using microarray technique
- To confirm the changes in gene expression profile using quantitative real-time polymerase chain reaction (qPCR)
- To define biological pathways associated with WS defect in broiler breast muscle

## 3.3 EXPERIMENTAL DESIGN AND METHODOLOGY

### 3.3.1 Sample information

The specimens of 49d broilers utilized in this experiment were the subset collected and used in chapter 2. The skeletal muscle was snap-frozen within 20 min postmortem and kept in liquid nitrogen while transporting back to FFBT lab (Pathum Thani, Thailand). Upon arrival, the muscle samples were immediately stored at -80°C until total RNA isolation. Defect severity was classified based on numbers of white lines and thickness appeared on the surface of breast meat as mentioned in Chapter 2. Six biological replicates were randomly selected from "mild" and "moderate" groups. For "severe" samples, only four samples exhibiting severe WS were used. The other two severe WS samples were excluded from the microarray hybridization as they appeared to exhibit other meat quality defects which potentially confound with the transcriptional results.

### 3.3.2 Microarray platform and experimental design

The Agilent SurePrint G3 Custom GE 8×60K chicken gene expression microarrays (Agilent Technologies, Inc.) used in this study was designed based on the National Center for Biotechnology Information (NCBI) Gallus gallus Annotation Release 103 (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Gallus_gallus/103/). The detailed information of the array is made available at NCBI Gene Expression Omnibus (GEO) with the platform accession GPL24307.

To determine differential gene expression associated with WS severity, expression patterns obtained among the WS levels were compared against one another (Figure 3.1). A total of 16 arrays (2 slides) were hybridized in order to accomplish three comparisons (WS 1; severe against mild, WS 2; severe against moderate, WS 3; moderate against mild). It must be noted that the initial aim was to compare severe WS against non-defective muscle samples. However, as no non-defective samples were obtained from the 49d broilers vice versa, the experimental design was adjusted.

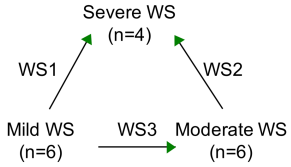


**Figure 3.1** Microarray experimental design. Gene expression patterns between severe WS against mild or moderate WS were compared as displayed. The arrow heads illustrate comparison of each counterpart.

### 3.3.3 Total RNA isolation

Total RNA was isolated using TRIzol™ Reagent (Invitrogen) according to the manufacturer's instruction. The isolated RNA was treated with DNase I (Thermo Scientific, Inc.) to remove any contaminated

genomic DNA, and re-purified using GeneJET RNA Cleanup and Concentration Micro Kit (Thermo Scientific, Inc.). Concentration of total RNA was measured using a Nanodrop ND8000 spectrophotometer (Thermo Fisher Scientific). Integrity of total RNA was confirmed using an Agilent 2100 Bioanalyzer (Santa Clara, CA). Samples with an RNA Integrity Number (RIN) equal to or exceeding 7.0 (RIN = 10 is the best) were proceeded in microarray hybridization at Molecular Genomics Pte. Ltd. (Singapore) and also used in qPCR.

**3.3.4 Microarray hybridizations**

Total RNA was labeled using One-color Low Input Quick Amp Labeling Kit (Agilent) following the company's instruction. In brief, 100 ng total RNA was reverse transcribed into double-stranded cDNA by priming with Oligo(dT) primer. The synthesized cDNA was subjected to an in vitro transcription using T7 RNA polymerases to produce cyanine 3CTP labeled complementary RNA (cRNA). The cRNA was purified using Qiagen RNeasy kit (Qiagen) and quantified using Nanodrop. Afterwards, 600 ng of purified cRNA was hybridized onto the Aglient SurePrint array at 65°C for 17 h. The array was washed for 1 min at room temperature in Gene Expression Washing buffer I (Agilent), followed by another minute at 37°C in Washing buffer II (Agilent). The array was gently blotted dry and scanned using Agilent High Resolution Microarray Scanner (C Model, Agilent). The TIFF image was saved and analyzed using Agilent Feature Extraction Software version V10.7.1.1 (Agilent).

**3.3.5 Microarray data analysis and gene annotation**

Raw microarray signal values were generated by Agilent Feature Extraction Software version V10.7.1.1 (Agilent). Feature extraction and probe quality control were processed using GeneSpring software. The data were exported as text file for further analysis in R version 3.4.0. The signal values of probes deemed to be suspicious or faulty were quarantined using flags and expression values. Flags are categorical indicators including 'detected', 'compromised', and 'not detected' from the scanner. Probes that were flagged as 'not detected' and 'compromised' were assigned to not available values (NA). Signal intensity of probes found to be below 20 raw signal intensities of that microarray were assigned as NA values. Probes that have intensity values at least three microarrays of WS group were further analyzed. The filtered raw data were normalized in R using quantile normalization (Bolstad, 2017). Due to different batches of WS samples, the filtered raw data of WS group were further normalized using combat normalization (Muller et al., 2016; Leek et al., 2017). Log2 transformation was applied to normalized data for statistical analysis. Statistically significant difference of each transcript between two treatments was identified by analysis of independent 2-group t-test with a cutoff value of 0.05. All microarray data have been submitted to the NCBI GEO repository with GEO accession number GSE107362. Positive and negative fold change (FC) values represent increased and decreased expression of particular gene in defective relative to its counterparts. Coefficient of variation (CV) was calculated in percentage to signify variation in expression of the particular gene among all defective severity levels.

### 3.3.6 Confirmation of differential gene expression patterns

Fifteen differentially expressed genes identified in the current microarray study were chosen based upon their biological functions. Primers (Table 3.1) were designed using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primer specificity was verified by subjecting the designed primer sequence to an NCBI BLAST search. The potential of secondary structure formation was also predicted using OligoAnalyzer (https://www.idtdna.com/calc/analyzer). Only primers that specifically matched their respective genes and scores low potential self-complementarity and GC content were submitted for primer synthesis. Additional specificity was verified by electrophoretic patterns of PCR product on 2% agarose gel and by dissociation curves with a single peak at melting temperature.

**Table 3.1** Primers

| NCBI Accession# | Gene ID | Gene annotation | Sequence (5' → 3') | Amplicon length (bp) | Melting temperature (°C) |
|---|---|---|---|---|---|
| NM_001004405.2 | *CAPN3* | Calpain 3 | F: GAACAACCAGCTCTACGACA<br>R: TGGAATGCCCTGAACATAGC | 117 | 82.5 |
| NM_205303.1 | *CAPN11* | Calpain 11 | F: GGACTTGTTTTGCTTGCTCC<br>R: CGTGTAACTTCTCACCCTGG | 134 | 85.5 |
| XM_015296118.1 | *IMPG2* | Interphotoreceptor matrix proteoglycan 2 | F: ACCAGTACACAAAGAGCGAC<br>R: GTTAGCTGAGGGTATCTGCG | 145 | 83.5 |
| NM_205284.1 | *LDHA* | Lactate dehydrogenase A | F: TTCTCTGCCAGCTGAATAGCT<br>R: CGGGTCATTGTCTTGTTGCAT | 200 | 78.5 |
| XM_417132.5 | *MTMR6* | Myotubularin related protein 6 | F: GTCAGCAGTGAGAGATGGTC<br>R: ATTTACTTCACCGCAGGTCC | 109 | 83 |
| XM_015273127.1 | *PFKFB4* | 6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 4 | F: ATGCTACAAAGCCACCTACG<br>R: GTGTGTGTTCATCAGGTAGTAC | 151 | 88 |
| NM_001007831.1 | *PHKB* | Phosphorylase kinase regulatory subunit beta | F: GCACGGTGTAGTAATTGTTGC<br>R: GGGCACTTTGTGTCTCTAATG | 148 | 79.5 |
| XM_015293901.1 | *SGCD* | Sarcoglycan, delta (35kDa dystrophin-associated glycoprotein) | F: CCTGAAGCCTCATACAGCAA<br>R: TATTCTTCTGCTCCACAGCG | 133 | 81.5 |
| XM_414851.5 | *SLC9A3R2* | Solute carrier family9, subfamily A (NHE3, cation proton antiporter 3), member 3 regulator 2 | F: TATTGGGGACAAGACTGGCA<br>R: CGTCTTTGAAGTGGGGTAGG | 114 | 84.5 |
| XM_015277760.1 | *SLC9A7* | Solute carrier family9, subfamily A (NHE7, cation proton antiporter 7), member 7 | F: CCCCTCTCCTTCTTGCTGAATC<br>R: GTGCCTCCACCAACAATCCAC | 200 | 83.5 |
| NM_001031305.2 | *SLC9A9* | Solute carrier family9, subfamily A (NHE9, cation proton antiporter 9), member 9 | F: TCGCACTGGCTATTCAAGAC<br>R: CCTCCACCAAATACCCAGAC | 100 | 80 |
| XM_015275374.1 | *SMTN* | Smoothelin | F: TTGACCGAGAGGATGAGAGTG<br>R: TGCGAGCCTGTGAGGTTGAT | 129 | 86.5 |
| XM_015296211.1 | *SMTNL2* | Smoothelin-like 2 | F: ACCTCACTGTCTTCATGGTA<br>R: AAACCGTCTCTAGCCCAGTA | 115 | 78 |
| XM_015278722.1 | *TACR2* | Tachyinin receptor 2 | F: TTCTCTGCCAGCTGAATAGCT<br>R: CGGGTCATTGTCTTGTTGCAT | 139 | 83.5 |
| XM_015289894.1 | *THSD7B* | Thrombospodin, type I, domain containing 7B | F: GCAGGTGTGTGATCCGAGTA<br>R: TGCTCCAGGCTGTTAGATTG | 152 | 82.5 |

Changes in expression patterns of the chosen genes observed in the current microarray analysis were confirmed using qPCR. In brief, 1.5 μg total RNA, the same materials used in the microarray hybridization process, was reverse transcribed into cDNA using ImPromII™ Reverse Transcriptase System kit (Promega) according to the manufacturer's protocol. The 20-μL reactions consisted of 40 ng cDNA, 250 nM of each primer, and SsoAdvanced™ Universal SYBR® Green supermix (BioRad) and were performed in a Real Time PCR model BIORAD CFX96 (BioRad). For no template control, cDNA was substituted by an equal volume of nuclease-free water. Threshold cycle (Ct) was analyzed using BioRad CFX Manager 2.1 software (BioRad). Hypoxanthine-guanine phosphoribosyltransferase (HPRT) was chosen as the reference gene due to its unchanged expression across all WS groups (data not shown). Relative mRNA abundance of each gene in the sample group relative to its designated counterparts was calculated based on $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Student's t-test was performed to identify statistical difference ($p<0.05$) in the abundance using the R package version 3.2.1.

### 3.3.7 Functional and pathway analyses

All differentially expressed (DE) transcripts ($p<0.05$) with CV>10% were subjected to classification system of Pathway Analysis THrough Evolutionary Relationships (PANTHER™) Version 12.0 (Thomas et al., 2003; Mi et al., 2017), available at http://pantherdb.org/. The system established Gene Ontology (GO) classification categories, including cellular component, molecular functions, biological processes and the GO pathways, that were significantly enriched in the genes from each condition. The DE transcripts ($p<0.05$, CV>10%) were also mapped onto Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (Kanehisa et al., 2012) to identify biological interactions among the DE genes. The KEGG orthology (KO) of genes were converted from RefSeq nucleotide ID by Retrieve/ID mapping in UniProt (The UniProt Consortium, 2017).

### 3.3.8 Determination of glycogen and lactic acid in skeletal muscle

To compare glycogen and lactic acid content in skeletal muscle, the same frozen 20-min postmortem tissues which were subjected for RNA isolation were used. Glycogen content was determined using glycogen assay kit (Sigma-Aldrich, MO) following the company's recommendation. Prior the recommended colorimetric assay, crude glycogen in the frozen muscle was extracted as previously stated (Abasht et al., 2016). Briefly, 80 mg of the frozen tissue was heated in 300 μL of 30% KOH at 100°C for 2 h. Afterwards, 3 volumes of 95% ethanol was added to precipitate crude glycogen. The mixture was centrifuged at 3,000 rpm for 10 min. The resulting pellet was resuspended in 150 μL deionized water and acidified to pH 3 using HCl. The extracted glycogen was re-precipitated in 95% ethanol twice to remove any impurities. The final pellet was air-dried and subsequently dissolved in 300 μL deionized water.

For lactic acid, 500 mg of the frozen tissue were homogenized with 10 mL of 1 M perchloric acid for 2 min. The pH of the homogenate was adjusted to pH 8.0 using KOH followed by volume

adjustment to 25 mL using distilled water. The solution was then incubated on ice for 20 min to precipitate potassium perchlorate and subsequently centrifuged at 13,000×g for 10 min. Lactic acid content in the supernatant was determined using L-lactic acid assay kit (Megazyme, Ireland). Lactic acid content in 24-h postmortem meat samples relevant to their respective deep-frozen muscle specimens was also determined.

All measurements were done in triplicates. Differences among WS levels were analyzed using ANOVA and mean difference was determined using Duncan's multiple range test ($p<0.05$) in R package.

## 3.4 RESULTS AND DISCUSSION

### 3.4.1 Differentially expressed transcripts associated with WS severity

The microarray analysis revealed that 2,517, 1,615 and 2,483 transcripts were differentially expressed in comparisons between severe WS and mild WS (WS 1), between severe WS and moderate WS (WS 2), and between moderate WS and mild WS (WS 3), respectively (Figure 3.2a). Approximately 80% of the DE transcripts in all comparisons showed absolute FC (|FC|) ranging between 1.0 and 1.5, except for the WS 1 comparison of which nearly 90% of the down-regulated transcripts fell within such |FC| range (Figure 3.2b). Numbers of up-regulated and down-regulated transcripts were comparable within each comparison. The most up-regulated transcripts in the comparisons of WS 1, WS 2 and WS 3 were dead (Asp-Glu-Ala-Asp) box polypeptide 60 (FC = 6.61, p = 0.04), cathepsin D-like (FC = 2.48, p = 0.04) and sperm-associated antigen 4 protein-like (FC = 9.54, p = 0.02), respectively. Among down-regulated transcripts, uncharacterized non-coding RNA showed the most decreased abundance in severe WS relative to mild or moderate WS samples (WS 1, NCBI# LOC107055185, FC = -2.36, p = 0.04; WS 2, NCBI#LOC101749734, FC = -4.75, p = 2.0×10-3). Between moderate and mild WS (WS 3), cystathionine beta-synthase-like showed the most down-regulation (FC = -4.03, p = 0.04). Top ten up-regulated and down-regulated transcripts within each comparison were shown in Table A1 (Appendix A). Twelve DE transcripts, listed in Table 3.2, were overlapped among all comparisons. Among those overlapping transcripts, there were two corresponding to oligos annotated as delta sarcoglycan (SGCD) and one uncharacterized non-coding RNA. In addition, most of the transcripts in severe WS compared with either mild and moderate WS were down-regulated. it was quite unexpected that changing direction of several transcripts among the comparisons was inconsistent. Only exportin 1 (*XPO1*), a protein transporter of RNA and proteins across the nuclear membrane (Nguyen et al., 2012), and pleckstrin homolog (*PHLDB2*), the small protein constituent of cytoskeleton involved in intracellular signaling (Haslam et al., 1993), showed similarly decreased direction in all comparisons.

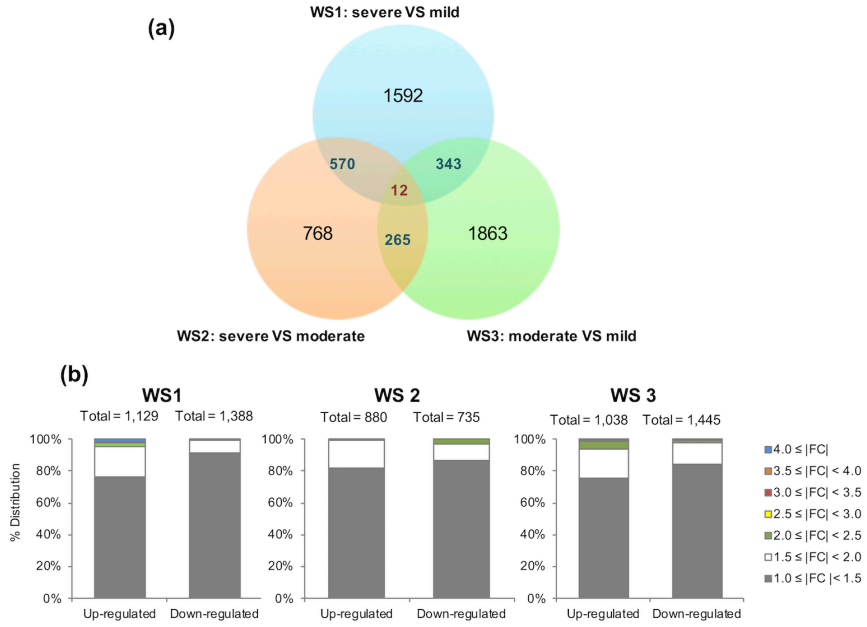


**Figure 3.2** Differential expression profiles associated with increasing white striping severity in broiler breast meat. (a) Venn diagrams represent total numbers of differentially expressed (DE) transcripts ($p<0.05$). The numbers in blue, orange and green diagrams present the DE transcripts within the comparisons between severe and mild (WS 1), severe and moderate (WS 2) and moderate and mild (WS 3), respectively. (b) Bar graphs indicate distribution (%) of the DE transcripts at different fold change (FC) ranges within each comparison. Different colors on bar graphs depicted ranges of absolute FC (|FC|) values.

**Table 3.2** Overlapped differentially expressed genes in chicken skeletal muscle (*pectoralis major*) associated with development of white striping

| NCBI Accession# | Gene ID | Description | Fold change (FC) | | |
|---|---|---|---|---|---|
| | | | WS 1 | WS 2 | WS 3 |
| NM_001290134.1 | *XPO1* | Exportin 1 | -1.24 | -1.12 | -1.10 |
| XM_003641774.3 | *GNB4* | Guanine nucleotide binding protein (G protein), beta polypeptide 4, transcript variant X1 | -1.09 | -1.22 | 1.12 |
| XM_004943587.2 | *COMMD2* | COMM domain containing 2 transcript variant X3 | -1.15 | -1.30 | 1.13 |
| XM_015284105.1 | *RPS12* | Ribosomal protein S12, transcript variant X1 | 1.09 | 1.19 | -1.09 |
| XM_015292896.1 | *IP6K1* | Inositol hexakisphosphate kinase 1, transcript variant X6 | 1.16 | -1.22 | 1.41 |
| XM_015293901.1 | *SGCD* | Sarcoglycan, delta (35kDa dystrophin-associated glycoprotein), transcript variant X2 | -1.17 | -1.39 | 1.18 |
| XM_015293902.1 | *SGCD* | Sarcoglycan, delta (35kDa dystrophin-associated glycoprotein), transcript variant X3 | -1.20 | -1.42 | 1.18 |
| XM_015297598.1 | *PHLDB2* | Pleckstrin homology-like domain, family B, member 2, transcript variant X2 | -1.30 | -1.13 | -1.15 |
| XM_415553.4 | *NDOR1* | NADPH dependent diflavin oxidoreductase 1 | -1.18 | -1.42 | 1.20 |
| XM_015279090.1 | *MTMR6* | Myotubularin related protein 6, transcript variant X1 | -1.13 | -1.29 | 1.14 |
| XR_001464428.1 | *MRRF* | Mitochondrial ribosome recycling factor, transcript variant X2, misc_RNA | -1.19 | -1.38 | 1.16 |
| XR_001468873.1 | LOC101751942 | Uncharacterized LOC101751942 (ncRNA) | 1.42 | -1.61 | 2.29 |

Among top ten up-regulated and down-regulated DE transcripts (Appendix A), numbers of transcripts encoding the same gene but different variants were appeared repeatedly. Majority of those was identified as uncharacterized non-coding RNAs (ncRNA). The ncRNAs play roles as regulators in various biological processes. Previous genome-wide systematic study (Zhang et al., 2009) demonstrated specific functions of chicken ncRNAs in lineage/species specification during evolution and post-hatching skeletal tissue development. Changes in transcript abundance of numerous ncRNAs suggested problematic regulatory mechanisms of tissue development in response to WS defect. Abundances of transcripts encoded DEAD (Asp-Glu-Ala-Asp) box polypeptide 60 (DDX60), a member of subset RNA helicases, and TruB (psi) pseudouridine synthase 1 (TRUB1) markedly increased in severe WS compared to its expression in mild WS. Both enzymes involved in numbers of cellular processes including RNA and modifications of RNA secondary structure (Zucchini et al., 2003; Oshiumi et al., 2015). Decreased abundance of transcripts encoded tubulin typrosine ligase (TTLL9) was found in severe WS compared to both mild and moderate WS. This cytosolic enzyme catalyzes post-translational tyrosination at C-terminus of disassembled **α**-tubulin (Erck et al., 2003), which was crucially required for tubulin to properly form protofilaments, the fundamental structure of microtubule cytoskeletons (Marcos et al., 2009; Janke and Bulinski, 2011). Down-regulation of various transcripts of which their encoded stress response proteins, including cystathionine beta synthase (CBS), heat shock protein 40 (DnaJ), glutathione S-transferase alpha 4 (GSTA4), and oxidative stress induced growth inhibitor 1 (OSGIN1), was observed. In comparison of moderate and mild WS, the catalytic CBS enzyme (LOC418544), responsible for conversion of serine and homocysteine into cystathionin in transsulfuration pathways, was down-regulated about 4 fold. The absence of the CBS activity in some tissues has been discussed to be associated with incapability of those tissues to synthesize cysteine or such tissues might have increased sensitivity to homocysteine toxicity (Jhee and Kruger, 2005). Heat shock protein 40, belonging to protein chaperones family with average molecular size of about 40 kDa (HSP40), function to prevent aggregation of proteins during protein synthesis or under cellular stress condition (Thomas and Baneyx, 1996; Qiu et al., 2006). The OSGIN1 regulates differentiation and proliferation of normal cells through regulation of apoptosis under oxidative stress and inflammatory conditions. Decreased expression of this protein correlates with uncontrolled cell growth and tumor formation (Liu et al., 2014). A glimpse of the top changed transcripts was implicated altered structural organization and cell division as well as irregular cellular responses under stress in defective WS chicken skeletal muscle.

### 3.4.2 Confirmation of expression patterns using qPCR

Differences in gene expression detected in the microarray analysis were confirmed by qPCR (Figure 3.3). Most of the selected genes exhibited changes in expression with a similar direction as observed in the microarray study. For those showing disagreement, it could be because mature RNA of those target genes presented in several isoforms. The primers designed and utilized in the qPCR might amplify different isoforms of the target genes from the microarray but those isoforms were annotated as the same gene.

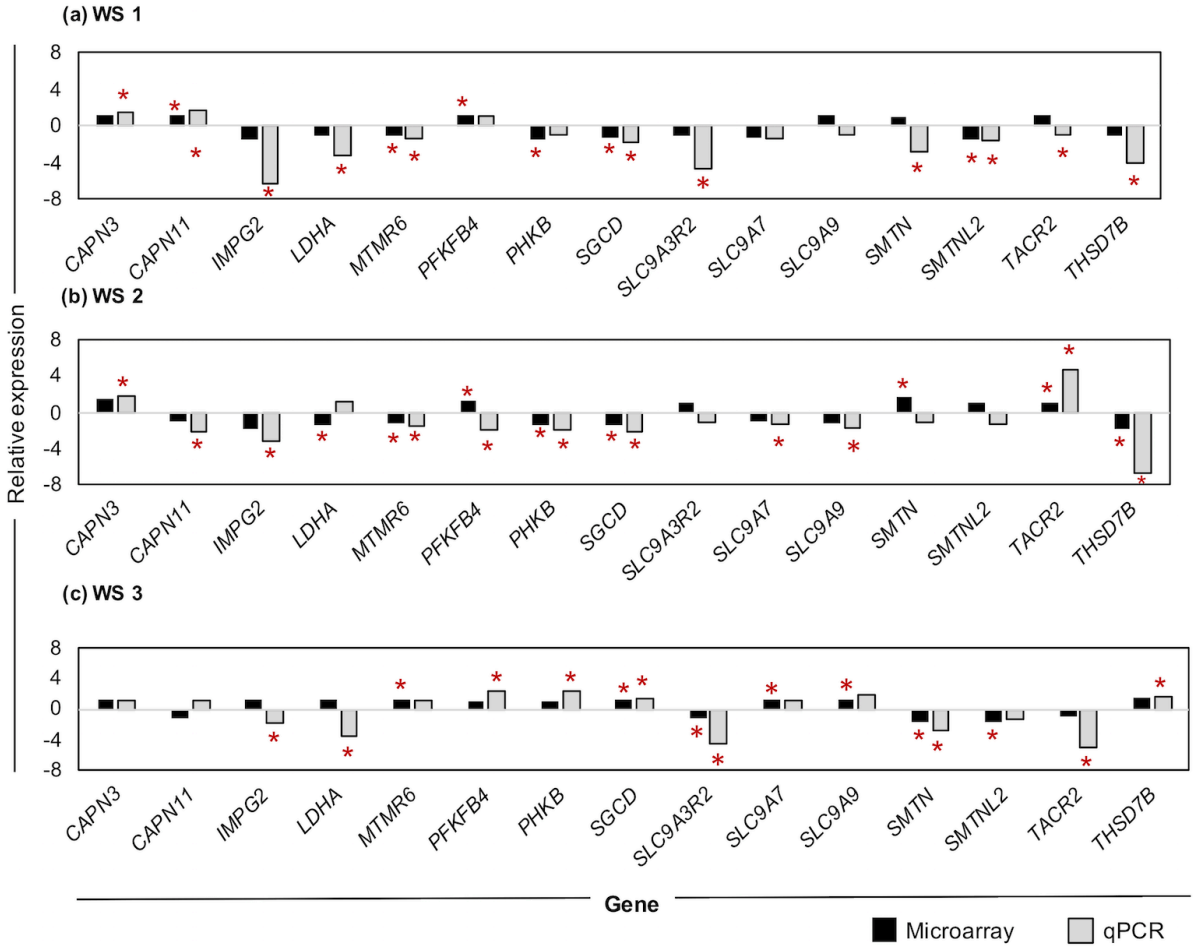


**Figure 3.3 Confirmation of gene expression analyzed by microarray using qPCR.** Results are displayed as relative expression or fold change for gene expression extreme WS severity relative to its counterparts. Expression comparisons include (a) severe WS vs. mild WS (WS 1), (b) severe vs moderate WS (WS 2) and (c) moderate vs mild WS (WS 3). Bars above the origin illustrated up-regulation; on the other hand, bars below the origin represent down-regulation within each comparison. Asterisks indicate statistical changes in expression of particular gene identified by either microarray or qPCR ($p<0.05$).

### 3.4.3 Altered biological functions and pathways

To review biological functions of the DE transcripts identified in the microarray study, those DE transcripts with CV > 10% (98 for WS 1, 43 for WS 2, 142 for WS 3) were submitted to the PANTHER™ classification system. The software designated each gene into one or more groups according to relevant biological roles of its respective proteins. Among the submitted transcripts, 32, 22 and 49 in the comparison of WS 1, WS 2 and WS 3, respectively, were recognized by the software and assigned into the GO classification categories (Figure 3.4). Regarding the GO cellular component category (Figure 3.4a), approximately 40% of the DE transcripts identified in all WS comparisons were allocated into cell part cluster. Considering molecular function (Figure 3.4b), an enrichment of the DE transcripts across the entire

comparisons exhibited catalytic and binding activities. In addition, the majority of the identified DE transcripts were categorized into cellular and metabolic processes (Figure 3.4c), indicating associations between development of WS defect and dysregulation occurred in those molecular processes. The PANTHER™ classification highlighted angiogenesis, the process responsible for formation of new blood vessels from the pre-existing vasculature (Nishida et al., 2006), in all comparisons (Figure 3.4d).

One limitation of PANTHER™ is that the program does not provide interactions among the DE genes themselves and with other key DE genes in particular pathway. In this regard, the DE genes were secondarily subjected to KEGG pathway knowledge database. KEGG complements PANTHER™ demonstrating complex biological interactions among the genes in the form of visualized schematic diagrams. To analyze KEGG pathways associated with WS defect, only the DE genes from the comparison of severe and mild, severe and moderate and moderate and mild were used. There were 110 pathways recognized by KEGG database (Table B1, Appendix B). The top pathway identified by KEGG was metabolic pathway (Table 3.3, Figure S.1, Appendix C), followed by necroptosis (Table 3.4, Figure S.2, Appendix C) and vascular smooth muscle contraction (Table 3.5, Figure S.3, Appendix C). Among the altered metabolic pathways, regulations of glycolysis/gluconeogenesis (Table 3.3, Figure 3.5), phosphatidylinositol (Table 3.3, Figure 3.6) and the groups of pathway networks associated with metabolism of lipids (Table 3.3) were revealed. In addition to necrosis, the other pathways mediating programmed cell death including autophagy and apoptosis were identified (Table 3.4). Abundance of transcript encoding glutathione S-transferase mu 2 (GSTM2) increased for 1.4 fold and 1.5 fold in severe WS relative to mild (WS 1) and moderate (WS 2), respectively (Table 3.6). This GST enzyme expressed in skeletal muscle catalyzes conjugation of glutathione in detoxification mechanisms and govern intracellular [$Ca2^{+}$] through negative regulation of ryanodine calcium release channel activity (Abdellatif et al., 2007). Expression of GSTs genes was induced in response to oxidative stress (Nebert and Vasiliou, 2004) generally by c-Jun N-terminal kinase transcription factor (Hayes and Pulford, 1995) and was shown to be manifested by insulin through PI3K/AKT/mTOR pathway (Franco et al., 2007). Opposite from *GSTM2* expression, *GSTA4* was one of the ten most down-regulated transcripts found in the WS 2 comparison (Table A1). This gene encodes an alpha class of GST enzyme which acts against cellular stresses by detoxifing lipid and protein oxidative products (Yang et al., 2003). Suppression of *GSTA4* by proinflammatory cytokines in an insulin-resistant mice adipose tissues attributed to an elevated protein carbonylation, altered glucose and lipid metabolism and decreased mitochondrial oxidation (Curtis et al., 2010). Overall, the pathways suggested by KEGG well agreed with ones reported by PANTHER™. The current outcomes from both pathway analysis databases underlined the cascades of responsive mechanisms for oxidative stress, muscle degeneration and formations of muscle and blood vessels along with metabolic shift in association with increasing WS severity.

| (a) Cellular component<br>Category name (Accession) | WS 1<br>#genes | WS 2<br>#genes | WS 3<br>#genes |
|---|---|---|---|
| Cell junction (GO:0030054) | 1 | 0 | 0 |
| Cell part (GO:0044464) | 8 | 4 | 10 |
| Extracellular matrix (GO:0031012) | 1 | 1 | 0 |
| Extracellular region (GO:0005576) | 1 | 3 | 3 |
| Macromolecular complex (GO:0032991) | 2 | 0 | 2 |
| Membrane (GO:0016020) | 2 | 0 | 4 |
| Organelle (GO:0043226) | 4 | 1 | 3 |

| (b) Molecular function<br>Category name (Accession) | WS 1<br>#genes | WS 2<br>#genes | WS 3<br>#genes |
|---|---|---|---|
| Binding (GO:0005488) | 5 | 5 | 16 |
| Catalytic activity (GO:0003824) | 10 | 5 | 14 |
| Receptor activity (GO:0004872) | 0 | 1 | 3 |
| Signal transducer activity (GO:0004871) | 0 | 0 | 2 |
| Transporter activity (GO:0005215) | 3 | 1 | 4 |

| (c) Biological process<br>Category name (Accession) | WS 1<br>#genes | WS 2<br>#genes | WS 3<br>#genes |
|---|---|---|---|
| Biological adhesion (GO:0022610) | 1 | 0 | 3 |
| Biological regulation (GO:0065007) | 3 | 1 | 7 |
| Cellular component organization or biogenesis (GO:0071840) | 2 | 3 | 1 |
| Cellular process (GO:0009987) | 8 | 8 | 18 |
| Developmental process (GO:0032502) | 3 | 3 | 4 |
| Immune system process (GO:0002376) | 1 | 0 | 1 |
| Localization (GO:0051179) | 5 | 2 | 6 |
| Locomotion (GO:0040011) | 0 | 1 | 0 |
| Metabolic process (GO:0008152) | 7 | 8 | 14 |
| Multicellular organismal process (GO:0032501) | 4 | 2 | 2 |
| Reproduction (GO:0000003) | 0 | 0 | 1 |
| Response to stimulus (GO:0050896) | 2 | 2 | 6 |

| (d) PANTHER Pathway<br>Category name (Accession) | WS 1<br>#genes | WS 2<br>#genes | WS 3<br>#genes |
|---|---|---|---|
| 5-Hydroxytryptamine degredation (P04372) | 0 | 0 | 1 |
| Alzheimer disease-presenilin pathway (P00004) | 0 | 0 | 1 |
| Angiogenesis (P00005) | 2 | 1 | 2 |
| Axon guidance mediated by Slit/Robo (P00008) | 0 | 0 | 1 |
| Blood coagulation (P00011) | 0 | 1 | 1 |
| Cadherin signaling pathway (P00012) | 0 | 0 | 1 |
| Circadian clock system (P00015) | 1 | 0 | 1 |
| De novo purine biosynthesis (P02738) | 0 | 0 | 1 |
| De novo pyrimidine deoxyribonucleotide biosynthesis (P02739) | 0 | 0 | 1 |
| De novo pyrimidine ribonucleotides biosythesis (P02740) | 0 | 0 | 1 |
| EGF receptor signaling pathway (P00018) | 1 | 0 | 0 |
| Flavin biosynthesis (P02741) | 1 | 0 | 0 |
| Huntington disease (P00029) | 0 | 0 | 1 |
| Inflammation mediated by chemokine and cytokine signaling pathway (P00031) | 1 | 0 | 1 |
| Integrin signalling pathway (P00034) | 1 | 0 | 2 |
| Oxidative stress response (P00046) | 1 | 0 | 1 |
| Parkinson disease (P00049) | 0 | 0 | 1 |
| VEGF signaling pathway (P00056) | 0 | 0 | 1 |
| Wnt signaling pathway (P00057) | 0 | 0 | 2 |

**Figure 3.4. Heat maps illustrate numbers of differentially expressed transcripts (p<0.05, CV > 10%) classified according to PANTHER™ Classification system database into categories of cellular component (a), molecular function (b), biological process (c) or pathways (d).** Expression comparisons include severe WS vs. mild WS (WS 1), severe vs moderate WS (WS 2) and moderate vs mild WS (WS 3).

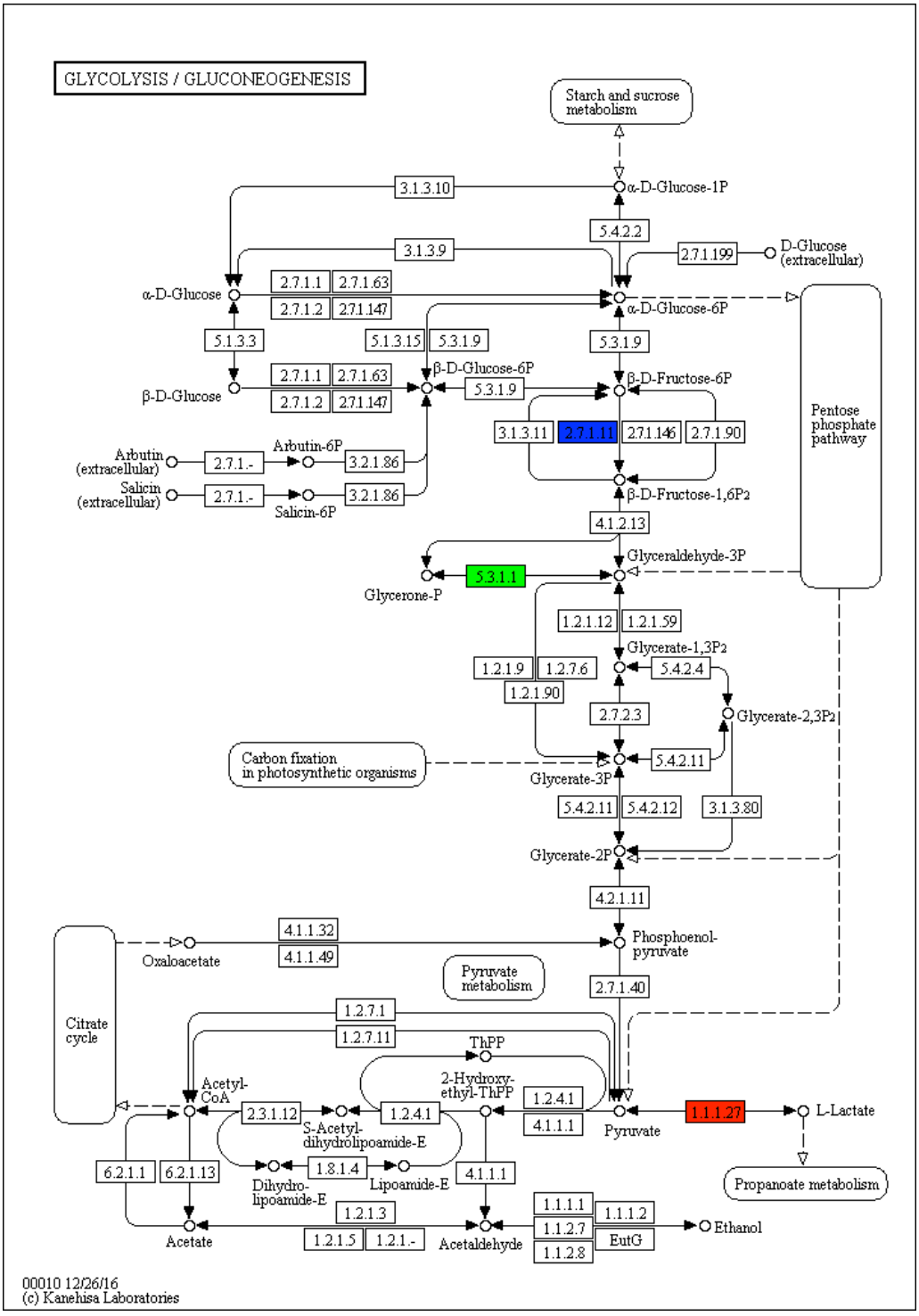


**Figure 3.5 Schematic diagram of glycolysis and gluconeogenesis associated with white striping (WS) defect in commercial broilers.** The pathway, suggested by KEGG database (id: gga00010), illustrates biological interactions among differentially expressed genes (n = 6) in chicken skeletal muscle. Green, red and blue lines indicate where the differentially expressed transcripts from comparisons of severe and mild (WS1), severe and moderate (WS2), and moderate and mild (WS3), respectively, were mapped into the pathways. 2.7.1.11 = Phosphofructokinase platelet, 5.3.1.1 = Triosephosphate isomerase 1, 1.1.1.27 = Lactate dehydrogenase A

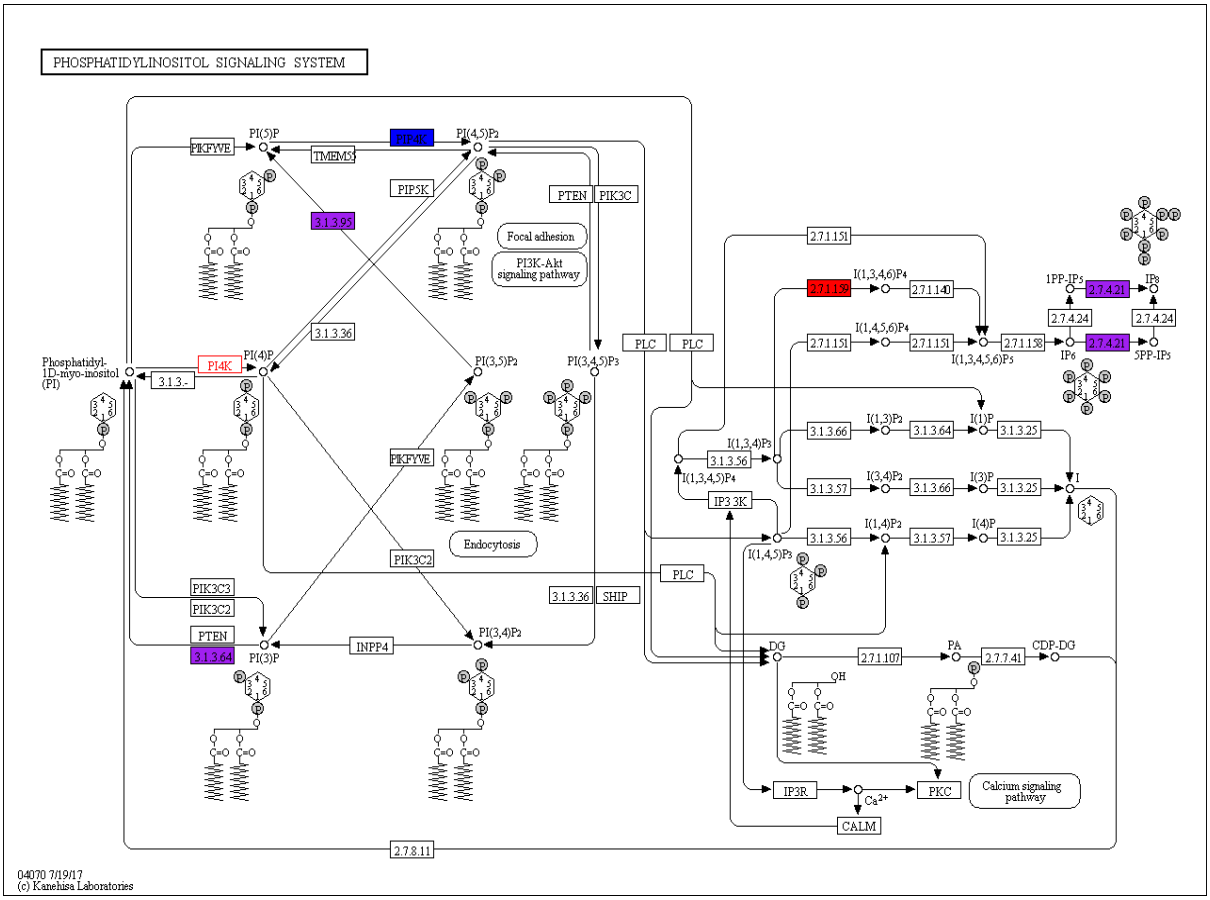


**Figure 3.6 Schematic diagram of phosphatidylinositol signaling system associated with white striping (WS) defect in commercial broilers.** The pathway, suggested by KEGG database (id: gga04070), illustrates biological interactions among differentially expressed genes (n = 9) in chicken skeletal muscle. The proteins highlighted in solid red and blue indicate were potentially translated from the differentially expressed transcripts from comparisons of severe and moderate (WS2), moderate and mild (WS3), respectively. The solid purple depicted the differentially expressed transcripts found in all comparisons. 2.7.4.21 = inositol-hexakisphosphate 5-kinase (IP6K), 2.7.1.159 = inositol-1,3,4-trisphosphate 5/6-kinase/inositol-tetrakisphosphate 1-kinase (ITPK1), 3.1.3.95 = myotubularin-related protein 6/7/8 (MTMR6_7_8), PIP4K2 = 1-phosphatidylinositol-5-phosphate 4-kinase [EC:2.7.1.149], PI4K2 = phosphatidylinositol 4-kinase type 2 [EC:2.7.1.67]

**Table 3.3** Differentially expressed transcripts recognized by KEGG to be associated with metabolic processes[1]

(a) Metabolic processes (gga01100)

| KO | NCBI Accession# | Gene ID | WS 1 | | WS 2 | | WS 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | FC | p-val | FC | p-val | FC | p-val |
| K00016 | NM_205284.1 | *LDHA* | -1.15 | 0.40 | -1.40 | 0.04 | 1.22 | 0.12 |
| K00016 | NM_204177.2 | *LDHB* | 1.06 | 0.75 | -1.12 | 0.52 | 1.19 | 0.24 |
| K00044 | NM_204837.1 | *HSD17B1* | -1.01 | 0.99 | -1.32 | 0.01 | 1.30 | 0.02 |
| K00234 | NM_001277398.1 | *SDHA* | -1.19 | 0.14 | 1.12 | 0.30 | -1.33 | 0.03 |
| K00237 | NM_001006321.1 | *SDHD* | -1.13 | 0.13 | -1.02 | 0.72 | -1.11 | 0.02 |
| K00764 | NM_001004401.1 | *PPAT* | -1.35 | 0.04 | -1.28 | 0.10 | -1.06 | 0.59 |
| K00850 | NM_001031511.1 | *PFKP* | -1.08 | 0.52 | -1.30 | 0.07 | 1.20 | 0.04 |
| K00913 | NM_001012588.1 | *ITPK1* | -1.14 | 0.21 | -1.32 | 0.00 | 1.16 | 0.09 |
| K01047 | NM_001184757.1 | *PLA2G10* | 1.41 | 0.01 | 1.05 | 0.56 | 1.35 | 0.06 |
| K01217 | NM_001031433.1 | *IDUA* | -1.18 | 0.25 | -1.43 | 0.02 | 1.21 | 0.05 |
| K01611 | NM_001012569.1 | *AMD1* | -1.10 | 0.41 | 1.17 | 0.17 | -1.29 | 0.02 |
| K01679 | NM_001006382.1 | *FH* | -1.13 | 0.18 | 1.01 | 0.94 | -1.13 | 0.05 |
| K01755 | NM_001030714.1 | *ASL2* | 1.00 | 0.85 | -1.56 | 0.02 | 1.56 | 0.02 |
| K01803 | NM_205451.1 | *TPI1* | 1.08 | 0.05 | 1.05 | 0.16 | 1.03 | 0.36 |
| K01830 | NM_204259.1 | *PTGDS* | 1.46 | 0.06 | 1.57 | 0.02 | -1.08 | 0.92 |
| K01900 | NM_001006271.2 | *SUCLA2* | -1.14 | 0.40 | 1.09 | 0.79 | -1.24 | 0.05 |
| | NM_001006141.1 | *SUCLG2* | -1.13 | 0.31 | -1.14 | 0.29 | 1.01 | 0.91 |
| K01966 | XM_001231793.4 | *PCCB* | -1.15 | 0.03 | -1.17 | 0.02 | 1.01 | 0.77 |
| K02129 | NM_001097534.2 | *ATP5I* | -1.15 | 0.23 | 1.00 | 0.96 | -1.15 | 0.04 |
| K03018 | NM_001006500.1 | *POLR3A* | -1.21 | 0.20 | 1.03 | 0.88 | -1.25 | 0.02 |
| K03848 | NM_204435.1 | *ALG6* | 1.07 | 0.70 | -1.08 | 0.51 | 1.16 | 0.04 |
| K03953 | NM_001006281.1 | *NDUFA9* | -1.21 | 0.10 | 1.03 | 0.80 | -1.25 | 0.00 |
| K03961 | NM_001006553.1 | *NDUFB5* | -1.08 | 0.43 | 1.10 | 0.28 | -1.19 | 0.02 |
| K05304 | NM_001007975.1 | *NANS* | 1.11 | 0.31 | -1.11 | 0.39 | 1.23 | 0.05 |
| K05917 | NM_001048077.1 | *CYP51A1* | 1.20 | 0.16 | -1.29 | 0.09 | 1.55 | 0.01 |
| K07249 | NM_204577.4 | *ALDH1A1* | -1.30 | 0.51 | 1.57 | 0.44 | -2.05 | 0.01 |
| | NM_204995.1 | *ALDH1A2* | 1.23 | 0.14 | 1.22 | 0.19 | 1.01 | 0.97 |
| K08729 | NM_001031505.1 | *PTDSS1* | -1.22 | 0.04 | -1.00 | 0.99 | -1.21 | 0.03 |
| K11188 | NM_001039329.2 | *PRDX6* | -1.09 | 0.08 | -1.02 | 0.65 | -1.07 | 0.02 |
| K11787 | NM_001001469.1 | *GART* | -1.25 | 0.78 | 1.58 | 0.05 | -1.98 | 0.11 |
| K13241 | NM_204961.1 | *NOS2* | -1.54 | 0.03 | -1.10 | 0.33 | -1.41 | 0.07 |
| K13403 | NM_001031360.1 | *mthfd2* | 1.14 | 0.19 | 1.74 | 0.01 | -1.53 | 0.04 |
| K13644 | NM_001006392.2 | *CEPT1* | -1.20 | 0.02 | -1.21 | 0.20 | 1.01 | 0.88 |
| K13711 | NM_001031157.1 | *PI4K2B* | -1.18 | 0.02 | -1.25 | 0.004 | 1.06 | 0.33 |
| K14455 | NM_205523.1 | *GOT2* | 1.18 | 0.04 | 1.06 | 0.36 | 1.11 | 0.26 |
| K17398 | NM_001024832.1 | *DNMT3A* | 1.07 | 0.35 | 1.23 | 0.004 | -1.15 | 0.19 |
| K18083 | NM_001012699.1 | *MTMR8* | -1.01 | 0.88 | -1.14 | 0.20 | 1.13 | 0.03 |
| | XM_417132.5 | *MTMR6* | -1.13 | 0.04 | -1.29 | 0.0001 | 1.14 | 0.03 |
| | XR_001464404.1 | *MTMR6* | -1.12 | 0.07 | -1.28 | 0.001 | 1.14 | 0.05 |
| | XM_015279090.1 | *MTMR6* | -1.12 | 0.05 | -1.28 | 0.001 | 1.15 | 0.01 |

**Table 3.3 (continued)**

(b) Phosphatidylinositol signaling system (gga04070)

| KO | NCBI Accession# | Gene ID | WS 1 | | WS 2 | | WS 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | FC | p-val | FC | p-val | FC | p-val |
| K01803 | NM_205451.1 | *TPI1* | 1.08 | 0.05 | 1.05 | 0.16 | 1.03 | 0.36 |
| K13711 | NM_001031157.1 | *PI4K2B* | -1.18 | 0.02 | -1.25 | 0.00 | 1.06 | 0.33 |
| K18083 | NM_001012699.1 | *MTMR8* | -1.01 | 0.88 | -1.14 | 0.20 | 1.13 | 0.03 |
| | XM_417132.5 | *MTMR6* | -1.13 | 0.04 | -1.29 | 0.00 | 1.14 | 0.03 |
| | XR_001464404.1 | *MTMR6* | -1.12 | 0.07 | -1.28 | 0.00 | 1.14 | 0.05 |
| | XM_015279090.1 | *MTMR6* | -1.13 | 0.04 | -1.29 | 0.00 | 1.14 | 0.01 |
| K00920 | NM_001030971.1 | *PIP4K2A* | 1.28 | 0.15 | -1.04 | 0.79 | 1.33 | 0.03 |
| K07756 | XM_015292896.1 | *IP6K1* | 1.16 | 0.03 | -1.22 | 0.01 | 1.41 | 0.00 |

(c) Glycerophospholipid metabolism (gga00564)

| KO | NCBI Accession# | Gene ID | WS 1 | | WS 2 | | WS 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | FC | p-val | FC | p-val | FC | p-val |
| K01047 | NM_001184757.1 | *PLA2G10* | 1.41 | 0.01 | 1.05 | 0.56 | 1.35 | 0.06 |
| K08729 | NM_001031505.1 | *PTDSS1* | -1.22 | 0.04 | -1.00 | 0.99 | -1.21 | 0.03 |
| K13644 | NM_001006392.2 | *CEPT1* | -1.20 | 0.02 | -1.21 | 0.20 | 1.01 | 0.88 |

(d) Carbon metabolism (gga01200)

| KO | NCBI Accession# | Gene ID | WS 1 | | WS 2 | | WS 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | FC | p-val | FC | p-val | FC | p-val |
| K00234 | NM_001277398.1 | *SDHA* | -1.19 | 0.14 | 1.12 | 0.30 | -1.33 | 0.03 |
| K00237 | NM_001006321.1 | *SDHD* | -1.13 | 0.13 | -1.02 | 0.72 | -1.11 | 0.02 |
| K01803 | NM_205451.1 | *TPI1* | 1.08 | 0.05 | 1.05 | 0.16 | 1.03 | 0.36 |
| K14455 | NM_205523.1 | *GOT2* | 1.18 | 0.04 | 1.06 | 0.36 | 1.11 | 0.26 |
| K00850 | NM_001031511.1 | *PFKP* | -1.08 | 0.52 | -1.30 | 0.07 | 1.20 | 0.04 |
| K01679 | NM_001006382.1 | *FH* | -1.13 | 0.18 | 1.01 | 0.94 | -1.13 | 0.05 |
| K01900 | NM_001006271.2 | *SUCLA2* | -1.14 | 0.40 | 1.09 | 0.79 | -1.24 | 0.05 |
| | NM_001006141.1 | *SUCLG2* | -1.13 | 0.31 | -1.14 | 0.29 | 1.01 | 0.91 |
| K01966 | XM_001231793.4 | *PCCB* | -1.15 | 0.03 | -1.17 | 0.02 | 1.01 | 0.77 |

(e) Arachidonic acid metabolism (gga00590)

| KO | NCBI Accession# | Gene ID | WS 1 | | WS 2 | | WS 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | FC | p-val | FC | p-val | FC | p-val |
| K01047 | NM_001184757.1 | *PLA2G10* | 1.41 | 0.01 | 1.05 | 0.56 | 1.35 | 0.06 |
| K13644 | NM_001006392.2 | *CEPT1* | -1.20 | 0.02 | -1.21 | 0.20 | 1.01 | 0.88 |

[1] FC = foldchange, positive FC indicates increased expression in severe WS relative to mild in WS 1 or relative to moderate in WS 2, and increased expression in moderate relative to mild in WS 3. Negative FC suggests opposite expression direction.

**Table 3.4** Differentially expressed transcripts recognized by KEGG to be associated with biological pathways responsible for programmed cell death[1]

| Pathway (ID) | KO | NCBI Accession # | Gene ID | WS1 | | WS 2 | | WS 3 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | FC | p-val | FC | p-val | FC | p-val |
| Necroptosis | K02161 | NM_205339.2 | *BCL2* | -1.04 | 0.55 | -1.32 | 0.04 | 1.27 | 0.03 |
| (gga04217) | K04440 | NM_205095.1 | *MAPK9* | -1.13 | 0.04 | -1.09 | 0.13 | -1.03 | 0.52 |
| | K05133 | NM_001008676.2 | *IFNGR2* | 1.32 | 0.08 | 1.45 | 0.04 | -1.10 | 0.51 |
| | K05863 | NM_204231.2 | *SLC25A6* | -1.11 | 0.62 | -1.33 | 0.03 | 1.20 | 0.19 |
| | K11251 | NM_001030753.1 | *H2AFJ* | 1.45 | 0.001 | 1.24 | 0.06 | 1.17 | 0.25 |
| | K15040 | NM_204741.1 | *VDAC2* | -1.08 | 0.16 | 1.12 | 0.06 | -1.21 | 0.004 |
| Autophagy | K01379 | NM_205177.1 | *CTSD* | 1.33 | 0.003 | 1.19 | 0.23 | 1.12 | 0.78 |
| (gga04140) | K02161 | NM_205339.2 | *BCL2* | -1.04 | 0.55 | -1.32 | 0.04 | 1.27 | 0.03 |
| | K04382 | NM_205124.1 | *PPP2CB* | -1.05 | 0.26 | -1.01 | 0.91 | -1.04 | 0.57 |
| | K04382 | NM_001006152.1 | *PPP2CA* | -1.03 | 0.68 | 1.08 | 0.31 | -1.11 | 0.04 |
| | K04440 | NM_205095.1 | *MAPK9* | -1.13 | 0.04 | -1.09 | 0.13 | -1.03 | 0.52 |
| | K07298 | NM_001045833.1 | *STK11* | -1.39 | 0.04 | -1.71 | 0.15 | 1.23 | 0.73 |
| | K07828 | NM_001012549.1 | *NRAS* | -1.13 | 0.05 | -1.09 | 0.04 | -1.03 | 0.53 |
| | K08334 | NM_001006332.1 | *BECN1* | 1.14 | 0.05 | 1.13 | 0.02 | 1.00 | 0.91 |
| Apoptosis | K01379 | NM_205177.1 | *CTSD* | 1.33 | 0.78 | 1.19 | 0.23 | 1.12 | 0.003 |
| (gga04210) | K02161 | NM_205339.2 | *BCL2* | -1.04 | 0.03 | -1.32 | 0.04 | 1.27 | 0.55 |
| | K04440 | NM_205095.1 | *MAPK9* | -1.13 | 0.52 | -1.09 | 0.13 | -1.03 | 0.04 |
| | K07828 | NM_001012549.1 | *NRAS* | -1.13 | 0.53 | -1.09 | 0.04 | -1.03 | 0.05 |
| | K04374 | NM_204880.2 | *ATF4* | -1.07 | 0.04 | 1.17 | 0.15 | -1.25 | 0.35 |
| | K06114 | NM_001042538.1 | *SPTAN1* | -1.12 | 0.38 | -1.07 | 0.18 | -1.05 | 0.01 |
| | K14021 | NM_001030920.1 | *BAK1* | 1.30 | 0.04 | -1.09 | 0.67 | 1.42 | 0.19 |

[1] FC = foldchange, positive FC indicates increased expression in severe WS relative to mild in WS 1 or relative to moderate in WS 2, and increased expression in moderate relative to mild in WS 3. Negative FC suggests opposite expression direction.

**Table 3.5** Differentially expressed transcripts recognized by KEGG to be associated with vascular smooth muscle contraction (gga04270) [1]

| KO | NCBI accession | Gene ID | WS 1 | | WS 2 | | WS 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | FC | p-val | FC | p-val | FC | p-val |
| K01047 | NM_001184757.1 | *PLA2G10* | 1.41 | 0.01 | 1.05 | 0.56 | 1.35 | 0.06 |
| K04267 | NM_205087.1 | *ADORA2B* | -1.35 | 0.05 | 1.14 | 0.27 | -1.53 | 0.03 |
| K06269 | NM_205122.1 | *PPP1CB* | 1.09 | 0.39 | -1.06 | 0.52 | 1.15 | 0.02 |
| K12329 | NM_204727.1 | *PPP1R12B* | 1.18 | 0.21 | 1.48 | 0.02 | -1.26 | 0.49 |

[1] FC = foldchange, positive FC indicates increased expression in severe WS relative to mild in WS 1 or relative to moderate in WS 2, and increased expression in moderate relative to mild in WS 3. Negative FC suggests opposite expression direction.

**Table 3.6** Differentially expressed transcripts recognized by KEGG to be associated with biological pathways responsible for stress responses [1]

| Pathway | KO | NCBI Accession # | Gene ID | WS1 | | WS 2 | | WS 3 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | FC | p-val | FC | p-val | FC | p-val |
| Phagosome (gga04145) | K06487 | NM_205439.1 | *ITGAV* | 1.34 | 0.11 | 1.03 | 0.69 | 1.30 | 0.02 |
| | K07375 | NM_205445.1 | *TUBB1* | -1.41 | 0.03 | -1.00 | 0.94 | -1.41 | 0.03 |
| Lysosome (gga04142) | K01217 | NM_001031433.1 | *IDUA* | -1.18 | 0.05 | -1.43 | 0.02 | 1.21 | 0.25 |
| | K01379 | NM_205177.1 | *CTSD* | 1.33 | 0.78 | 1.19 | 0.23 | 1.12 | 0.00 |
| | K12301 | NM_001031086.1 | *SLC17A5* | 1.32 | 0.01 | -1.26 | 0.32 | 1.67 | 0.22 |
| | K12387 | NM_001031092.1 | *laptm4a* | -1.31 | 0.48 | -1.44 | 0.05 | 1.10 | 0.09 |
| Cytochrome P450 | K00799 | NM_205090.1 | *GSTM2* | 1.37 | 0.04 | 1.54 | 0.01 | -1.12 | 0.55 |
| (gga00980, gga00982) | | NM_001001777.1 | *GSTA3* | 1.95 | 0.31 | -1.05 | 0.69 | 2.05 | 0.66 |
| Glutathione metabolism | | XM_015284817.1 | *GSTA4* | -1.55 | 0.10 | 1.69 | 0.09 | -2.61 | 0.0003 |
| (gga00480) | | XM_015284816.1 | *GSTA4* | -1.63 | 0.09 | 1.47 | 0.24 | -2.41 | 0.0005 |

[1] FC = foldchange, positive FC indicates increased expression in severe WS relative to mild in WS 1 or relative to moderate in WS 2, and increased expression in moderate relative to mild in WS 3. Negative FC suggests opposite expression direction.

The origin of WS defect remain unclear. Several studies have suggested the link between WS myopathy and the modern breeding selection for maximizing breast muscle mass (Kuttappan et al., 2013a; 2013b; Petracci et al., 2013a; Lorenzi et al., 2014; Bailey et al., 2015; Mazzoni et al., 2015; Mutryn et al., 2015; Alnahhas et al., 2016; Clark and Velleman, 2017; Griffin et al., 2018). The hypertrophied muscle fibers occupied areas originally maintained by connective networks reducing spaces for capillaries (Sosnicki and Wilson, 1991; Velleman, 2015; Kindlein et al., 2017). Insufficient vascularization affected efficiency of waste removal, leading to accumulation of metabolic wastes that could induce oxidative stress and necrosis within the muscle (Mutryn et al., 2015; Clark and Velleman, 2017). In addition, the hypertrophied fibers are more likely outgrown their supportive connective networks (Wilson et al., 1990). In response to the damage, satellite cells, the muscle stem cells responsible for posthatch muscle growth, are generally induced and begin to fuse with the existing myofibers or synthesize the new fibers, hence muscle repair. During muscle regeneration, sufficient vascularization is crucial for simultaneous formations of muscle and blood vessels (Velleman, 2015). However, as capillary supply became limited in the muscle of birds with high breast yield (Sosnicki and Wilson, 1991; Kindlein et al., 2017), satellite cell-mediated muscle regeneration was undergone impairment.

Previously addressed by Kuttappan et al. (2013a), microscopic lesions of WS myophatic breasts included floccular/ vacuolar degeneration, lysis, mild mineralization, occasional regeneration and interstitial inflammation along with fibrosis. Multiple rounded hypereosinophilic fibers with loss of cross striation and internalization of nuclei were also observed in the WS samples. The interstitium showed multifocal edema with infiltration by lymphocytes and macrophages. Several muscle fibers were fragmented and undergoing phagocytosis. Although both acute and chronic changes were found in the

same tissue sections, chronicity of myopathic lesions increased as WS severity increased. The biological pathways identified in the current study well supported the previous histological changes suggesting that muscle fibers might undergo degeneration inducing activities of necroptosis, apoptosis and autophagy. Modifications in chemical compositions of the WS breast proposed to be a consequence of muscle degeneration also corresponded with differences in gene expression associated with glycolysis/gluconeogenesis and carbon metabolism. The activated necroptosis in the defective samples potentially affected the downstream cascades manifesting homeostasis of metabolic compounds as illustrated in Figure S.2 (Appendix C). The necroptosis cascades link with the pathways regulating some metabolic compounds such as alanine, aspartate and glutamate as well as glycogen through glucagon signaling. Particularly, glucagon, the pancreatic enzyme, promotes gluconeogenesis and glycogenolysis, regulates rate of glucose production through lipolysis and generates energy in tissues such as skeletal muscle when required. Altered carbohydrate metabolism in accordance with increasing WS severity is discussed in detail in the following section.

### 3.4.4 Abnormal carbohydrate utilization associated with WS defect

In the early investigation regarding impact of WS on meat quality, an aberrantly high ultimate pH was consistently detected in WS meat (Mazzoni et al., 2015; Alnahhas et al., 2016; Kuttappan et al., 2017a; Baldi et al., 2018; Zambonelli et al., 2016). Such observations point out a compromised carbohydrate metabolism within the defective myofibers. Zambonelli et al., (2016), exploiting microarrays, identified differential expressions of genes encoding enzymes involved in polysaccharide metabolic processes in skeletal muscle of broilers exhibiting combined defective characteristics of WS and wooden breast (WB). In addition, based on the proteomic profiling, Kuttappan et al. (2017b) observed a decreased LDHA protein abundance and predicted declined activities of glycolysis and gluconeogenesis in WS/WB samples.

Herein, as WS severity increased, changes in transcriptional level of genes encoding glycolytic enzymes were observed. Those transcripts included phosphofructokinase platelet (*PFKP*; WS 2, FC = -1.29, p = 0.02; WS3, FC = 1.26, p = 0.01) and triosephosphate isomerase (*TPI1*; WS 1, FC = 1.08, p = 0.04). In particular, *LDHA* mRNA level decreased for approximately 1.3 fold in the severe WS relative to mild WS (p = 0.02) and moderate WS ($p = 5.0\times10^{-3}$), corresponding to the lowest lactic acid content and the greatest ultimate pH in the 24-h postmortem severe WS meat (Figure 3.8a). Expression of *LDHA* gene was shown to be activated under an experimentally hypoxic condition (Firth et al., 1995; Maftouh et al., 2014) primarily through binding of hypoxia-inducible transcription factor (HIF-1) (Firth et al.,1995; Lee et al., 2015) as one of the responsive mechanisms to compensate the reduced mitochondrial energy production. At the later state of hypoxic response, lactate, the primary metabolite of anaerobic metabolism of skeletal muscle, could play roles in promoting angiogenesis and cell growth under prolonged hypoxia (Lee et al., 2015). Based on the present microarray data, however, transcript abundance of gene encoding HIF-1 alpha subunit (*HIF1A*) significantly decreased in the severe WS (WS 1, FC = -1.20, $p = 7.0\times10^{-3}$; WS 2, FC = -1.17, p = 0.03),

suggesting a potential disrupted hypoxic responsiveness of the myofibers at the early hypoxic exposure and pyruvate dehydrogenase kinase isoform 1 (*PDK1*; WS 2, FC = -1.17, p = 0.02). An association between WS severity and changes in *PFKFB4* expression was also observed. This gene encodes bifunctional kinase/phosphatase enzyme that regulates glucose flux through manifesting fructose-2,6-bisphosphate (F2,6BP) concentration (Michenko et al., 2003; Chesney et al., 2014). An up-regulation of *PFKFB4* has been reported to be required for survival of cancer cell as a response to hypoxia (Chesney et al., 2014). Based on this microarray study, *PFKFB4* was 1.3-fold up-regulated in severe WS relative to mild ($p = 7.0\times10^{-3}$) and moderate ($p = 9.0\times10^{-3}$).

Initially, a reduction of glycogen reserved in the WS breast has been widely anticipated (Alnahhas et al., 2016; Kuttappan et al., 2017b). Based on the current findings, decreased expression of *LDHA* at transcriptional level corresponded with a reduction of stored glycogen in the muscle of the broilers exhibiting severe WS defect ($p<0.05$, Figure 3.8b). To our knowledge, this study was the first to demonstrate a significantly declined glycogen content in the severe WS than the other WS degrees ($p<0.05$), supporting the earlier hypothesis. The current results were in accordance with the previous chicken muscle metabolomics profile of Beauclercq et al. (2016), indicating low energy preserved in the form of glycogen in skeletal muscle of chickens divergently selected for high ultimate pH. At transcriptional level, *PHKB* was down-regulated for approximately -1.2 fold in the severe relative to mild WS (p = 0.02) and moderate WS (p = 0.02). This gene encodes regulatory beta subunit of phosphorylase b kinase which activates the glycogen degradation cascade. The findings agreed with the previous observation of Beauclercq et al. (2017) indicating a decreased *PHKB* expression in chicken skeletal muscle showing extremely high ultimate pH.

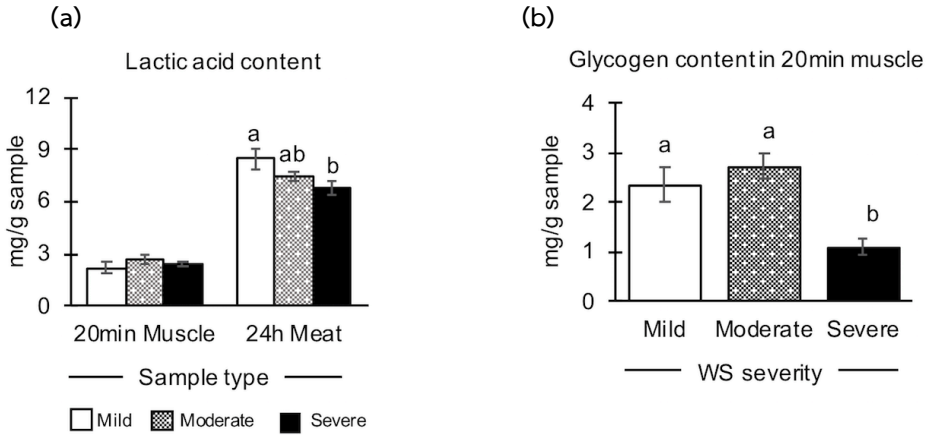


**Figure 3.8. Amounts of (a) lactic acid and (b) glycogen in chicken breasts as affected by different white striping severity levels.** Bars depicted mean whereas error bars represent standard deviation. Different letters above bars indicate significant difference ($p<0.05$).

Suppressions of *SCGD*, the δ-sarcoglycan encoding gene, for approximately 1.2 fold and 1.4 fold were observed in the severe WS samples in comparison with mild and moderate samples, respectively (Table 3.2). The δ-sarcoglycan, one of the five transmembrane sarcoglycans primarily expressed in skeletal muscle, plays roles in stabilizing sarcolemma structure during muscle contraction and relaxation (Ehmsen et al., 2002). In mice, *SCGD*-knockout skeletal muscle developed fibrosis as well as impaired activities of various enzymes required for encountering cellular oxidative stress (Ramirez-Sanchez et al., FEBS 2014). In addition to membrane stabilization, the sarcoglycan complex regulated glucose homeostasis (Groh et al., 2009) and maintained cytosolic $Ca^{2+}$ level within the muscle cells (Solares-Perez et al., 2010). Decreased abundance level of α-sarcoglycan was observed in turkey skeletal muscle with pale, soft and exudative (PSE) defect of which exhibited abnormal regulated cellular glucose breakdown (Malila et al., 2013).

Transcript abundances of *SMTN* and *SMTNL2*, the genes encoding smoothelin isoform C and smoothelin-like 2, respectively, were also significantly reduced as WS defect became more severe. Differential expression of *SMTN* between non-defective and the WS/WB skeletal muscle was also reported by Zambonelli et al. (2016). Primary studies regarding smoothelin revealed two muscle-specific smoothelin isoforms, including isoform A (59 kDa) and B (95 kDa) co-localized with actin cytoskeleton in fully-differentiated visceral or vascular smooth muscle cells, respectively (van Eys et al., 1997; Wehrens et al., 1997). Afterwards, protein expression of 62-kDa smoothelin, corresponded with the differentially expressed *SMTN* identified in this microarray study, was observed in skeletal muscle of White Leghorn during embryogenesis (Deruiter et al., 2001). Recently, smoothelin-like proteins isoform 1 (SMTNL1) and isoform 2 (SMTNL2) were identified. High expressions of SMTNL2 at both mRNA and protein levels were detected in adult mouse skeletal muscle tissues (Gordon et al., 2013). A slight decreased *SMTNL2* abundance has been associated with low exercise aerobic capacity which has been linked with increased risk of metabolic disease (Timmons et al., 1985). Although functions of SMTNL2 remained unclear, the previous study of Gordon et al. (2013) revealed specific docking sites for c-Jun N-terminal kinase (JNK) mitogen-activated protein kinases (MAPKs) on SMTNL2. Upon activation, the JNK-family MAPKs regulate metabolic homeostasis and cellular stress-response cascades, including necroptosis.

The current findings affirmed a dysregulation of glucose utilization at transcriptional level potentially under limiting oxygen condition within the skeletal muscle severely affected with WS defect. Differential gene expression may influence the reduction of glycogen reserved within the muscle cells restricting the magnitude of lactic acid production within the severe WS breast.

**3.5 SUMMARY**

- The microarray analysis revealed that 2,517, 1,615 and 2,483 transcripts were differentially expressed in comparisons between severe WS against mild WS, against moderate WS, and between moderate WS and mild WS (WS 3), respectively.
- Majority of the DE transcripts in all comparisons showed absolute FC ranging between 1.0 and 1.5.

- Based on PANTHER$^{TM}$ classification system, 40% of the DE transcripts identified in all WS comparisons were allocated into cell part cluster with biological functions of catalytic and binding activities involved in cellular and metabolic processes.
- KEGG pathway highlighted metabolic processes, necroptosis and vascular smooth muscle contraction as the top three pathways associated with differential gene expression according to increasing WS severity.
- Decreased abundances of transcripts, particularly *LDHA* (FC = -1.3; WS1, p = 0.02; WS2, p = $5.0\times10^{-3}$) and *PHKB* (FC = -1.2; WS 1, p = 0.02; WS 2, p = 0.02), were associated with dysregulated glucose utilization, impaired glycogen reservation and lactic acid postmortem formation, hence high ultimate pH in the severe WS meat.

# CHAPTER 5

# CONCLUSIONS

In conclusion, the occurrence of WS defect in broiler breasts revealed a steadily increasing trend over the past few years, in which the defect in commercial broilers was evidently found associated among the birds with ages of 39d to 49d. Of 184, only 4 (2.2%) samples were grouped as non-defective whereas 102 (55%), 71 (39%) and 7 (3.8%) were mild, moderate and severe WS, respectively. While the origins of WS defect are still under investigation, our findings were congruent with those of previous studies that the causal of WS defect might be linked to the intensive broiler breeding selection for massive breast yield. Delaying slaughter ages could result in an increasing WS severity among the birds. The majority of the breasts was classified as mild WS of which chemical composition and technological properties remained unchanged. Although prevalence of the severe WS was approximately 4% of the broiler population, the defective meat showed an apparent technological impairment and diminishing nutritional quality in terms of low in protein but high in fat content that would adversely affect overall value of the broiler breast.

Transcriptome profiling was conducted with an attempt to obtain better understanding regarding molecular mechanisms associated with development of WS defect. The results indicated changes in expression of thousands of transcripts ($p<0.05$) associated with increasing WS severity. Pathway analysis highlighted alterations of metabolic processes, angiogenesis, necroptosis and vascular smooth muscle signaling. The muscle fibers exhibiting WS defect were prone to a disrupted hypoxic responsiveness of the myofibers at the early hypoxic exposure. Reduced muscular reserved glycogen along with declined meat lactic acid in the severe WS breast were corresponded with decreased abundances of lactate dehydrogenase isoform A, phosphorylase b kinase and the other relevant DE transcripts indicating impaired abilities of the cells to reserve glycogen as well as to produce lactic acid postmortem. Differences in expression patterns of transcripts associated with muscle regeneration and blood vessel formations suggested myodegeneration and regeneration in the WS breasts.

# APPENDICES

# APPENDIX A

**Table A1** Top ten up-regulated and down-regulated transcripts (p<0.05, coefficient of variation > 10%) in chicken skeletal muscle associated with development of white striping (WS) defect

(A) Severe WS relative to mild WS

| Symbol | Gene name | Fold change | P-value |
|---|---|---|---|
| **Up-regulated** | | | |
| *DDX60* | DEAD (Asp-Glu-Ala-Asp) box polypeptide 60, transcript variant X1 | 6.61 | 0.045 |
| *DDX60* | DEAD (Asp-Glu-Ala-Asp) box polypeptide 60, transcript variant X2 | 6.58 | 0.049 |
| *TRUB1* | TruB pseudouridine (psi) synthase family member 1 (TRUB1), transcript variant X1 | 6.15 | 0.027 |
| *NPTX2* | Neuronal pentraxin II (NPTX2) | 5.93 | 0.043 |
| *TRUB1* | TruB pseudouridine (psi) synthase family member 1 (TRUB1), transcript variant X2 | 4.92 | 0.041 |
| *DKK3* | Dickkopf homolog 3 | 4.84 | 0.039 |
| *PER3* | Period homolog 3 (Drosophila), transcript variant X5 | 4.83 | 0.036 |
| LOC107049794 | Uncharacterized LOC107049794, transcript variant X2 | 4.82 | 0.037 |
| LOC107050518 | Cohesin subunit SA3-like, partial mRNA | 4.81 | 0.042 |
| *DKK3* | Dickkopf homolog 3 (Xenopus laevis), transcript variant X1 | 4.79 | 0.036 |
| **Down-regulated** | | | |
| LOC107055185 | Uncharacterized LOC107055185 (ncRNA) | -2.35 | 0.045 |
| LOC101749734 | Uncharacterized LOC101749734, transcript variant X3, ncRNA | -2.31 | 0.039 |
| *TTLL9* | Tubulin tyrosine ligase-like family member 9, transcript variant X1 | -2.29 | 0.012 |
| LOC101751974 | Uncharacterized LOC101751974, transcript variant X2, ncRNA | -2.23 | 0.043 |
| LOC101750025 | Uncharacterized LOC101750025, transcript variant X1, ncRNA | -2.20 | 0.009 |
| LOC107051346 | Uncharacterized (ncRNA) | -2.18 | 0.040 |
| LOC107050807 | Uncharacterized, transcript variant X2, ncRNA | -2.16 | 0.030 |
| LOC101749734 | Uncharacterized, transcript variant X2, ncRNA | -2.15 | 0.032 |
| *PLEKHA6* | Pleckstrin homology domain containing, family A member 6 transcript variant X2 | -2.09 | 0.015 |
| *DNAJC11* | Dnaj (Hsp40) homolog, subfamily C, member 11 (DNAJC11), transcript variant X1 | -1.97 | 0.013 |

**Table A1 (continued)**

(B) Severe WS relative to moderate WS

| Symbol | Gene name | Fold change | P-value |
|---|---|---|---|
| **Up-regulated** | | | |
| LOC107050543 | Cathepsin D-like, partial mRNA | 2.48 | 0.038 |
| F10 | Coagulation factor X, transcript variant X1 | 2.32 | 0.013 |
| MTIF2 | Mitochondrial translational initiation factor 2, transcript variant X2 | 2.25 | 0.002 |
| GAL3ST1 | Galactose-3-O-sulfotransferase 1, transcript variant X2 | 1.96 | 0.021 |
| P3H3 | Prolyl 3-hydroxylase 3 (P3H3), transcript variant X5 | 1.95 | 0.019 |
| PADI1 | Peptidyl arginine deiminase, type I (PADI1) | 1.93 | 0.036 |
| DPF3 | D4, zinc and double PHD fingers, family 3, transcript variant X6, misc_RNA | 1.91 | 0.040 |
| LOC107054227 | Uncharacterized LOC107054227, transcript variant X3, ncRNA | 1.88 | 0.037 |
| LOC101748134 | Uncharacterized LOC101748134, ncRNA | 1.87 | 0.014 |
| PDCL3 | Phosducin-like 3 | 1.86 | 0.010 |
| **Down-regulated** | | | |
| LOC101749734 | Uncharacterized LOC101749734, transcript variant X2, ncRNA | -4.75 | 0.002 |
| *C12ORF43* | Chromosome 15 open reading frame, human C12orf43, transcript variant X9, misc_RNA | -2.77 | 0.002 |
| LOC107049981 | Uncharacterized LOC107049981, ncRNA | -2.56 | 0.043 |
| LOC107049954 | Uncharacterized LOC107049954, ncRNA | -2.25 | 0.003 |
| LOC107049645 | Uncharacterized LOC107049645, ncRNA | -2.06 | 0.019 |
| LOC101749278 | Uncharacterized LOC101749278, ncRNA | -2.05 | 0.035 |
| LOC107056485 | Uncharacterized LOC107056485, transcript variant X2, ncRNA | -2.04 | 0.031 |
| *TTLL9* | Tubulin tyrosine ligase-like family member 9, transcript variant X6, misc_RNA | -1.98 | 0.028 |
| LOC107054795 | Uncharacterized LOC107054795, transcript variant X2, ncRNA | -1.95 | 0.035 |
| LOC107054227 | Uncharacterized LOC107054227, transcript variant X7, ncRNA | -1.89 | 0.041 |

**Table A1 (continued)**

(C) Moderate WS relative to mild WS

| Symbol | Gene name | Fold change | P-value |
|---|---|---|---|
| **Up-regulated** | | | |
| LOC107057467 | Sperm-associated antigen 4 protein-like, transcript variant X1 | 9.54 | 0.016 |
| LOC107051545 | Myosin-7-like (LOC107051545), partial ncRNA | 5.64 | 0.034 |
| LOC107051448 | Uncharacterized LOC107051448 (ncRNA) | 3.55 | 0.020 |
| LOC107049645 | Uncharacterized LOC107049645 (ncRNA) | 3.10 | 0.009 |
| *CDKN1C* | Uncharacterized LOC101751827 | 2.91 | 0.035 |
| LOC107054720 | Uncharacterized LOC107054720 (ncRNA) | 2.87 | 0.023 |
| LOC101750767 | Uncharacterized LOC101750767, transcript variant X4 | 2.82 | 0.028 |
| LOC769704 | Fatty acyl-co A hydrolase precursor, medium chain-like, transcript variant X2 | 2.80 | 0.007 |
| LOC776146 | SUN domain-containing protein 3-like | 2.77 | 0.025 |
| *CSF3R* | Colony stimulating factor 3 receptor (granulocyte), transcript variant X | 2.76 | 0.037 |
| **Down-regulated** | | | |
| LOC418544 | Cystathionine beta-synthase-like | -4.03 | 0.042 |
| *PHF11* | PHD finger protein 11, transcript variant X2 | -3.38 | 0.032 |
| *PHF11* | PHD finger protein 11, transcript variant X1 | -3.35 | 0.046 |
| *AQP4* | Aquaporin 4, transcript variant X1 | -3.24 | 0.013 |
| *OSGIN1* | Oxidative stress induced growth inhibitor 1, transcript variant X3 | -2.70 | 0.033 |
| *AGBL1* | ATP/GTP binding protein-like 1, transcript variant X1 | -2.64 | 0.032 |
| LOC101751974 | Uncharacterized LOC101751974, transcript variant X3, ncRNA | -2.63 | 0.011 |
| *GSTA4* | Glutathione S-transferase alpha 4, transcript variant X2 | -2.61 | 0.0003 |
| *Mettl21ep* | Methyltransferase-like 21E, pseudogene, transcript variant X3 | -2.59 | 0.013 |

# APPENDIX B

**Table B1** Biological pathways, revealed by KEGG pathway knowledge database, associated with increasing white striping severity in commercial broilers

| KEGG ID | Pathways | #mapped genes | KEGG ID | Pathways | #mapped genes |
|---|---|---|---|---|---|
| gga01100 | Metabolic pathways | 54 | gga04310 | Wnt signaling pathway | 5 |
| gga04217 | Necroptosis | 22 | gga00020 | Citrate cycle (TCA cycle) | 5 |
| gga04270 | Vascular smooth muscle contraction | 15 | gga04080 | Neuroactive ligand-receptor interaction | 5 |
| gga04261 | Adrenergic signaling in cardiomyocytes | 14 | gga00620 | Pyruvate metabolism | 5 |
| gga00980 | Metabolism of xenobiotics by cytochrome P450 | 13 | gga05132 | Salmonella infection | 5 |
| gga00982 | Drug metabolism - cytochrome P450 | 13 | gga00270 | Cysteine and methionine metabolism | 5 |
| gga00480 | Glutathione metabolism | 13 | gga04371 | Apelin signaling pathway | 5 |
| gga00564 | Glycerophospholipid metabolism | 12 | gga00640 | Propanoate metabolism | 5 |
| gga03015 | mRNA surveillance pathway | 12 | gga00220 | Arginine biosynthesis | 4 |
| gga03018 | RNA degradation | 11 | gga04620 | Toll-like receptor signaling pathway | 4 |
| gga04530 | Tight junction | 11 | gga04120 | Ubiquitin mediated proteolysis | 4 |
| gga04510 | Focal adhesion | 11 | gga04060 | Cytokine-cytokine receptor interaction | 4 |
| gga01200 | Carbon metabolism | 11 | gga04144 | Endocytosis | 4 |
| gga00565 | Ether lipid metabolism | 11 | gga03022 | Basal transcription factors | 4 |
| gga00590 | Arachidonic acid metabolism | 11 | gga00670 | One carbon pool by folate | 4 |
| gga00592 | alpha-Linolenic acid metabolism | 10 | gga00250 | Alanine, aspartate and glutamate metabolism | 4 |
| gga05168 | Herpes simplex infection | 10 | gga04914 | Progesterone-mediated oocyte maturation | 4 |
| gga00591 | Linoleic acid metabolism | 10 | gga04012 | ErbB signaling pathway | 4 |
| gga04140 | Autophagy - animal | 10 | gga00230 | Purine metabolism | 4 |
| gga00562 | Inositol phosphate metabolism | 10 | gga00051 | Fructose and mannose metabolism | 4 |
| gga04910 | Insulin signaling pathway | 10 | gga03040 | Spliceosome | 4 |
| gga04218 | Cellular senescence | 10 | gga04622 | RIG-I-like receptor signaling pathway | 3 |
| gga04020 | Calcium signaling pathway | 10 | gga03460 | Fanconi anemia pathway | 3 |
| gga04070 | Phosphatidylinositol signaling system | 9 | gga04150 | mTOR signaling pathway | 3 |
| gga04810 | Regulation of actin cytoskeleton | 9 | gga00330 | Arginine and proline metabolism | 3 |
| gga04141 | Protein processing in endoplasmic reticulum | 9 | gga04260 | Cardiac muscle contraction | 3 |
| gga04210 | Apoptosis | 9 | gga04110 | Cell cycle | 3 |
| gga04145 | Phagosome | 8 | gga00030 | Pentose phosphate pathway | 3 |
| gga04540 | Gap junction | 8 | gga04136 | Autophagy - other | 3 |
| gga04114 | Oocyte meiosis | 7 | gga00052 | Galactose metabolism | 3 |
| gga01230 | Biosynthesis of amino acids | 7 | gga03050 | Proteasome | 3 |
| gga04933 | AGE-RAGE signaling pathway in diabetic complications | 6 | gga03008 | Ribosome biogenesis in eukaryotes | 2 |
| gga04621 | NOD-like receptor signaling pathway | 6 | gga04623 | Cytosolic DNA-sensing pathway | 2 |
| gga03010 | Ribosome | 6 | gga00140 | Steroid hormone biosynthesis | 2 |
| gga04010 | MAPK signaling pathway | 6 | gga03013 | RNA transport | 2 |
| gga04137 | Mitophagy - animal | 6 | gga03450 | Non-homologous end-joining | 2 |
| gga04068 | FoxO signaling pathway | 6 | gga00970 | Aminoacyl-tRNA biosynthesis | 2 |
| gga05164 | Influenza A | 6 | gga00830 | Retinol metabolism | 2 |

**Table B1** (continued)

| KEGG ID | Pathways | #mapped genes | KEGG ID | Pathways | #mapped genes |
|---|---|---|---|---|---|
| gga04142 | Lysosome | 6 | gga04916 | Melanogenesis | 2 |
| gga00010 | Glycolysis / Gluconeogenesis | 6 | gga04340 | Hedgehog signaling pathway | 2 |
| gga00190 | Oxidative phosphorylation | 5 | gga04330 | Notch signaling pathway | 2 |
| gga04920 | Adipocytokine signaling pathway | 5 | gga04350 | TGF-beta signaling pathway | 2 |
| gga04912 | GnRH signaling pathway | 5 | gga03320 | PPAR signaling pathway | 2 |
| gga00520 | Amino sugar and nucleotide sugar metabolism | 1 | gga00860 | Porphyrin and chlorophyll metabolism | 1 |
| gga04216 | Ferroptosis | 1 | gga00350 | Tyrosine metabolism | 1 |
| gga00360 | Phenylalanine metabolism | 1 | gga00510 | N-Glycan biosynthesis | 1 |
| gga03030 | DNA replication | 1 | gga00100 | Steroid biosynthesis | 1 |
| gga01210 | 2-Oxocarboxylic acid metabolism | 1 | gga00310 | Lysine degradation | 1 |
| gga00400 | Phenylalanine, tyrosine and tryptophan biosynthesis | 1 | gga04512 | ECM-receptor interaction | 1 |
| gga00240 | Pyrimidine metabolism | 1 | gga03020 | RNA polymerase | 1 |
| gga00531 | Glycosaminoglycan degradation | 1 | gga00280 | Valine, leucine and isoleucine degradation | 1 |
| gga00440 | Phosphonate and phosphinate metabolism | 1 | gga04514 | Cell adhesion molecules (CAMs) | 1 |
| gga04115 | p53 signaling pathway | 1 | gga04370 | VEGF signaling pathway | 1 |
| gga00630 | Glyoxylate and dicarboxylate metabolism | 1 | gga00514 | Other types of O-glycan biosynthesis | 1 |
| gga04146 | Peroxisome | 1 | gga00532 | Glycosaminoglycan biosynthesis – chondroitin sulfate / dermatan sulfate | 1 |

# APPENDIX C

Figures depicted top three biological pathways, mapped by KEGG pathway knowledge base, associated with WS defect in chicken skeletal muscle

**1. Metabolic pathways**

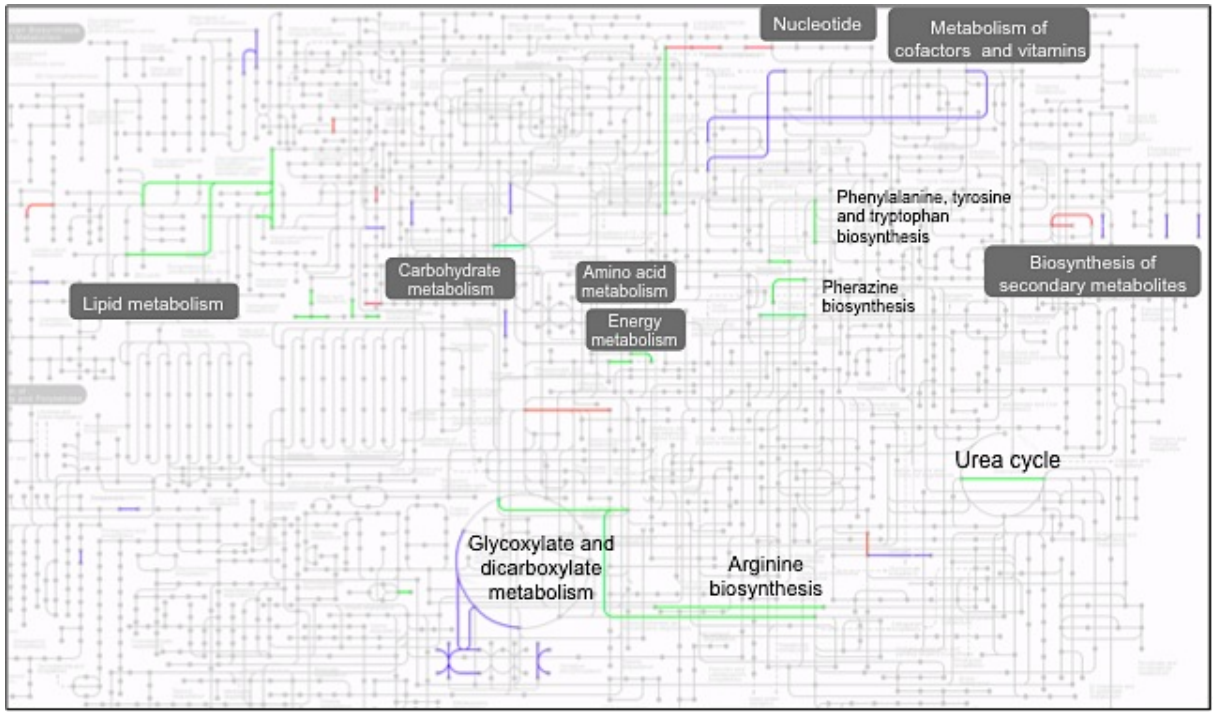


**Figure S.1 Schematic diagram of metabolic pathways associated with white striping (WS) defect in commercial broilers.** The pathway, suggested by KEGG database (id: gga01100), illustrates biological interactions among differentially expressed genes (n = 54) in chicken skeletal muscle. Green, red and blue lines indicate where the differentially expressed transcripts from comparisons of severe and mild (WS1), severe and moderate (WS2), and moderate and mild (WS3), respectively, were mapped into the pathways.

## 2. Necroptosis

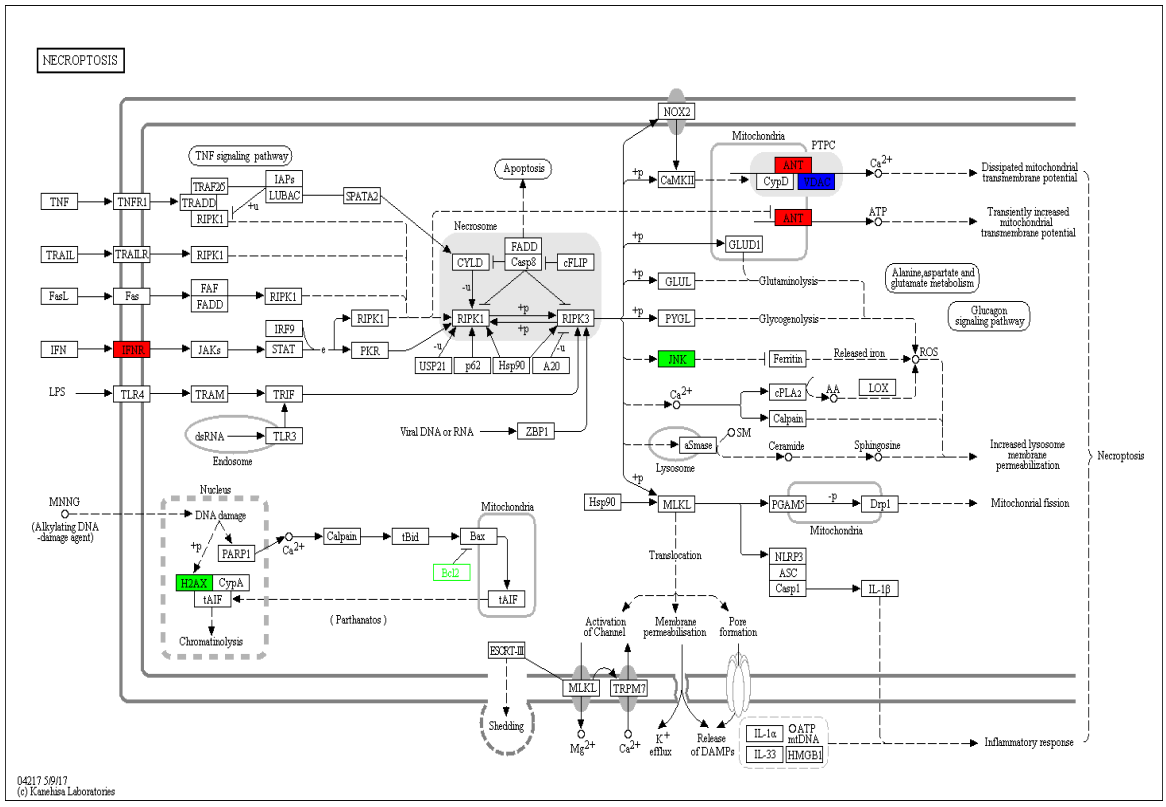


**Figure S.2 Schematic diagram of necroptosis pathway associated with white striping (WS) defect in commercial broilers.** The pathway, suggested by KEGG database (id: gga04217), illustrates biological interactions among differentially expressed (DE) genes (n = 22) in chicken skeletal muscle affected with WS defect. Boxes highlighted in solid green, red and blue indicate where the differentially expressed transcripts from comparisons of severe and mild (WS1), severe and moderate (WS2), and moderate and mild (WS3), respectively, were mapped into the pathways. The gene with green letter was the DE gene found in both WS2 and WS3. The translated proteins of the DE transcripts are as follows; apoptosis regulator Bcl-2 (BCL2), c-Jun N-terminal kinase [EC:2.7.11.24] (JNK), interferon gamma receptor 2 (IFNGR2), solute carrier family 25 (mitochondrial adenine nucleotide translocator), member 4/5/6/31 (SLC25A4S), histone H2A (H2A) and voltage-dependent anion channel protein 2 (VDAC2).

### 3. Vascular smooth muscle contraction

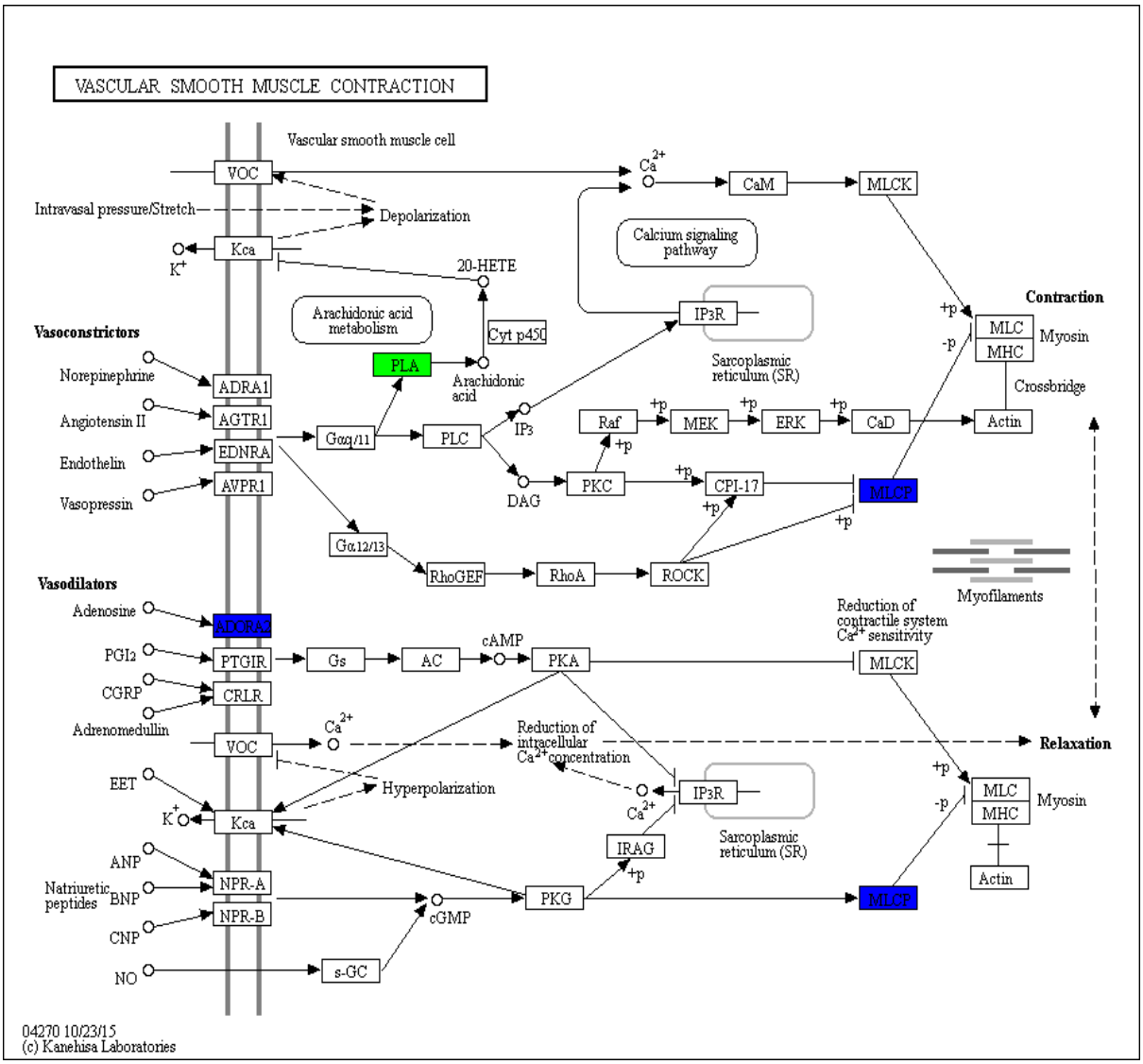


**Figure S.3 Schematic diagram of vascular smooth muscle contraction pathway associated with white striping (WS) defect in commercial broilers.** The pathway, suggested by KEGG database (id: gga04270), illustrates biological interactions among differentially expressed (DE) genes (n = 15) in chicken skeletal muscle affected with WS defect. Boxes highlighted in solid green and blue indicate where the differentially expressed transcripts from comparisons of severe and mild (WS1) and moderate and mild (WS3), respectively, were mapped into the pathways. The translated proteins of the DE transcripts include secretory phospholipase A2 [EC:3.1.1.4] (PLA2G), adenosine receptor A2b (ADORA2B), serine/threonine-protein phosphatase PP1 catalytic subunit [EC:3.1.3.16] (PPP1C) and protein phosphatase 1 regulatory subunit 12B (PPP1R12B).

# APPENDIX D

Copies of publicly available articles.

1. เอกสารตีพิมพ์ใน proceedings งานประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีเนื้อสัตว์ ครั้งที่ 6 จัดโดยสถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง (สจล.) ระหว่างวันที่ 18 – 19 มิถุนายน 2561 โดยได้รับเชิญให้เป็นผู้บรรยายในหัวข้อ “White striping defect: สถานการณ์ที่พบในประเทศและผลกระทบต่อคุณภาพของเนื้อไก่”

**White striping defect: สถานการณ์ที่พบในประเทศ และผลกระทบต่อคุณภาพของเนื้อไก่**

**White striping defect: Incidence in Thailand and its impact on broiler meat quality**

**ญาณี ศรีมารุต[1] จุฑาวุฒิ อุชุปัจ[1] เปรมศักดิ์ ชัยวิวัฒน์ตระกูล[1] ธนพร อึ้งเวชวานิช[2] โศภชา อารยเมธากร[2] วีระศักดิ์ ปัญญาพรวิทยา[3] ชลัทวรรณ แสนเสมอ[3] วณิลดา รุ่งรัศมี[2] วรรณพ วิเศษสงวน[1] และ ยุวเรศ มลิลา[1*]**

**Y. Srimarut[1], J. U-chupaj[1], P. Chaiwiwattrakul[1], T. Uengwetwanich[2], S. Arayamethakorn[2], V. Punyapornwithaya[3], C. Sansamur[3], W. Rungrassamee[2], W. Visessanguan[1] and Y. Malila[1*]**

[1]หน่วยวิจัยเทคโนโลยีชีวภาพอาหาร [2]หน่วยวิจัยเทคโนโลยีการตรวจวินิจฉัยทางชีวภาพ ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ 113 อุทยานวิทยาศาสตร์ประเทศไทย ถ.พหลโยธิน ต.คลองหนึ่ง อ.คลองหลวง จ.ปทุมธานี 12120 [3]คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ 155 ต.แม่เหียะ อ.เมือง จ.เชียงใหม่ 50100

[1]Food Biotechnology Research Unit, [2]Bio-sensing Technology Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phahonyothin Rd., Khlong Nueng, Khlong Luang, Pathum Thani 12120, Thailand. [3]Faculty of Veterinary Medicine, Chiang Mai University, 155 Mae Hia, Muang, Chiang Mai, 50100, Thailand.

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## บทคัดย่อ

การเกิดตำหนิแบบแถบลายสีขาว (white striping; WS) บนเนื้ออกไก่เป็นลักษณะของความผิดปกติรูปแบบหนึ่งในไก่เนื้อ ผลการสำรวจการเกิดตำหนิบนเนื้ออกไก่จากไก่เนื้อเพศผู้สายพันธุ์ Ross 308 อายุระหว่าง 39-49 วัน จำนวน 184 ตัว พบไก่ที่มีตำหนิ 180 ตัว (97.8%) แบ่งตามระดับความรุนแรงของการเกิดตำหนิเป็น 3 กลุ่ม คือเล็กน้อย 102 ตัว (55%) ปานกลาง 71 ตัว (39%) และมาก 7 ตัว (3.8%) จากการวิเคราะห์ ordinal logistic regression model (OLR) พบว่าอายุและเปอร์เซ็นต์เนื้ออกส่งผลต่อระดับความรุนแรงในการเกิดตำหนิ ไก่ที่มีอายุมากมีแนวโน้มที่เกิดตำหนิที่รุนแรงกว่าไก่อายุน้อย ไก่ที่มีเปอร์เซ็นต์เนื้ออกมากมีโอกาสเกิดตำหนิสูงขึ้น เนื้ออกไก่ที่มีตำหนิรุนแรงมากมีสัดส่วนของไขมันสูง แต่โปรตีนและเถ้าต่ำกว่ากลุ่มอื่น ($p<0.05$) การเกิดตำหนิระดับรุนแรงมากยังส่งผลต่อลักษณะเนื้อสัมผัสของเนื้อไก่ โดยลักษณะเนื้อสัมผัสเมื่อปรุงสุกมีค่า hardness springiness และ chewiness สูงขึ้น ($p<0.05$)

**คำสำคัญ**: ตำหนิแบบแถบลายสีขาว (White striping defect), ไก่กระทง, เนื้ออกไก่, สมการถดถอยโลจิสติค (ordinal logistic regression)

## Abstract

Development of white striping (WS) defect has been in great concern of poultry industry. The objective of this study was to estimate incidence of WS defect in breast meat collected from commercial broilers (n=184, male Ross 308) slaughtered at the ages varying from 39d to 49d. Impact of WS development on meat quality indices was also evaluated. The meat samples were classified as non-defective, mild WS, moderate WS and severe WS based upon numbers and thickness of white striation appeared on the surface of the meat. Of 184, only 4 (2.2%) samples were grouped as non-defective whereas 102 (55%), 71 (39%) and 7 (3.8%) were mild, moderate and severe, respectively. Based on ordinal logistic regression analysis, slaughter age and percent breast weight were significantly related with increasing WS severity ($p<0.05$). Severe WS samples showed elevated fat content but reduced protein and ash content ($p<0.05$). Increases in hardness, springiness, and chewiness were observed in cooked severe WS samples ($p<0.05$).


**Keywords**: white striping, commercial broiler, breast meat, ordinal logistic regression

## บทนำ

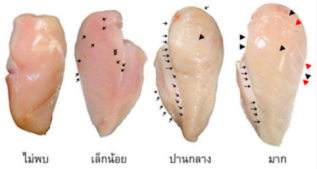
ความผิดปกติของเนื้อไก่แบบแถบลายสีขาว หรือ White striping defect (WS) มักพบบนเนื้อส่วนอกของไก่เนื้อ โดยเห็นเป็นเส้นสีขาวขนานกับเส้นใยกล้ามเนื้ออก การเกิดตำหนิแบบ WS เริ่มเป็นที่สนใจในกลุ่มประเทศแถบในยุโรปเมื่อช่วง ค.ศ. 2000 เป็นต้นมา (Petracci et al., 2013; Tasoniero et al., 2016) และมีรายงานการตรวจพบ WS ในสหรัฐอเมริกา (Kuttappan et al., 2012a) และบราซิล (Ferreira et al., 2014) จากการทบทวนวรรณกรรมพบว่า ในช่วงต้นของการพบตำหนิ WS มีความชุกของตำหนิมีประมาณ 10% ขึ้นอยู่กับน้ำหนักของไก่ อย่างไรก็ตาม จากนั้นไม่นานนักมีรายงานว่าพบความชุกของโรคเพิ่มมากขึ้นตั้งแต่ 20% ถึง 96% โดยรายงานระบุว่าความผิดปกติที่เกิดขึ้นนี้ส่งผลให้เกิดการเปลี่ยนแปลงขององค์ประกอบทางเคมีและคุณสมบัติของเนื้ออกไก่ รวมถึงการยอมรับของผู้บริโภค (Kuttappan et al., 2012b) ซึ่งอาจเป็นปัญหาที่น่าวิตกของทางโรงงานผู้ผลิตเนื้อไก่ อย่างไรก็ตาม ในส่วนของประเทศแถบเอเชียตะวันออกและเอเชียตะวันออกเฉียงใต้ รวมถึงประเทศไทยซึ่งเป็นผู้ผลิตและส่งออกเนื้อไก่และผลิตภัณฑ์ไก่เนื้อไปยังประเทศในแถบยุโรปเอง ยังไม่มีรายงานการศึกษาการเกิดความผิดปกตินี้ วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาอุบัติการณ์ (occurrence) ของ WS ในไก่เนื้อในประเทศ และศึกษาผลกระทบตำหนิต่อตัวชี้วัดคุณภาพของเนื้อไก่ส่วนอก

## อุปกรณ์และวิธีการ

***ตัวอย่างเนื้อไก่ และการจำแนกความรุนแรงของ WS***

ตัวอย่างที่ใช้เป็นไก่เพศผู้สายพันธุ์ ROSS 308 อายุระหว่าง 39-49 วัน จำนวน 184 ตัว สุ่มเก็บตัวอย่างซากภายหลังขั้นตอนการถอนขนจากโรงเชือดที่ได้มาตรฐานอุตสาหกรรมในจังหวัดสระบุรี วิเคราะห์คุณภาพเนื้อสัตว์ภายหลังจากเชือด 24 ชั่วโมง (24 h postmortem)

การจำแนกความรุนแรงของ WS ดัดแปลงจาก Kuttappan et al. (2012b) โดยจำแนกระดับ WS ตามจำนวนและความหนาของเส้นสีขาวบนเนื้ออก สามารถแบ่งตัวอย่างออกได้เป็น 4 กลุ่ม (ภาพที่ 1) ได้แก่ กลุ่มที่ไม่พบความผิดปกติ (ไม่พบแถบลายสีขาวบนเนื้ออก) กลุ่มที่ผิดปกติเล็กน้อย (พบแถบลายสีขาวตั้งแต่ 1-40 เส้นและความหนาของเส้นไม่เกิน 0.5 มม.) กลุ่มที่ผิดปกติปานกลาง (พบแถบลายสีขาวมากกว่า 40 เส้น หรือพบแถบลายสีขาวที่มีความหนาของเส้น 1.0-1.9 มม.ตั้งแต่ 1-5 เส้น) กลุ่มที่ผิดปกติระดับรุนแรงมาก (พบแถบลายสีขาวที่มีความหนาของเส้น 1.0-1.9 มม. มากกว่า 5 เส้น หรือพบว่ามีแถบลายสีขาวที่มีความหนาของเส้นมากกว่า 2.0 มม.)




**ภาพที่ 1** การจำแนกระดับความรุนแรงของ WS

***การวิเคราะห์คุณภาพของเนื้ออกไก่***

ความชื้น โปรตีน และเถ้าวิเคราะห์ตามวิธีของ AOAC (2000) ปริมาณไขมันสกัดด้วยคลอโรฟอร์มและเมทานอลตามวิธีของ Bligh and Dyer (1959) ความสามารถในการอุ้มน้ำของเนื้อไก่บ่งชี้ด้วยค่าการสูญเสียน้ำระหว่างเก็บรักษา (drip loss) ค่าการสูญเสียน้ำจากการทำให้สุก (cook loss) โดยวิเคราะห์ตามวิธีของ Chiang et al. (2008) เนื้อสัมผัส texture profile analysis (TPA) ด้วยเครื่อง texture analyzer (TA-XTi) ตามวิธีของ U-chupaj et al. (2017)

***การวิเคราะห์ค่าทางสถิติ***

วิเคราะห์ค่าทางสถิติโดยใช้โปรแกรม R เวอร์ชัน 3.2.1 วิเคราะห์ลักษณะการกระจายตัวของข้อมูลด้วย Shapiro-Wilk normality Test เนื่องจากข้อมูลกระจายตัวแบบ non-parametric วิเคราะห์ผลของระดับความรุนแรงของ WS ต่อคุณภาพของตัวอย่างตามวิธีของ Kruskal-Wallis Test เปรียบเทียบค่าเฉลี่ยด้วยวิธี Dunn's Test และใช้ ordinal logistic regression model (OLR) ในการศึกษาผลของอายุและเปอร์เซ็นต์น้ำหนักเนื้ออกต่อความรุนแรงในการเกิดตำหนิ

## ผลการศึกษาและวิจารณ์

***อุบัติการณ์ของ WS ในไก่เนื้อที่พบในประเทศ***

จากการสำรวจตัวอย่างไก่ อายุระหว่าง 39-49 วัน จำนวน 184 ตัว น้ำหนักซากอยู่ระหว่าง 2.03-3.94 กิโลกรัม พบตัวอย่างเนื้ออกที่มีตำหนิถึง 180 ตัวอย่าง คิดเป็น 97.8% ของตัวอย่างที่สุ่มเก็บ มีเพียง 4 ตัวอย่างที่จัดเป็นเนื้อปกติ (2.2%) เป็นกลุ่มที่มีตำหนิระดับเล็กน้อย 102 ตัว (55%) ปานกลาง 71 ตัว (39%) และรุนแรงมากจำนวน 7 ตัว (3.8%)

***ความสัมพันธ์ระหว่างการเกิดความผิดปกติ อายุ และขนาดของอกไก่***

น้ำหนักซาก น้ำหนักเนื้อส่วนอก และเปอร์เซ็นต์เนื้ออกแสดงในตารางที่ 1 พบว่า ตัวอย่างที่มีตำหนิ WS ในระดับรุนแรงมากมีน้ำหนักซาก น้ำหนักเนื้ออก และเปอร์เซ็นต์เนื้ออกสูงกว่าตัวอย่างที่ไม่มีตำหนิ หรือมีตำหนิระดับเล็กน้อยอย่างมีนัยสำคัญ ($p<0.05$) ตัวอย่างไก่ที่มีตำหนิระดับปานกลางมีน้ำหนักซาก น้ำหนักเนื้ออกสูงกว่าตัวอย่างกลุ่มที่มีตำหนิระดับเล็กน้อย ($p<0.05$) และไม่ต่างจากตัวอย่างระดับรุนแรง ($p\geq0.05$) ในขณะที่ดัชนีคุณภาพซากของตัวอย่างระดับเล็กน้อยไม่แตกต่างจากตัวอย่างที่ไม่มีตำหนิ ($p\geq0.05$) เมื่อวิเคราะห์ความสัมพันธ์ระหว่างระดับความรุนแรงของ WS กับอายุและเปอร์เซ็นต์เนื้ออกโดย OLR (ตารางที่ 2) พบว่า ปัจจัยทั้งสองให้อัตราส่วนปัจจัยเสี่ยง (odds ratios) 1.118 และ 1.515 ตามลำดับ

**ตารางที่ 1** คุณภาพซากในตัวอย่างไก่ที่มีตำหนิแบบ WS ในระดับต่างๆ

| คุณสมบัติ | ระดับความรุนแรง | | | |
|---|---|---|---|---|
| | ไม่พบ (n=4) | เล็กน้อย (n=102) | ปานกลาง (n=71) | มาก (n=7) |
| น้ำหนักซาก (กิโลกรัม) | 2.58$^{b}$ ± 0.43 | 2.80$^{b}$ ± 0.43 | 3.07$^{a}$ ± 0.42 | 3.16$^{a}$ ± 0.46 |
| น้ำหนักเนื้ออก (กรัม) | 188.36$^{b}$ ± 32.73 | 240.07$^{b}$ ± 48.64 | 278.48$^{a}$ ± 47.07 | 303.08$^{a}$ ± 50.30 |
| เปอร์เซ็นต์เนื้ออก | 7.35$^{c}$ ± 1.04 | 8.54$^{b}$ ± 0.85 | 9.06$^{ab}$ ± 0.80 | 9.58$^{a}$ ±0.66 |

* ตัวอักษรภาษาอังกฤษที่ต่างกันแสดงถึงความแตกต่างอย่างมีนัยสำคัญ ($p<0.05$)

**ตารางที่ 2** ค่าสัมประสิทธิ์ ค่าคลาดเคลื่อนมาตรฐาน และอัตราส่วนปัจจัยเสี่ยงของตัวแปรใน ordinal logistic model

| ปัจจัย | ค่าสัมประสิทธิ์ | ค่าคลาดเคลื่อนมาตรฐาน | อัตราส่วนปัจจัยเสี่ยง (95% CI)[1] |
|---|---|---|---|
| อายุ | 0.112 | 0.044 | 1.118 *** (1.024, 1.221) |
| เปอร์เซ็นต์เนื้ออก | 0.415 | 0.098 | 1.515 *** (1.249, 1.836) |

[1] CI = ช่วงความเชื่อมั่น (confidence interval) ***$p<0.01$

***ผลของการเกิดตำหนิต่อคุณภาพเนื้อไก่***

จากผลการวิเคราะห์องค์ประกอบในเนื้อไก่ พบว่าตัวอย่างกลุ่มที่มีตำหนิ WS ระดับรุนแรงมากมีไขมันสูงที่สุด แต่มีโปรตีนและเถ้าต่ำที่สุด ($p<0.05$) ส่วนตัวอย่างกลุ่มที่ไม่มีตำหนิ มีตำหนิเล็กน้อยและปานกลางนั้นมีปริมาณองค์ประกอบดังกล่าวไม่แตกต่างกัน ($p\geq0.05$) ตัวอย่างกลุ่มที่เกิดความผิดปกติมากมีค่าการสูญเสียน้ำจากการทำให้สุกมากกว่าตัวอย่างกลุ่มที่เกิดความผิดปกติเล็กน้อยและปานกลาง ($p<0.05$) แต่ค่าดังกล่าวของตัวอย่างกลุ่มที่ไม่พบความผิดปกตินั้นมีค่าไม่แตกต่างจากกลุ่มอื่น นอกจากนี้ตัวอย่างกลุ่มที่เกิด WS ระดับรุนแรงมากมีค่า hardness และ chewiness สูงที่สุด ($p<0.05$) แม้ว่าค่า springiness และ gumminess จะไม่แตกต่างจากกลุ่มที่เกิดความผิดปกติเล็กน้อยและปานกลาง ($p\geq0.05$) แต่ก็มีค่าสูงกว่าตัวอย่างกลุ่มที่ไม่พบความผิดปกติ ($p<0.05$) บ่งชี้ว่าตัวอย่างเนื้อไก่สุกของกลุ่มที่เกิดความผิดปกติมากมีความแข็งและเหนียวกว่าตัวอย่างอื่น

**ตารางที่ 3** ผลของการเกิด WS และระดับความรุนแรงต่อคุณภาพเนื้ออกไก่

| คุณสมบัติ | ระดับความรุนแรงของ WS | | | |
|---|---|---|---|---|
| | ไม่พบ (n=4) | เล็กน้อย (n=102) | ปานกลาง (n=71) | มาก (n=7) |
| ความชื้น (%) | 75.01 ± 0.68 | 75.14 ± 0.98 | 75.33 ± 1.03 | 75.76 ± 1.95 |
| ไขมัน (%) | 1.34$^{b}$ ± 0.19 | 1.54$^{ab}$ ± 0.32 | 1.63$^{ab}$ ± 0.40 | 1.88$^{a}$ ± 0.98 |
| โปรตีน (%) | 23.02$^{a}$ ± 1.04 | 22.07$^{ab}$ ± 1.08 | 21.77$^{b}$ ± 1.20 | 21.07$^{b}$ ± 1.50 |
| เถ้า (%) | 1.22$^{a}$ ± 0.04 | 1.17$^{ab}$ ± 0.09 | 1.16$^{ab}$ ± 0.07 | 1.12$^{b}$ ± 0.08 |
| การสูญเสียน้ำระหว่างเก็บรักษา (%) | 1.02 ± 0.30 | 0.82 ± 0.43 | 0.86 ± 0.50 | 0.82 ± 0.29 |
| การสูญเสียน้ำจากการทำให้สุก (%) | 18.81$^{ab}$ ± 2.24 | 17.86$^{b}$ ± 4.33 | 17.33$^{b}$ ± 5.29 | 22.77$^{a}$ ± 7.06 |
| Hardness (N) | 23.10$^{b}$ ± 2.80 | 26.03$^{b}$ ± 5.17 | 26.99$^{b}$ ± 5.29 | 34.92$^{a}$ ± 8.16 |
| Springiness (ratio) | 0.61$^{b}$ ± 0.03 | 0.64$^{ab}$ ± 0.04 | 0.63$^{ab}$ ± 0.04 | 0.67$^{a}$ ± 0.04 |
| Cohesiveness | 0.41 ± 0.01 | 0.42 ± 0.06 | 0.43 ± 0.07 | 0.39 ± 0.04 |
| Gumminess (N) | 9.50$^{b}$ ± 1.02 | 10.23$^{b}$ ± 3.31 | 11.55$^{ab}$ ± 2.70 | 13.83$^{a}$ ± 4.33 |
| Chewiness (N×mm) | 5.88$^{b}$ ± 0.88 | 6.63$^{b}$ ± 2.16 | 7.33$^{b}$ ± 1.75 | 9.37$^{a}$ ± 3.53 |

* ตัวอักษรภาษาอังกฤษที่ต่างกันแสดงถึงความแตกต่างอย่างมีนัยสำคัญ ($p<0.05$)

## สรุปและข้อเสนอแนะ

งานวิจัยนี้แสดงให้เห็นถึงอุบัติการณ์ WS ที่พบในไก่เนื้อในประเทศ ที่อาจเป็นสาเหตุของปัญหาคุณภาพของผลิตภัณฑ์จากเนื้ออกไก่ในอุตสาหกรรม อย่างไรก็ตาม เนื่องจากข้อจำกัดบางประการในงานนี้จึงสุ่มเก็บตัวอย่างไก่ตัวผู้เพียง 184 ตัวอย่าง แต่ก็เพียงพอที่แสดงให้เห็นถึงผลกระทบของการเกิด WS ระดับรุนแรงต่อคุณค่าทางโภชนาการ ความสามารถในการอุ้มน้ำ และเนื้อสัมผัสของเนื้ออกไก่ โดยพบว่ามีไก่ถึง 180 ตัวที่มีตำหนิแบบ WS และความรุนแรงของ WS มี

ความสัมพันธ์กับอายุของไก่และเปอร์เซ็นต์เนื้ออกอย่างมีนัยสำคัญ สำหรับในงานวิจัยต่อเนื่องจะศึกษาการเกิดตำหนิแบบ WS ในกลุ่มประชากรที่มากขึ้น

## กิตติกรรมประกาศ

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๒๑ มีนาคม ๒๕๖๑

เรื่อง ขอเชิญเป็นวิทยากรและส่งบทความในการบรรยายเข้าร่วมการประชุมวิชาการวิทยาศาสตร์เทคโนโลยีเนื้อสัตว์ ครั้งที่ ๖ ปี ๒๕๖๑

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สิ่งที่แนบมาด้วย ๑. รายละเอียดการประชุมวิชาการ
๒. แบบตอบรับ
๓. แบบฟอร์มประวัติวิทยากร

ด้วย ศูนย์เครือข่ายการวิจัยเทคโนโลยีเนื้อสัตว์ ภาควิชาเทคโนโลยีการผลิตสัตว์และประมง คณะเทคโนโลยีการเกษตร สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง (สจล.) ซึ่งเป็นศูนย์เครือข่ายฯ จัดตั้งขึ้นจากความร่วมมือระหว่าง สจล. กับสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) มีความประสงค์ที่จะจัดงานประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีเนื้อสัตว์ครั้งที่ ๖ ปี ๒๕๖๑ ในวันที่ ๑๘ - ๑๙ มิถุนายน ๒๕๖๑ ณ โรงแรมรามาการ์เด้นส์ หลักสี่ กรุงเทพฯ โดยมีวัตถุประสงค์ เพื่อนำเสนอความรู้ความสำเร็จของการผลิตเนื้อสัตว์บนพื้นฐานของเทคโนโลยีนวัตกรรม นำเสนอผลงานวิจัยที่เกี่ยวข้องกับวิทยาศาสตร์และเทคโนโลยีเนื้อสัตว์ตลอดจนผลงานวิชาการอื่นๆ ที่เกี่ยวข้องกับการผลิตเนื้อสัตว์และการเพิ่มมูลค่าตลอดห่วงโซ่การผลิต แลกเปลี่ยน ความรู้ และประสบการณ์ทางวิชาการและสร้างเครือข่ายความร่วมมือทางวิชาการระหว่างหน่วยงานที่เกี่ยวข้องทั้งภาครัฐและเอกชน แสดงศักยภาพของศูนย์เครือข่ายการวิจัยเทคโนโลยีเนื้อสัตว์ในความพร้อมของการเป็นแม่ข่ายในการวิจัยด้านเทคโนโลยีเนื้อสัตว์ เผยแพร่องค์ความรู้จากงานวิจัยสู่สาธารณชน ด้วยการชมผลงานทางวิชาการและการฟังบรรยายทางวิชาการด้านวิทยาศาสตร์และเทคโนโลยีเนื้อสัตว์จากผู้ทรงคุณวุฒิรับเชิญทั้งในและต่างประเทศ

ในการนี้ จึงใคร่ขอเรียนเชิญท่านเป็นวิทยากรในการบรรยาย เรื่อง White Striping: สถานการณ์ในประเทศและผลกระทบต่อคุณภาพของเนื้อไก่ และส่งบทความในการบรรยาย พร้อมแบบตอบรับและแบบฟอร์มประวัติวิทยากรมายังคณะกรรมการผู้จัดงานภายในวันที่ ๓๐ เมษายน ๒๕๖๑ ผ่านทางอีเมล์ meatcongress2018@gmail.com เพื่อถ่ายทอดองค์ความรู้และแนวคิดที่เป็นประโยชน์ต่อผู้เข้าร่วมประชุม และหน่วยงานที่เกี่ยวข้อง

จึงเรียนมาเพื่อโปรดพิจารณาและขอความอนุเคราะห์เป็นวิทยากรในการประชุมครั้งนี้

ขอแสดงความนับถือ

(รองศาสตราจารย์ ดร. จุฑารัตน์ เศรษฐกุล)
ประธานคณะกรรมการดำเนินงานจัดการประชุมวิชาการฯ

2. One manuscript has been submitted to Asian-Australasian Journal of Animal Science (AJAS) for consideration for publication.

*Note:

1) AJAS is a peer reviewed journal which is indexed in SciSearch (SCI Exp.), Biosciences Information Service of Biological Abstracts (BIOSIS), Chemical Abstracts, CABI, AGRIS, Bibliography of Agriculture (AGRICOLA) and clustered in 2nd Quartiles (Q2) in Food Science and Animal Science and Zoology.

2) Due to the contracted with TRF, another manuscript tentatively entitled "Transcriptional profiling revealed metabolic disturbance in broiler skeletal muscle associated with white striping severity" has been under preparation and will be submitted to PLoS One for consideration for publication.

AJAS Asian-Australasian Journal of Animal Sciences

ONLINE MANUSCRIPT SUBMISSION

**Completion of manuscript submission to AJAS**

| | |
|---|---|
| **Manuscript ID :** | AJAS-18-0355 |
| **Date Submitted :** | May 04, 2018 |
| **Title :** | Monitoring of white striping and wooden breast cases and impacts on quality of breast meat collected from commercial broilers (Gallus gallus) |
| **Corresponding Author :** | Yuwares Malila |

Dear Dr. Yuwares Malila:

Thank you for submitting your manuscript to *Asian-Australasian Journal of Animal Sciences*.

Your manuscript titled "Monitoring of white striping and wooden breast cases and impacts on quality of breast meat collected from commercial broilers (Gallus gallus)" has been received by the Editorial Office of *Asian-Australasian Journal of Animal Sciences*. Your manuscript I.D. is AJAS-18-0355. Please refer to this number in future communications.

Your manuscript will be evaluated by reviewers and editors. The Editorial Office will inform you of the review outcome as soon as possible.
Please understand that at least 4 weeks are required for the first evaluation of most manuscripts submitted to *Asian-Australasian Journal of Animal Sciences*.
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Sincerely,

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# รายงานสรุปการนำผลงานวิจัยไปใช้ประโยชน์

สัญญาเลขที่ TRG5980007 ชื่อโครงการ โพรไฟล์ทรานสคริปโตมที่เกี่ยวข้องกับการเกิดตำหนิแบบ white striping ในไก่เนื้อส่วนอก

หัวหน้าโครงการ ยุวเรศ มลิลา หน่วยงาน หน่วยวิจัยเทคโนโลยีชีวภาพอาหาร ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ

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สถานะผลงาน ☑ ปกปิด ☐ ไม่ปกปิด

**ความสำคัญ/ความเป็นมา**

จากการทบทวนวรรณกรรม การเกิดตำหนิแบบ white striping ในไก่เนื้อส่งผลกระทบต่อคุณค่าทางโภชนาการ คุณภาพทางเทคโนโลยีของเนื้อไก่ส่วนอก

**วัตถุประสงค์โครงการ**

1) ศึกษาอุบัติการณ์ของตำหนิแบบ WS ในไก่ที่เก็บจากโรงเชือดอุตสาหกรรม รวมทั้งวิเคราะห์ผลของการเกิดตำหนิต่อคุณภาพของเนื้ออกไก่

2) เปรียบเทียบการแสดงออกของยีนในกล้ามเนื้อไก่ *pectoralis major* ในกลุ่มตัวอย่างที่มีตำหนิ WS ระดับต่างๆ

**ผลการวิจัย**

ผลการศึกษาตัวอย่างไก่เนื้อตัวผู้สายพันธุ์ Ross 308 (n=184) ที่มีอายุระหว่าง 39 วัน ถึง 49 วัน พบว่า มีเนื้อไก่ 4 ชิ้น (คิดเป็น 2.2%) ที่ไม่พบตำหนิดังกล่าว แต่พบว่ามีตัวอย่างเนื้อไก่จำนวน 102 ชิ้น (55%) 71 ชิ้น (39%) และ 7 ชิ้น (3.8%) จัดเป็น WS ระดับเล็กน้อย ปานกลาง และรุนแรงมาก ตามลำดับ ตัวอย่างเนื้อไก่ที่เป็น WS ระดับรุนแรงมากประกอบด้วยไขมันมากขึ้น แต่มีโปรตีนและเถ้าลดลง ($p<0.05$) ตัวอย่างที่มีตำหนิระดับรุนแรงมากเมื่อทำให้สุกมีค่า hardness springiness และ chewiness สูง แต่มีค่าแรงและพลังงานที่ใช้เฉือนผ่านตัวอย่างต่ำกว่าตัวอย่างที่ไม่มีตำหนิอย่างมีนัยสำคัญ จากการวิเคราะห์ ordinal logistic regression model (OLR) พบว่าหากเพิ่มอายุของไก่ 1 วัน หรือเพิ่มสัดส่วนเนื้ออกขึ้น 1 % จะทำให้มีโอกาสเกิด WS ระดับรุนแรงขึ้น 11.8% และ 51.5% ตามลำดับ จาก pathway analysis พบ metabolic pathway necroptosis และ vascular smooth muscle contraction เป็นกลไกทางชีวภาพสามอันดับแรก ที่เกี่ยวข้องกับการเกิดตำหนิแบบ WS ที่รุนแรงต่างกัน การแสดงออกของยีน lactate dehydrogenase A และ phosphorylase kinase regulatory subunit beta ลดลงอย่างมีนัยสำคัญในตัวอย่างที่มี WS ระดับรุนแรงมาก สัมพันธ์กับปริมาณไกลโคเจนและกรดแลคติกที่ลดลง รวมถึงค่า pH ที่สูงขึ้นในเนื้อสัตว์

**คำสืบค้น**

ตำหนิแบบแถบลายสีขาว, ไก่เนื้อทางการค้า, เนื้ออกไก่, การถดถอยโลจิสติค (ordinal logistic regression) ไมโครอะเรย์ โพรไฟล์ทรานสคริปโตม

**การนำผลงานวิจัยไปใช้ประโยชน์**

☐ ด้านนโยบาย โดยใคร.....

นำไปใช้อย่างไร.....

☐ ด้านสาธารณะ โดยใคร.....

นำไปใช้อย่างไร.....

☐ ด้านชุมชนและพื้นที่ โดยใคร.....

นำไปใช้อย่างไร.....

☐ ด้านการพาณิชย์ โดยใคร.....

นำไปใช้อย่างไร.....

☑ ด้านวิชาการ โดยใคร จัดโดยสถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง (สจล.).....

นำไปใช้อย่างไร เอกสารตีพิมพ์เผยแพร่ใน proceedings งานประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีเนื้อสัตว์ ครั้งที่ 6 และ นำเสนอผลงานในรูปแบบ oral presentation ในงานประชุมวิชาการฯ ระหว่างวันที่ 18 – 19 มิถุนายน 2561 โดยได้รับเชิญให้เป็นผู้บรรยายในหัวข้อ “White striping defect: สถานการณ์ที่พบในประเทศและผลกระทบต่อคุณภาพของเนื้อไก่”.....

☐ ยังไม่มีการนำไปใช้

**การเผยแพร่/ประชาสัมพันธ์**

1. สิ่งพิมพ์ หรือสื่อทั่วไป

.....

2. สิ่งพิมพ์ทางวิชาการ

1) ญาณี ศรีมารุต และคณะ “White striping defect: สถานการณ์ที่พบในประเทศและผลกระทบต่อคุณภาพของเนื้อไก่” เอกสารตีพิมพ์เผยแพร่ใน proceedings งานประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีเนื้อสัตว์ ครั้งที่ 6 ระหว่างวันที่ 18 – 19 มิถุนายน 2561 โรงแรมรามาการ์เด้น กรุงเทพมหานคร.....

2). Malila Y et al. Monitoring of white striping and wooden breast cases and impacts on quality of breast meat collected from commercial broilers (*Gallus gallus*). Asian-Australasian Journal of Animal Science (submitted).....