# Research Note: Comparison of histochemical characteristics, chicken meat quality, and heat shock protein expressions between PSE-like condition and white-stripping features of *pectoralis major* muscle

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**ABSTRACT** The present study compared the histochemical and meat quality characteristics of broiler *pectoralis major* (**PM**) muscle among the groups categorized according to muscle abnormalities, including pale, soft, and exudative (**PSE**)-like condition and white-striping (**WS**) feature. Additionally, this study investigated the associations between muscular abnormalities and expression levels of heat shock proteins (**HSPs**), including  $\alpha\beta$ -crystallin, HSP70, and HSP90, at the early postmortem period. The WS breasts with normal quality condition showed greater PM muscle weight and were more associated with fiber hypertrophy, compared to the no WS breasts with PSE-like condition (P < 0.05). The PSE-like group exhibited paler surface color and tougher meat, causing more fluid loss after cooking, compared to the normal quality group (P < 0.05). However, there were no significant differences in the quality traits between the WS groups (P > 0.05), except for lightness and cooking loss. Higher  $\alpha\beta$ -crystallin and HSP90 expression levels were observed in PSE-like breast compared to normal quality breast (P < 0.05), whereas WS pattern was not related with HSPs levels (P > 0.05). Therefore, HSP levels at the early postmortem period, especially those of  $\alpha\beta$ -crystallin and HSP90, were associated with the breast quality characteristics of PSE-like condition broilers.

Key words: heat shock proteins, PSE-like condition, white-striping feature, muscle fiber, chicken meat quality

#### INTRODUCTION

Modern broilers have been showing a rapid growth rate due to the effort of the poultry industry to maximize muscle development and growth (Livingston et al., 2019). However, increased growth rate and muscle mass were associated with modifications in skeletal muscle functionalities, especially morphological and biochemical traits (Livingston et al., 2019). Moreover, fast-growing broilers selected for higher body weight and breast yield showed more susceptibility to stressors before and after slaughter, including temperature, humidity, stunning methods, and chilling conditions (Petracci et al., 2015). These physical and chemical changes can lead to the development of muscular abnormalities (Kuttappan et al., 2012; Huang and Ahn, 2018). The pectoralis *major* (**PM**) muscles are especially prone to occur metabolic-associated defects and myopathies, and the recent major concerns regarding muscular abnormalities and 2021 Poultry Science 100:101260 https://doi.org/10.1016/j.psj.2021.101260

myopathies are pale, soft, and exudative (**PSE**)-like condition and white-striping (**WS**) feature (Petracci et al., 2013). Incidence of both abnormalities can negatively affect fresh meat quality and sensory characteristics, thus affecting the functional properties, especially water-binding capacity, of processed products (Kuttappan et al., 2012).

Under stress conditions as well as normal and apoptotic conditions, heat shock proteins (**HSPs**), as molecular chaperones, are expressed to protect and repair cells and tissues (Lomiwes et al., 2014). Generally, HSPs are classified into three main families based on their functions and molecular weights (small HSPs, HSP70, and HSP90), and are differentially expressed according to animal species, breed, age, and muscle location (Lomiwes et al., 2014; Picard et al., 2014). These molecular chaperones play important roles in normal folding of various polypeptides, assisting misfolded polypeptides to attain or regain their native states, and regulating protein degradation (Oh et al., 2019). HSP expression levels at the pre- and/or postmortem periods can affect the fresh meat and sensory quality characteristics in beef, pork, and chicken (Lomiwes et al., 2014). Oh et al. (2019) reported that beef steaks with a lower expression level of  $\alpha\beta$ -crystallin were more tender and left less

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Received August 31, 2020.

Accepted May 10, 2021.

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perceptible residue in the mouth compared to steaks with a higher expression level of  $\alpha\beta$ -crystallin, as this protein inhibits proteolytic degradation due to its chaperoning properties (Contreras-Castillo et al., 2016). In chicken breast muscles, similar to porcine muscles, HSP90 and HSP70 expression levels are greatly increased after stress, and these proteins maintain the structural network of muscle fibers (Picard et al., 2014). Hao and Gu(2014) reported that these expression levels were negatively correlated with muscle pH at 30 min and 24 h postmortem, respectively. However, there is a lack of information on the association between HSPs levels and muscular abnormalities that shows diminished meat quality in broilers. The objective of the present study was to compare the muscle fiber and meat quality characteristics of broiler PM muscle among the groups categorized by muscle abnormalities, including the PSElike condition and WS feature. Additionally, this study investigated the associations between the expression levels of HSPs, including  $\alpha\beta$ -crystallin, HSP70, and HSP90, and muscular abnormalities.

# MATERIALS AND METHODS

# Muscle Sample and Treatments

Commercial broilers (Ross 308; mean live weight of  $1792 \pm 301$  g) were randomly selected and obtained at the local slaughterhouse (Gyeongsangbuk-do, South Korea) in three batches (30 to 31 chickens per batch) during Autumn season following the standard slaughter procedures of the Korea Institute for Animal Products Quality Evaluation (KAPE, 2020). The body weight of selected broiler was obtained before slaughter at the slaughterhouse. At 15 min postmortem, the left and right PM muscles of 91 broiler carcasses were removed and weighed in a 4°C cold room. The pH at the early postmortem period (pH<sub>15 min</sub>) was immediately measured on the right side of the PM muscle. The cross-sectional area (CSA) of PM muscle was measured in the area cut from the lower right to the upper left at the 1/2point of the right PM muscle (Scheuermann et al., 2004). At the same time, the right breast muscle was cut into  $0.5 \times 0.5 \times 1.0$  cm<sup>3</sup> pieces to evaluate muscle fiber characteristics, and the muscle samples (approximately 20 g per sample) were taken and subjected to quantitative real-time polymerase chain reaction (RT-PCR) and Western blot analysis. These muscle pieces were immediately frozen using liquid nitrogen and stored at  $-80^{\circ}$ C. The remaining entire left PM muscle was immediately cooled using ice-cold water, and stored at 4°C until further analysis.

After 24 h postmortem, the whole left PM muscle was visually screened and classified according to the degree of WS on the muscle surface (Kuttappan et al., 2013). The no WS group had no striping, and the WS group had moderate or severe striping. These muscles were immediately assessed in terms of meat quality characteristics, including ultimate pH (pH<sub>24 h</sub>), meat color, water holding capacity (**WHC**), and Warner-Bratzler shear

force (**WBS**). The quality classes were determined by the lightness ( $L^*$ ) value among the measured meat color according to modifying the previous study (Qiao et al., 2002; Carvalho et al., 2014). The normal quality group had a  $L^*$  value range of 48 to 53, and the PSE-like quality group had a  $L^*$  value range of more than 53 (Carvalho et al., 2014). Thus, a total of four quality and WS groups were used in this study as follows: normal quality breast without WS group (NN, n = 57, 19 per replication), normal quality breast with WS group (NW, n = 3, 1 per replication), PSE-like quality breast without WS group (PN, n = 19, 6 to 7 per replication), and PSE-like quality breast with WS group (PW, n = 12, 4 per replication).

# Histochemical Analysis

Serial transverse muscle sections (10  $\mu$ m thickness) were obtained from each right PM muscle sample of 91 broilers using a cryostat (CM1510S, Leica, Nussloch, Germany) at -25°C. Hematoxylin and eosin staining method was used to stain the muscle fiber cross-section (Cardiff et al., 2014). These stained muscle sections were assessed in terms of muscle fiber characteristics, including average CSA and total fiber number. All stained sections from each sample were examined using the image analysis (Image-Pro Plus software, Media Cybernetics, Silver Spring, MD). At least 500 fibers per sample were evaluated, and the average of fiber CSA was determined by dividing the total fiber number was determined by multiplying muscle fiber density by the CSA of PM muscle.

## Meat Quality Characteristics

The  $pH_{15 \text{ min}}$  and  $pH_{24 \text{ h}}$  of PM muscle samples were determined using a portable pH meter with a penetration probe (Testo 206-pH2, Test Inc., Lenzkirch, Germany). Meat surface color, including lightness  $(L^*)$ , redness  $(a^*)$ , and yellowness  $(b^*)$ , was assessed in cold room (4°C) using a Minolta chromameter (CR-400, Minolta Camera Co., Osaka, Japan) with 8 mm port/viewing area according to the recommendations of the Commission Internationale de l'Eclairage (1978). Saturation index  $[(a^{*2} + b^{*2})^{0.5}]$  was also calculated. Percentage of drip loss was measured according to previously described procedure (Honikel, 1998). To assess cooking loss, each muscle sample weighed was put into a polyethylene bag, and then placed in a temperature-controlled water bath (80°C) until the core temperature reached 71°C (Honikel, 1998). Each sample was then cooled in an ice-slurry until equilibration, and then weighed again, and cooking loss was calculated as a percentage of weight loss. After measuring cooking loss, the cooked samples (at least six cores; 1.27 cm diameter) were cut parallel to the fiber orientation, and then cuts were used for WBS analysis. WBS measurement was performed using an Instron Universal Testing machine (crosshead

speed, 200 mm/min; Model 1011, Instron Corp., Canton, MA).

# **Quantitative RT-PCR**

Total RNA was isolated according to the instructions of the manufacturer. A quantity of total RNA was measured using an ABI 7300 real-time PCR instrument (Applied Biosystems, Foster City, CA), and RNA quality was assessed through gel electrophoresis and normalized accordingly. Complementary DNA (cDNA) was synthesized using 1 ng of total RNA. Relative mRNA expression levels of heat shock protein beta (HSPB) 5  $(\alpha\beta$ -crystallin) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the PM muscles among the four groups were assessed through quantitative RT-PCR. The sequences of HSPB5 and GAPDH for forward and reverse primers were as follows: 5'-GGC TTC ATC TCC AGG TGC TT-3' and 5'-GTG ACG GGG ATG GTG ATC TC-3'; and 5'-CGT CCT CTC TGG CAA AGT CC-3' and 5'-AAG ATA GTG ATG GCG TGC CC-3', respectively. RT-PCR was carried out using SYBR green dye (A25741, Applied Biosystems) and ABI 7300 real-time PCR instrument (Applied Biosystems). The comparative  $2^{-\Delta\Delta Ct}$  method for relative quantification was used to calculate relative gene expression. GADPH, which a housekeeping gene, was used to normalize the RT-PCR calculation.

#### Western Blot Analysis

Muscle samples were homogenized in radio immunoprecipitation assay (RIPA) buffer, and whole muscle proteins were extracted, including myofibrillar and sarcoplasmic

proteins. Whole proteins were separated through SDS-PAGE using a Mini-PROTEAN system (Bio-Rad Laboratory Inc., Richmond, CA). For Western blot analysis, the primary antibodies were HSP70 (1:1,000 dilution; SPA-820, StressGen Biotechnology Corp., Victoria, Canada), HSP90 (1:2,000 dilution; SPA-835, StressGen Biotechnology Corp.), and  $\beta$ -actin (1:1,000 dilution; sc-47778, Santa Cruz Biotechnology Inc., Santa Cruz, CA). The secondary antibody was antimouse immunoglobulin G (IgG) horseradish peroxidase (HRP)-linked antibody (1:3,000 dilution for HSP70 and HSP90; Cell Signaling Technology Inc., Beverly, MA). Proteins were detected using the WesternBright ECL kit (Advansta Inc., Menlo Park, CA), and image was taken using the ImageQuant LAS 500 (GE Healthcare Ltd., Freiburg, Germany). Image analysis for each protein band was performed using a one-dimensional (1D) image analysis software (Eastman Kodak Co., Rochester, NY). The intensities of protein bands were compared among the groups, and they were normalized by the band intensities of  $\beta$ -actin.

#### Statistical Analysis

The general linear mixed model (SAS Institute, Cary, NC) procedure was performed for comparing carcass traits, histochemical characteristics, meat quality characteristics, and HSP expression levels among the groups classified by the meat quality class and extent of WS. Significant differences in the least-squares means (LSM) for investigated parameters among the groups were evaluated through probability difference, with the significance level set at 5%. Data were presented as LSM with standard errors.

 Table 1. Characteristics of carcass, muscle fiber, and meat quality of broiler in groups categorized according to pale, soft, and exudative (PSE)-like condition and white-striping feature.

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Quality class (Q) White-striping (W)	Normal		PSE-like		Level of significance		
$\begin{array}{c c} Carcass traits \\ Body weight (g) & 1606^{c} (21.6)^{1} & 2355^{a} (114.5) & 1998^{b} (37.2) & 2251^{a} (46.8) & * & *** & *** \\ Pectoralis major muscle (g) & 264.4^{b} (4.43) & 299.5^{ab} (23.3) & 271.7^{b} (7.54) & 322.2^{a} (9.49) & NS & * & N \\ Muscle CSA (cm2) & 19.4^{b} (0.35) & 21.6^{ab} (1.81) & 19.6^{b} (0.59) & 22.1^{a} (0.74) & NS & * & N \\ Muscle fiber characteristics \\ Fiber CSA (\mu m2) & 2565^{b} (92.9) & 3436^{a} (341.4) & 2829^{b} (124.7) & 3285^{a} (152.7) & NS & * & N \\ Total fiber number (\times 1,000) & 762.0 (24.4) & 635.4 (89.8) & 714.8 (32.8) & 702.6 (38.3) & NS & NS \\ Meat quality traits \\ pH_{15 \min} & 6.43 (0.03) & 6.28 (0.12) & 6.47 (0.05) & 6.53 (0.09) & NS & NS \\ pH_{24 h} & 6.04^{a} (0.02) & 5.77^{b} (0.11) & 5.65^{b} (0.04) & 5.63^{b} (0.04) & *** & NS \\ pH change value2 & 0.39^{b} (0.04) & 0.47^{b} (0.19) & 0.85^{a} (0.07) & 0.90^{a} (0.08) & *** & NS \\ Lightness (L*) & 49.2^{b} (0.28) & 51.5^{b} (1.22) & 55.4^{a} (0.48) & 56.2^{a} (0.61) & *** & * \\ Redness (a*) & 1.86^{ab} (0.14) & 2.74^{a} (0.60) & 1.62^{ab} (0.24) & 1.29^{b} (0.30) & * & NS \\ Yellowness (b*) & 7.08 (0.20) & 5.86 (0.87) & 5.58 (0.35) & 4.91 (0.43) & NS \\ Saturation index^{3} & 7.47^{a} (0.18) & 8.06a (0.94) & 5.89^{b} (0.31) & 4.87^{c} (0.40) & *** & NS \\ \end{array}$		None $(N = 57)$	WS $(N=3)$	None $(N = 19)$	WS $(N=12)$	Q	W	$Q^*W$
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Carcass traits							
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Body weight (g)	$1606^{\rm c} (21.6)^{\rm 1}$	$2355^{\rm a}$ (114.5)	$1998^{\rm b}(37.2)$	$2251^{\rm a}$ (46.8)	*	***	***
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Pectoralis major muscle (g)	$264.4^{\rm b}(4.43)$	$299.5^{ab}$ (23.3)	$271.7^{\rm b}(7.54)$	$322.2^{a}(9.49)$	NS	*	NS
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Muscle CSA $(cm^2)$	$19.4^{\rm b}(0.35)$	$21.6^{ab}(1.81)$	$19.6^{\rm b}(0.59)$	$22.1^{a}(0.74)$	NS	*	NS
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Muscle fiber characteristics							
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Fiber $CSA (\mu m^2)$	$2565^{\rm b}(92.9)$	$3436^{\rm a}(341.4)$	$2829^{\rm b}$ (124.7)	$3285^{\rm a}$ (152.7)	NS	*	NS
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Total fiber number $(\times 1.000)$	762.0 (24.4)	635.4 (89.8)	714.8 (32.8)	702.6 (38.3)	NS	$\mathbf{NS}$	NS
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Meat quality traits							
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	pH <sub>15 min</sub>	6.43(0.03)	6.28(0.12)	6.47(0.05)	6.53(0.09)	NS	$\mathbf{NS}$	NS
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$pH_{24 h}$	$6.04^{a}(0.02)$	$5.77^{b}(0.11)$	$5.65^{b}(0.04)$	$5.63^{b}(0.04)$	***	$\mathbf{NS}$	NS
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	pH change value <sup>2</sup>	$0.39^{\rm b}(0.04)$	$0.47^{\rm b}(0.19)$	$0.85^{\rm a}(0.07)$	$0.90^{\rm a}$ (0.08)	***	$\mathbf{NS}$	NS
Redness $(a^*)$ $1.86^{ab}(0.14)$ $2.74^{a}(0.60)$ $1.62^{ab}(0.24)$ $1.29^{b}(0.30)$ *         NS         N           Yellowness $(b^*)$ $7.08(0.20)$ $5.86(0.87)$ $5.58(0.35)$ $4.91(0.43)$ NS         NS         NS           Saturation index <sup>3</sup> $7.47^{a}(0.18)$ $8.06a(0.94)$ $5.89^{b}(0.31)$ $4.87^{c}(0.40)$ ***         NS         NS	Lightness (L*)	$49.2^{\rm b}$ (0.28)	$51.5^{\rm b}$ $(1.22)^{\prime}$	$55.4^{\rm a}$ (0.48)	$56.2^{\rm a}$ (0.61)	***	*	NS
Yellowness (b*) $7.08 (0.20)^{\prime}$ $5.86 (0.87)^{\prime}$ $5.58 (0.35)^{\prime}$ $4.91 (0.43)^{\prime}$ NS	Redness $(a^*)$	$1.86^{ab}$ (0.14)	$2.74^{a}(0.60)$	$1.62^{ab}$ (0.24)	$1.29^{\rm b}(0.30)$	*	$\mathbf{NS}$	NS
Saturation index <sup>3</sup> $7.47^{a}(0.18)$ $8.06a(0.94)$ $5.89^{b}(0.31)$ $4.87^{c}(0.40)$ *** NS N	Yellowness (b*)	7.08 (0.20)	5.86(0.87)	5.58(0.35)	4.91 (0.43)	NS	$\mathbf{NS}$	NS
	Saturation index <sup>3</sup>	$7.47^{a}(0.18)$	8.06a(0.94)	$5.89^{b}(0.31)$	$4.87^{\circ}(0.40)$	***	$\mathbf{NS}$	NS
Drip loss (%) $2.29$ ( $\dot{0}.18$ ) $2.36$ ( $\dot{0}.77$ ) $2.47$ ( $\dot{0}.31$ ) $2.61$ ( $\dot{0}.39$ ) NS NS N	Drip loss (%)	2.29(0.18)	2.36(0.77)	2.47(0.31)	2.61(0.39)	NS	$\mathbf{NS}$	NS
Cooking loss (%) $11.5^{\rm b}(0.52)$ $18.6^{\rm a}(2.25)$ $16.3^{\rm a}(0.89)$ $17.3^{\rm a}(1.13)$ NS ** *	Cooking loss (%)	$11.5^{\rm b}(0.52)$	$18.6^{\rm a}$ (2.25)	$16.3^{\rm a}(0.89)$	$17.3^{\rm a}$ (1.13)	NS	**	*
Warner-Bratzler shear force (N) $50.0^{b}(1.83)$ $44.5^{b}(7.98)$ $63.2^{a}(3.17)$ $55.7^{ab}(4.17)$ ** NS N	Warner-Bratzler shear force (N)	$50.0^{\rm b}(1.83)$	$44.5^{\rm b}(7.98)$	$63.2^{\mathrm{a}}(3.17)$	$55.7^{ab}(4.17)$	**	NS	NS

Levels of significance: NS, not significant; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001.

<sup>a-c</sup>Different superscripts in the same row represent significant differences (P < 0.05).

<sup>1</sup>Standard error of least-square means.

 $^{2}$ pH change value: pH<sub>15 min</sub> - pH<sub>24 h</sub>.

<sup>3</sup>Saturation index =  $(a^{*2} + b^{*2})^{0.5}$ .

Abbreviations: CSA, cross-sectional area; PSE, pale, soft, and exudative; WS, white-striping.

# Carcass, Histochemical, and Meat Quality Characteristics

Table 1 shows the comparison of carcass and histochemical characteristics among the groups categorized according to PSE-like condition and WS feature. The broilers showing normal meat quality without WS exhibited a lower body weight compared to the broilers showing PSE-like meat quality without WS (1,606 vs. 1,998 g, P < 0.05), and the highest body weight was observed in the NW and PW groups (2,355 and 2,251 g). The PW group showed greater PM weight (322.2 vs. 264.4 g, P < 0.05) and muscle CSA (22.1 vs. 19.4 cm<sup>2</sup>, P < 0.05) compared to the NN group, and was similar to the NW group (P > 0.05). For muscle fiber characteristics, the NW group with a greater body weight exhibited a greater fiber CSA compared to the PN groups with a lower body weight (3,436 vs. 2,829  $\mu m^2$ , P < 0.05), and there was no difference between the NN and PN groups (P > 0.05). The total muscle fiber did not differ among the groups (P > 0.05).

There was no significant difference in muscle  $pH_{15 \text{ min}}$ between the quality class and WS groups (P > 0.05); however, the NN group showed a higher  $pH_{24 h}$  value compared to the other groups (P < 0.001). The pH changes did not differ between the WS groups within the quality class (P > 0.05); however, the PN group exhibited a higher change value compared to the NN group (0.85 vs. 0.39, P < 0.001). The NW group showed a lower lightness value (51.5 vs. 56.2, P < 0.05) and a higher redness value (2.74 vs. 1.29, P < 0.05) compared to the PW group, but no significant difference was observed in yellowness among the groups (P > 0.05). Saturation index was a lower in the PW group compared to the other groups (P < 0.05). There was similar drip loss among the four groups (P > 0.05), and the NN group exhibited a lowest cooking loss compared to the other groups (P < 0.05). The NN and NW groups had lower WBS values compared to the PN group (50.0 and 44.5 vs. 63.2 N, P < 0.01).

# Expression Levels of HSPs

HSP levels in the PM muscles at the early postmortem period were assessed through quantitative RT-PCR and Western blot analysis, and the results are graphically shown in Figure 1. Expression of the  $\alpha\beta$ -crystallin that was determined through quantitative RT-PCR analysis was higher in the PN group compared to with the NN and NW groups (1.52 vs. 1.00 and 0.82, P < 0.05). On another note, when Western blot experiment was performed, there was no significant difference in the expression level of HSP70 among the groups (P > 0.05). However, a lower level of HSP90 was observed in the NW group compared to the PW group (0.98 vs. 1.85, P < 0.05), whereas the PN and NN groups exhibited a similar level of HSP90 (1.29 vs. 1.00, P > 0.05).

# DISCUSSION

PSE-like condition chicken has continued to be a major concern for the poultry industry since the early 1990s (Petracci et al., 2015), and this defect in poultry meat is the result of accelerated glycolysis during the postmortem period (Choi and Kim, 2009). In modern broilers, as a result of intense genetic selection, the PM muscles are entirely composed of larger-sized type IIB fibers (fast-twitch and glycolytic fiber), and this condition may increase the susceptibility to various stressors (Petracci et al., 2015). Generally, PSE-like chicken breast due to increased glycolytic properties can produce and accumulate great amount of lactate at the early postmortem period, which may result in rapid pH decline with higher muscle temperatures compared to normal breast (Choi and Kim, 2009). This combination causes a higher extent of protein denaturation including myofibrillar and sarcoplasmic proteins, which in turn can decrease the ability of fibers to hold water; thus, PSE-like breasts exhibit higher lightness value and reduced WHC (Choi et al., 2010). These PSE-like conditions were associated with the overabundance of proteins involved in glycolytic pathway and muscle contraction (Desai et al., 2016). In the current study, a greater body weight was observed in the PSE-like group compared to the normal group (2,090 vs. 1,632 g, P <0.001; Supplementary Table S1) with regardless of the presence of WS feature. In meat quality traits, the PSElike quality group had a pH change value that was approximately 2.2 times higher than the normal quality group (0.87 vs. 0.39, P < 0.001; Supplementary Table S1), although no difference was detected in muscle  $pH_{15}$ min between the quality groups (P > 0.05). As expected, the PSE-like breast showed a paler surface color, and also exhibited a higher level of WBS due to a higher cooking loss compared to the normal breast (P < 0.05; Supplementary Table S1).

The incidence of WS myopathy has been recently increasing, although the etiology of WS is still unknown (Kuttappan et al., 2012; Petracci et al., 2019). Generally, WS myopathy is easily recognized by the white striations on the breast muscle surface, which follows the direction of muscle fibers, and occurs primarily in heavier chicken with larger muscle fibers (Kuttappan et al., 2012). Similar result was observed in the present study, wherein broilers with WS exhibited greater body weight and fiber area compared to broilers without WS (P < 0.05) with regardless of PSE-like conditions. Additionally, within the PSE-like condition group, chicken breast with WS had greater PM weight and fiber area than breast without WS (P < 0.05). Therefore, the increase in both body weight and fiber area was more related to WS occurrence compared with PSE-like condition. Degrees of WS on breast muscles appear differently for each individual. Moderate or severe white striation, similar to PSE-like condition, can negatively affect visual acceptance, and consumers generally can distinguish and avoid purchasing these fillets (Kuttappan et al., 2012; Petracci et al., 2019). However,



Figure 1. Heat shock proteins (HSPs) expression levels of the *pectoralis major* muscle of broilers in groups categorized according to pale, soft, and exudative (PSE)-like condition and white-striping feature. (A) Expression levels of  $\alpha\beta$ -crystalline (*HSPB5*) were measured through real-time PCR with *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) as a control for normalization. (B) Relative intensities and Western blot images of the indicated HSP70 and HSP90 proteins were analyzed, and  $\beta$ -actin was used as a housekeeping gene for normalization. Bars indicate standard errors of the means. <sup>a-b</sup> Different letters denote significant differences (P < 0.05). Abbreviations: HSPB5, heat shock protein beta 5; NN, normal quality chicken with white-striping; PN, PSE-like chicken without white-striping; PW, PSE-like chicken with white-striping.

Kuttappan et al. (2012) and Tasoniero et al. (2016) reported that the development of WS has a limited effect on fresh and cooked meat quality traits. A similar result was observed in the current study, wherein unlike PSElike condition, meat quality traits did not differ between the WS and no WS groups (P > 0.05) within the normal quality condition, although a higher pH<sub>24 h</sub> value and a lower cooking loss were observed in the NN group compared to the NW group (P < 0.05). However, a marked difference was observed in the saturation index between the PN and PW groups. Generally, HSP expression levels dynamically increase after the onset of stress (Laskowaska et al., 2019). During the apoptotic cascade after slaughter, HSP expression particularly increases at the early postmortem period, and then rapidly disappears due to protein precipitation within 48 h postmortem (Pulford et al., 2009). Additionally, significantly higher levels of HSPs during the apoptotic process were also observed in stress-susceptible animals, especially fast-growing broilers and pigs, in response to various stressors, and individual stress susceptibility and stressor characteristics were associated with the development of muscular abnormalities, such as PSE-like condition (Xing et al., 2019). In the current study, PSE-like chicken breast showing higher levels of  $\alpha\beta$ -crystallin and HSP90 showed a lower muscle pH<sub>24 h</sub> and higher cooking loss and WBS values compared to normal breast showing lower levels of  $\alpha\beta$ -crystallin and HSP90 (P < 0.05).

On the other hand, muscle fibers exposed to various stress stimuli undergo biochemical and structural changes, and significantly alternations are accompanied with a collapse in cellular homeostasis, leading to myopathy development (Laskowaska et al., 2019). Kuttappan et al. (2017) reported that broiler with severe myopathy changes lead to increased protein synthesis associated with cellular stress and damaged cell repair. Thus, occurrence of stress-induced myopathies could associate with the expression levels of HSPs, as the compensatory mechanisms by HSPs are activated to limit cell damage (Lomiwes et al., 2014; Xing et al., 2019). However, in the present study, expression levels of HSPs did not depend on the presence or absence of WS patterns (P >0.05). This result can be explained by the characteristics of WS myopathy, because the WS formation is usually associated with degeneration, necrosis, and atrophy of muscle fibers in vivo during the lifetime, and not associated with stress conditions before and/or after slaughter (Kuttappan et al., 2013; Livingston et al., 2019).

In conclusion, fiber hypertrophy of chicken PM muscles was more associated with WS formation rather than PSE-like development. Meanwhile, PSE-like breasts, which are often seen in stress-susceptible chicken, have exhibited impaired meat quality and higher expression levels of  $\alpha\beta$ -crystallin and HSP90 at the early postmortem period compared to normal breasts. However, WS features were not related to HSP expression levels in chicken PM muscle at 15 min postmortem. Therefore, the results of the present study suggest that HSP expression levels at the early postmortem period can be useful stress biomarkers and indicators for the explanation of variations in the meat quality characteristics.

#### ACKNOWLEDGMENTS

This research was supported by the National Research Foundation of Korea (NRF-2020R1A2C1010756).

#### DISCLOSURES

The authors did not provide a conflict of interest statement.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j. psj.2021.101260.

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