Research Note: Expression level of heat shock protein 27 in PSE-like and fast-glycolyzing conditions of chicken *pectoralis major* muscle

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ABSTRACT This study compared the meat quality traits, histochemical characteristics, and heat shock protein (**HSP**) 27 expression levels of the broiler *Pectoralis major* (**PM**) muscles in groups classified by pale, soft, and exudative (**PSE**)-like and fast-glycolyzing conditions using the lightness and muscle pH change values. Chicken PM muscles showing higher pH change value and paler meat surface (as **HP** group) could be associated with the PSE-like condition, and exhibited a lower $pH_{24 h}$ value and higher cooking loss compared to PM muscles showing lower pH change value and normal color (as **LN** group) ($P < 0.05$). Greater PM muscle weight and fiber area were observed in the HP group compared to the other groups ($P < 0.05$); meanwhile, the PM muscles showing higher pH change value with normal color (as **HN** group) and the PM muscles showing lower pH change value with paler color (as **LP** group) did not differ in the water holding capacity, Warner-Bratzler shear force, and muscle fiber characteristics ($P > 0.05$). Muscle samples showing a higher pH change value exhibited a greater level of HSP27 compared to muscle samples showing a lower pH change value ($P < 0.05$). Therefore, the current findings suggested that the expression level of HSP27 can be a useful indicator for explaining variations in the glycolytic rate of chicken breast muscle.

Key words: heat shock protein 27, PSE-like condition, glycolytic rate, meat quality, chicken breast

INTRODUCTION

Heat shock proteins (**HSPs**) are well-known molecular chaperones, expressed under various conditions, including normal, stress, and apoptotic processes (Oh et al., 2019). A variety of functionally different HSPs plays important roles that shield and repair diverse cell types from all organisms in various damages from intrinsic and extrinsic factors (Lomiwes et al., 2014). HSPs are broadly classified by their molecular weights, and small HSPs, including $\alpha\beta$-crystallin, HSP20, and HSP27, have the most various molecular weights ranging from 12 to 43 kDa (Lomiwes et al., 2014). Small HSPs can enhance muscle cell survival, as these proteins interfere with cell signaling pathways regulating apoptotic cell death (Oh et al., 2019). Especially, HSP27 is located in the Z-line and I-band of myofilament, regulates intermediate filament interactions with cytoskeletal proteins, and maintains thin filament (Lomiwes et al., 2014). Thus, this protein is associated with the integrity of myofibrillar structures under stressed conditions as well as apoptotic process in postmortem muscles (Lomiwes et al., 2014). During 48 h postmortem, expression level of HSP27 positively correlated with the Warner-Bratzler shear force (**WBS**) of beef steak, as this protein can prevent the degradation of muscle proteins (Kim et al., 2008). Therefore, expression levels of HSP27 at the pre- and/or postmortem periods can influence the sensory and meat quality characteristics (Oh et al., 2019).

Muscular abnormalities caused by various stressors may be associated with alterations of the anaerobic glycolysis during postmortem period, and the rate and extent of glycolysis are dependent on the stress characteristics and susceptibility of the individual animal (Ferguson et al., 2001). Particularly, the *Pectoralis major* (**PM**) muscles of meat-type chickens are composed mostly of larger sized glycolytic fibers (Petracci et al., 2015). This characteristic of PM muscle, as more reveal glycolytic properties, can make prone to metabolic-related abnormalities, such as pale, soft, and exudative (**PSE**)-like condition, in chicken (Petracci et al., 2015). The occurrence of PSE-like chicken is generally associated with fast-acidification at the early postmortem (pH <6 within 1 h postmortem — due to accelerated glycolytic metabolism leading to increased muscle temperature and rapid development of rigor mortis) or greater extent of

acidification during the postmortem period (i.e., "acid meat" − ultimate pH < 5.8 without remarkable alterations in the acidification rate; Desai et al., 2016). Thus, in chicken breast meat, both early postmortem and ultimate pH values are important to classify the quality groups (Desai et al., 2016), as well as the change values of muscle pH can be one of the important indicators to determine the glycolytic traits of an individual muscle.

On the other hand, the expression level of HSP27 as stress-related chaperones may be associated with the extent and rate of glycolysis during the postmortem period (Kim et al., 2007). Thus, the occurrence of deteriorated meat quality could be related to the HSP27 expression level (Picard et al., 2015). However, there are no studies describing whether the HSP27 has an impact on a muscular abnormality, especially PSE-like condition, in broilers. Therefore, the objective of this study was to compare the meat quality traits, muscle fiber characteristics, and HSP27 expression levels of broiler PM muscles in groups categorized according to acidification capacity and lightness value through cluster analysis.

MATERIALS AND METHODS
Muscle Samples and Treatments

A total of 91 broiler carcasses (Ross 308; age of 4 wk; mean live weight of 1,784 ± 303 g) were randomly selected and obtained by the standard slaughtering process at the commercial slaughterhouse. Commercial slaughterhouse provided the body weight (**BW**) of each broiler before slaughter. At 15 min postmortem, both the left and right fillets of the PM muscles were removed and weighed in a cold room (4°C). The percentage of PM weight (**PMW**) was calculated as the ratio of the PMW to BW of each broiler. The $pH_{15 min}$ was immediately measured on the left PM muscle. The cross-sectional area (**CSA**) of the PM muscle was assessed in the area cut from the lower right to the upper left at the 1/2 point of the left PM muscle (Scheuermann et al., 2004). At the same time, the left breast muscles were cut into $0.5 \times 0.5 \times 1.0$ cm^3 pieces for histochemical analysis, and approximately 20 g of each muscle sample was taken for quantitative real-time polymerase chain reaction (**RT-PCR**) analysis. The remaining right breast muscles were immediately cooled using ice-cold water, and stored at 4°C until further analysis. After 24 h postmortem, the meat quality characteristics, including $pH_{24 h}$, lightness, drip loss, cooking loss, and WBS, were assessed using the right PM muscles.

Meat Quality Characteristics

The muscle $pH_{15 min}$ and $pH_{24 h}$ were determined using a portable pH instrument with a penetration probe (Testo 206-pH2, Test Inc., Lenzkirch, Germany). Minolta chromameter (CR-400, Minolta Camera Co., Osaka, Japan) was used to assess the surface color of the PM muscle upper part. The lightness (L^*), redness (a^*), and yellowness (b^*) were expressed as the recommendations of Commission Internationale de l'Eclairage (1978), and the average of triplicate measurement was used. For water-holding capacity (**WHC**), drip loss and cooking loss were measured. Drip loss percentage was calculated as the difference in sample weight before and after storage at 4°C for 48 h. To determine cooking loss, chicken breast samples were weighed and then put into a polyethylene bag and cooked in a temperature-controlled water bath (80°C) until the core internal temperature reached 71°C. Cooking loss was calculated as a percentage of weight loss after cooking. After cooking loss, samples were cut into above 6 cylindrical shapes (1.27 cm diameter, parallel to muscle fiber direction), and used for the WBS analysis. WBS values of samples were obtained by cutting the perpendicular to the muscle fiber direction with an Instron Universal Testing Machine (Model 1011, Instron Corp., Canton, MA) with a crosshead speed of 200 mm/min.

Histochemical Analysis

A cryostat (CM1510S, Leica, Nussloch, Germany) at −25°C was used to obtain a cross-section (10 μm thickness) of the muscle sample. The muscle samples were stained with a hematoxylin and eosin staining method. All stained sections were photographed at 100 × magnification using an optical microscope (DM500, Leica Microsystem, Heerbrugg, Switzerland) equipped with a charge-coupled device camera (ICC50, Leica Microsystems) linked to Leica software (LAS EZ) for a standard workstation computer. At least 500 fibers at histochemical images were analyzed for mean CSA and number of fibers using the image analysis (Image Pro Plus software, Media Cybernetics, Silver, Spring, MD). The mean fiber CSA was determined as the total area ratio measured to the total fiber number counted. Total fiber number was calculated by multiplying fiber density by the CSA of left breast muscle.

Quantitative RT-PCR

Total RNA was isolated using left PM muscle at 15 min postmortem following the manufacture instructions and the quality was assessed by electrophoresis and normalized accordingly. Then the complementary DNA (**cDNA**) was synthesized using 1 ng of total RNA. The synthesized cDNA was then used for quantitative RT-PCR to measure expressions of *heat shock protein beta* (**HSPB**) *1* (HSP27; forward 5′-CTG AGC AGC GGC ATC TCC-3′ and reverse 5′-AAG CAC CTG GAG ATG AAG CC-3′) and *glyceraldehyde-3-phosphate dehydrogenase* (**GAPDH**; forward 5′-CGT CCT CTC TGG CAA AGT CC-3′ and reverse 5′-AAG ATA GTG ATG GCG TGC CC-3′). RT-PCR was carried out using SYBR green dye (A25741, Applied Biosystems, Foster City, CA) and ABI 7300 real-time PCR instrument (Applied Biosystems). The relative gene expression was calculated according to the comparative

$2^{-\Delta\Delta Ct}$ method, and the housekeeping gene (*GAPDH*) was used in order to normalize the RT-PCR calculation.

Statistical Analysis

The pH change value was clustered (FASTCLUS procedure in SAS software 9.4) into 2 discrete classes corresponding to the high (range from 0.01 to 0.51, average of 0.31) and normal (range from 0.57 to 1.31, average of 0.85) groups. Additionally, cluster analysis was conducted to 2 clusters (the normal and pale groups) based on lightness value of muscle surface. The normal group had a range of 44.57 to 51.67 and an average of 48.9, and the pale group had a range of 51.96 to 59.93 and an average of 55.0. The FASTCLUS algorithm minimized the sum of squared distances from cluster means (Jain et al., 1999). To analyze the comparison of carcass traits, meat quality traits, muscle fiber characteristics, and HSP 27 expression level, the general linear model procedure was used to compare any associations. Significant differences in the least-squares means (**LSM**) for investigated parameters were evaluated through probability difference, with the significance level set at $P < 0.05$. All data were presented as LSM with standard errors.

RESULTS AND DISCUSSION

The breast meat quality, carcass, and muscle fiber characteristics among the groups categorized by lightness and pH change values are presented in Table 1. Breast muscles showing higher pH change value with normal color (**HN**) exhibited a higher $pH_{15\ min}$ compared to breast muscles showing lower pH change value and paler color (**LP**) (6.58 vs. 6.33, $P < 0.05$), and was similar to breast muscles showing higher pH change and paler color (**HP**) (6.51, $P > 0.05$). The lowest value of $pH_{24\ h}$ was observed in the HP group compared to the other groups ($P < 0.05$), and the HN and LP groups were the same ($P > 0.05$). The pH change value was not different between the lower pH change and normal color (**LN**) and LP groups (0.29 vs. 0.40, $P > 0.05$) and was higher in the HP group compared to the HN group (0.93 vs. 0.68, $P < 0.05$). Samples from the HP group showed a significantly higher lightness compared to samples from the LP group (55.5 vs. 53.3, $P < 0.05$), whereas no significant difference was found between the LN and HN groups (48.8 vs. 49.1, $P > 0.05$). There was a similar percentage of drip loss among the 4 groups ($P > 0.05$), and the highest cooking loss showed in the HP group compared to the other groups ($P < 0.05$). WBS value was not significantly different between the LP and HN groups (50.5 vs. 49.2 N, $P > 0.05$), and the lower WBS value was observed in the LN group compared to the HP group (47.0 vs. 56.8 N, $P < 0.05$).

All livestock will experience various stress before slaughter, and each individual has different stress susceptibility that can affect glycolytic properties during the postmortem periods. In stress susceptible animals, especially modern pigs and poultry, their metabolic traits can be altered, and then these animals may exhibit a stronger acidification capacity compared to stress-resistant animals (Petracci et al., 2015). Choi et al. (2012) reported that fast-glycolyzing porcine muscles exhibited a higher percentage of type IIB fibers and were more susceptible to protein denaturation as an increase in the rate and extent of pH decline at the early postmortem period compared to slow-glycolyzing muscles. Moreover, these fast-glycolyzing muscles also displayed lower WHC and paler surface color compared to slow-glycolyzing muscles (Choi et al., 2012). It is well known that lightness and ultimate pH values are used as

Table 1. Comparison of meat quality, carcass, and muscle fiber characteristics of chicken breast in groups categorized according to lightness and pH change values.

Acidification capacity (A) Meat color (C)	Low		High		Level of significance		
	Normal (N = 32)	Pale (N = 8)	Normal (N = 11)	Pale (N = 25)	A	C	A × C
Meat quality characteristics							
$pH_{15\ min}$	6.38[b] (0.03)[1]	6.33[b] (0.06)	6.58[a] (0.05)	6.51[a] (0.03)	***	NS	NS
$pH_{24\ h}$	6.08[a] (0.03)	5.93[b] (0.05)	5.92[b] (0.05)	5.62[c] (0.03)	***	***	*
pH change value[2]	0.29[c] (0.03)	0.40[c] (0.06)	0.68[b] (0.05)	0.93[a] (0.04)	***	***	NS
Lightness (L^*)	48.8[c] (0.34)	53.3[b] (0.69)	49.1[c] (0.59)	55.5[a] (0.39)	*	***	*
Drip loss (%)	2.40 (0.24)	1.84 (0.50)	2.30 (0.44)	2.54 (0.28)	NS	NS	NS
Cooking loss (%)	11.6[b] (0.69)	10.7[b] (1.37)	11.3[b] (1.17)	16.7[a] (0.78)	**	*	**
WBS (N)	47.0[b] (2.09)	50.5[ab] (4.24)	49.2[ab] (3.62)	56.8[a] (2.45)	NS	*	NS
Carcass traits							
BW (g)	1,631[b] (37.9)	1,612[b] (75.9)	1,647[b] (67.9)	2,090[a] (42.9)	***	***	***
PMW (g)	270[c] (6.60)	257[b] (13.2)	260[b] (11.8)	294[a] (7.46)	NS	NS	*
PMW/BW (%)	16.6[a] (0.32)	16.0[a] (0.64)	15.9[a] (0.57)	14.1[b] (0.36)	**	*	NS
MCSA (cm^2)	19.5 (0.49)	19.3 (0.97)	19.1 (0.92)	20.7 (0.55)	NS	NS	NS
Muscle fiber characteristics							
Muscle fiber area (μm^2)	2,566[b] (118)	2,690[ab] (333)	2,368[b] (192)	2,995[a] (100)	NS	*	NS
Total fiber number (× 1,000)	767 (31.3)	756 (88.7)	792 (51.2)	715 (26.1)	NS	NS	NS

Abbreviations: BW, body weight; MCSA, muscle cross-sectional area; PMW, *Pectoralis major* muscle weight; WBS, Warner-Bratzler shear force.

Level of significance: NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a–c]Different superscripts in the same row represent significant differences ($P < 0.05$).

[1]Standard error of least square means.

[2]pH change value: $pH_{15\ min} - pH_{24\ h}$.

criteria for classifying pale and normal breast muscles (Qiao et al., 2002; Carvalho et al., 2014), and previous studies have reported that the criteria values of pH and lightness at 24 h postmortem in PSE-like chicken meat were under 5.7 and above 53.0, respectively (Li et al., 2015; Desai et al., 2016). As expected, the HP group as classified by pH change value and lightness through cluster analysis showed a greater acidification capacity and a paler surface compared to the other groups. Thus, considering the meat characteristics of the HP group, chicken breast meat from the HP group belongs to the PSE-like condition. Meanwhile, the LP condition chicken has similar quality characteristics of the pale, firm, and non-exudative (**PFN**) condition pork, and the cause of PFN pork is related to the extent of denaturation of some sarcoplasmic proteins (Kazemi et al., 2011). In this study, there were not many chickens classified into the LP chicken (approximately 10.5%); however, this quality chicken may exhibit a lower consumer acceptability due to a paler surface color. Therefore, chicken breast meat from the LP group, like the PFN pork, is a milder form of PSE-like chicken.

Skeletal muscle growth and ultimate mass are mainly determined by the increase in muscle fiber number (hyperplasia) and size (hypertrophy) (Choi et al., 2014). In mammals and birds, heavy-weight animals compared to low-weight animals at the same age are associated with fiber hypertrophy rather than hyperplasia due to fiber number is generally fixed before birth (Choi et al., 2014). Similar results associated with muscle mass and fiber hypertrophy were observed in the current study. The HP group showed the highest BW and PMW compared to the other groups ($P < 0.05$), although a lower percentage of PMW was observed in the HP group compared to the other groups ($P < 0.05$). The HP group exhibited a greater fiber area compared to the LN and HN groups (2,995 vs. 2,566 and 2,368 μm^2, $P < 0.05$). However, the HP and LP groups did not differ in fiber area (2,690 μm^2, $P > 0.05$), and the total fiber number was no significant difference among the groups ($P > 0.05$). On the other hands, muscle mainly composed of larger sized fibers have less space between the fibers compared to muscle mainly composed of normal or smaller sized fibers (Petracci et al., 2017). Reduced space between muscle fibers can limit the space available to capillaries, which can reduce muscle pH by decreasing the amount of lactate removed from the muscle through the blood vessels (Petracci et al., 2017). These findings suggested that severely increased fiber size can lead to muscular abnormalities (Petracci et al., 2017). In the current study, the quality characteristics of the HP group as PSE-like chicken may be attributed to the muscle fiber hypertrophy.

Muscle fibers undergo cell death proceeding after slaughter and then appear biochemical and structural changes that can lead to loss of cell functions (Lomiwes et al., 2014). During cell apoptosis, chaperone proteins are induced to maintain cellular homeostasis resulting in controlled cell suicide (Lomiwes et al., 2014). Particularly, HSP27 is more
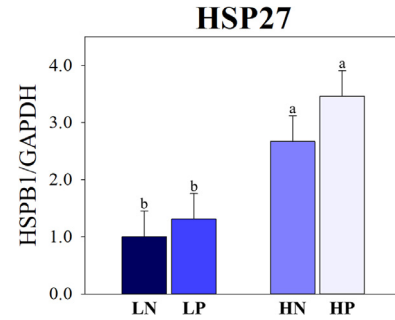
Figure 1. Quantitative real-time PCR for expression of HSP27 (*HSPB1*) of broiler *Pectoralis major* muscle in groups categorized according to lightness and pH change values. *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) was used to control for normalization. [a-b]Different letters denote significant differences ($P < 0.05$). Abbreviations: HN, higher pH change value and normal meat color; HP, higher pH change value and paler meat color; HSP, heat shock protein; *HSPB1, heat shock protein beta 1*; LN, lower pH change value and normal meat color; LP, lower pH change value and paler meat color.

expressed 10 to 20 times in cells after apoptotic and stress conditions compared to cells in normal condition (Liu and Steinacker, 2001). HSP27 has a role in regulating the metabolism-related products, such as the glucose-6-phosphate, and can be modified by methylglyoxal (glycolysis side-product) that induces the formation of large HSP27 oligomers and increases its activity (Garrido et al., 2012). Additionally, in ovine and swine muscles, the expression level of HSP27 increases in fast-twitch glycolytic fibers compared to slow-twitch oxidative fibers (Kim et al., 2007). Liu et al. (2012) reported that Korean native chickens showed greater glycolytic potential and upregulated HSP27 compared to commercial chickens (Ross). In the present study, no difference was observed in HSP27 level between the LN and HN groups representing a similar pH change value (1.00 vs. 1.31, $P > 0.05$; Figure 1). Higher pH change groups, which can be explained as fast-glycolyzing breast muscles, exhibited a higher level of HSP27 compared to lower pH change groups, which can be explained as slow-glycolyzing breast muscles ($P < 0.05$).

Overall, metabolic-related abnormalities, especially PSE-like condition, are associated with the glycolytic rate during the postmortem period. In the current study, a higher expression level of HSP27 at the early postmortem period was observed in chicken breasts exhibiting a higher pH change value compared to chicken breast exhibiting a lower pH change value. Therefore, the current findings suggested that the expression level of HSP27 could represent an available indicator for the explanation of variations in the chicken meat quality characteristics, especially the glycolytic rate.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

REFERENCES

Carvalho, H., A. L. Soares, D. C. B. Honorato, P. D. Guarnieri, M. R. Pedrao, F. G. Paiao, A. Oba, E. I. Ida, and M. Shimokomaki. 2014. The incidence of pale, soft, and exudative (PSE) turkey meat at a Brazilian commercial plant and the functional properties in its meat product. LWT 59:883–888.

Choi, Y. M., K. C. Jung, J. H. Choe, and B. C. Kim. 2012. Effects of muscle cortisol concentration on muscle fiber characteristics, pork quality, and sensory quality of cooked pork. Meat Sci. 91:490–498.

Choi, Y. M., Y. Suh, S. Chin, and K. Lee. 2014. Skeletal muscle characterization of Japanese quail line selectively bred for lower body weight as an avian model of delayed muscle growth with hypoplasia. PLoS One. 9:e95932.

Commission Internationale de l'Eclairage (C.I.E). 1978. Recommendations on uniform color spaces, color-difference equations, psychometric color terms. CIE Publication No. 15 (E-1.3.1) 1971, Supplement 2. Bureau Central del la CIE, Vienna.

Desai, M. A., V. Jackson, W. Zhai, S. P. Suman, M. N. Nair, C. M. Beach, and M. W. Schiling. 2016. Proteome basis of pale, soft, and exudative-like (PSE-like) broiler breast (*Pectoralis major*) meat. Poult. Sci. 95:2696–2706.

Ferguson, D. M., H. L. Bruce, J. M. Thompson, A. F. Egan, D. Perry, and W. R. Shorthose. 2001. Factors affecting beef palatability. Aust. J. Exp. Agric. 41:879–891.

Garrido, C., C. Paul, R. Seigneuric, and H. H. Kampinga. 2012. The small heat shock proteins family: the long forgotten chaperones. Int. J. Biochem. Cell B. 44:1588–1592.

Jain, A. K., M. N. Murty, and P. J. Flynn. 1999. Data clustering: a review. ACM Comput. Surv. 31:264–323.

Kazemi, S., M. O. Ngadi, and C. Gariepy. 2011. Protein denaturation in pork longissimus muscle of different quality groups. Food Bioprocess Technol. 4:102–106.

Kim, N. K., J. H. Lim, M. J. Song, O. H. Kim, B. Y. Park, M. J. Kim, I. H. Hwang, and C. S. Lee. 2007. Developmental proteomic profiling of porcine skeletal muscle during postnatal development. Asian-Aust. J. Anim. Sci. 20:1612–1617.

Kim, N. K., S. Cho, S. H. Lee, H. R. Park, C. S. Lee, Y. M. Cho, Y. H. Choy, D. Yoon, S. K. Im, and E. W. Park. 2008. Proteins in *longissimus* muscle of Korean native cattle and their relationship to meat quality. Meat Sci. 80:1068–1073.

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

Liu, X. D., D. D. Jayasena, Y. Jung, S. Jung, B. S. Kang, K. M. Heo, J. H. Lee, and H. Jo. 2012. Differential proteome analysis of breast and thigh muscles between Korean native chickens and commercial broilers. Asian-Aust. J. Anim. Sci. 25:895–902.

Liu, Y., and J. M. Steinacker. 2001. Changes in skeletal muscle heat shock proteins: pathological significance. Front. Biosci. 6D:12–25.

Lomiwes, D., M. M. Farouk, E. Wiklund, and O. A. Young. 2014. Small heat shock proteins and their role in meat tenderness: a review. Meat Sci. 96:26–40.

Oh, E., B. Lee, and Y. M. Choi. 2019. Associations of heat-shock protein expression with meat quality and sensory quality characteristics in highly marbled *longissimus thoracis* muscle from Hanwoo steers categorized by Warner-Bratzler shear force value. Foods 8:638.

Petracci, M., F. Soglia, and C. Berri. 2017. Muscle metabolism and meat quality abnormalities. Pages 51–75 in Poultry Quality Evaluation. M. Petracci and C. Berri, eds. Woodhead Publishing, Duxford, UK.

Petracci, M., S. Mudalal, F. Soglia, and C. Cavani. 2015. Meat quality in fast-growing broiler chickens. World Poult. Sci. J. 71:363–374.

Picard, B., B. Lebret, I. Cassar-Malek, L. Liaubet, C. Berri, E. Le Bihan-Duval, J. F. Hocquette, and G. Renand. 2015. Recent advances in omic technologies for meat quality management. Meat Sci. 109:18–26.

Qiao, M., D. L. Fletcher, J. K. Northcutt, and D. P. Smith. 2002. The relationship between raw broiler breast meat color and composition. Poult. Sci. 81:422–427.

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