Published July 5, 2018

Meat and Muscle Biology™

Characterizing the Ham Halo Condition: A Color Defect in Fresh Pork *Biceps Femoris* Muscle

David A. King[1]*, Steven. D. Shackelford[1], Tim Schnell[2], Lee Pierce[2], and Tommy. L. Wheeler[1]

[1]USDA-ARS, Roman L. Hruska US Meat Animal Research Center, Meat Safety and Quality Research Unit, Clay Center, NE 68933, USA
[2]Kraft Heinz Company, Glenview, IL 60025, USA
*Corresponding author. Email: andy.king@ars.usda.gov (D. King)

Abstract: This experiment characterized a color defect called halo, involving pale tissue in the superficial portion of ham muscles, causing a two-toned appearance. *Biceps femoris* muscles (n = 200) were obtained from the ham-boning line of a large processor. Instrumental color attributes were determined on the medial (inside) and lateral (halo) surfaces of the muscle. Muscles were sliced (2.54-cm-thick) perpendicular to the long axis of the muscle. Slices were numbered from the proximal end of the muscle so that slice numbers represented anatomical location. Severity of the defect was greatest on the distal end of the muscle corresponding to slices 6 and 7 (9 locations). The halo and inside portions of slices 6 and 7 were separated for myoglobin concentration and muscle pH determination. The inside portion of muscle had lower ($P < 0.001$) L^* (53.1 versus 63.4) and greater ($P < 0.001$) a^* (23.2 versus 15.3) and b^* (18.5 versus 15.4) values than the halo portion. Compared to the halo portion, the inside portion of the muscle had greater ($P < 0.001$) muscle pH (5.7 versus 5.5) and myoglobin concentration (1.97 versus 0.85 mg/g). Four minimally and 5 severely affected muscles were sampled in the halo and inside portions for muscle fiber typing. Fiber type distribution did not differ ($P > 0.05$) between locations within minimally affected muscles. In severely affected muscles, the inside portion had increased ($P < 0.001$) proportion of type I fibers, and concomitant decrease ($P < 0.001$) in type IIB fibers relative to the halo portion. These data indicate that the halo portion of the muscle is much lighter and less red in color, resulting from reduced myoglobin concentration in this portion of the muscle associated with a shift in muscle fiber type. These results should contribute to solutions to mitigate the ham halo color defect.

Keywords: color, ham halo, Intramuscular variation, pork
Meat and Muscle Biology 2(1):205–213 (2018) **doi:10.22175/mmb2018.02.0001**
Submitted 16 Feb. 2018 Accepted 19 May 2018

Introduction

Color is a primary determinant of consumer purchasing decisions regarding meat products. In cured meat products, consistent cured color development is essential to meeting consumer expectations. Most efforts to characterize variation in ham color have focused on differences across muscles (McKeith and Pringle, 2013; Stufft et al., 2017). Though color differences within a single muscle have been documented and associated with differences in muscle fiber type and myoglobin content (Stufft et al., 2017; Kim et al., 2018).

An investigation into consumer feedback regarding inconsistent color of cured ham products identified a defect that was commonly present in raw materials prior to processing into cured ham products. Muscles from the ham had a band of very pale lean color on the superficial portion of the muscles, coupled with normal lean color on the inside por-

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture The authors are grateful to Patty Beska, Kristen Ostdiek, Casey Trambly, and Pat Tammen of the U.S. Meat Animal Research Center for their assistance in the execution of this experiment and to Jody Gallagher of the U.S. Meat Animal Research Center for her secretarial assistance. USDA is an equal opportunity provider and employer

© American Meat Science Association.
This is an open access article distributed under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

tion of the muscle (Fig. 1). Further investigation of raw materials indicated that the condition was present in muscles from a wide variety of suppliers and from production lines differing widely in genetics and management from each supplier (King and Pierce, 2015). Moreover, the defect was found in perimortem ham muscles from pigs immediately following exsanguination in a commercial slaughter facility.

A working group assembled by the National Pork Board comprised of ham processors, packers, and researchers postulated that the defect, which was referred to as the halo condition, was present in the live animal rather than being induced by postmortem processing. Moreover, the working group determined that despite the pale color, the defect was likely not an incidence of the pale, soft, and exudative lean condition. The present experiment was conducted to characterize the muscle properties associated with the condition. It was hypothesized that myoglobin concentration would be the primary driver of the pale color in affected hams.

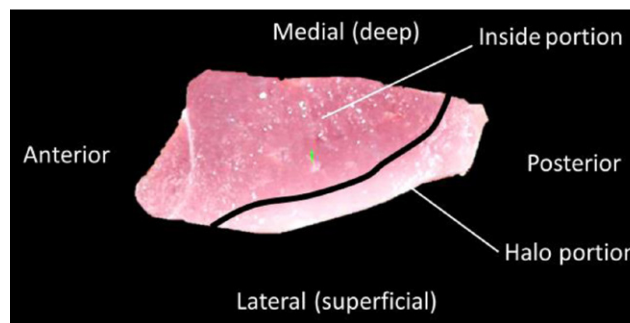

Figure 1. Picture of pork *biceps femoris* cross-section depicting the halo condition. Picture is of slice 6 of a muscle with a severe halo. Black curve depicts approximate separation during dissection between the halo sample and the sample of the unaffected portion.

Materials and Methods

Ham muscles were selected, postmortem, from the boning lines of a USDA inspected processing facility. Thus, animal care and use approval was not obtained for this experiment.

Sample selection and handling

Biceps femoris muscles (similar to IMPS #402E with gluteal superficialis removed (USDA, 2014; n = 200) were selected at approximately 24 h postmortem from the ham boning line of a large commercial processor. To avoid sampling duplicate muscles from the same animal, only muscles from the right carcass side were included in the study. All subcutaneous and seam fat were removed, exposing lean tissue, as part of normal plant procedures. To ensure wide representation of production system and genetics the muscles were sampled from hams produced from pigs of a lean line with aggressive growth promotion strategies (n = 50), pigs from a meat quality line with minimal growth promotion (essentially an all-natural line; n = 50), and pigs from lots of undetermined origin (n = 100). The study was not designed to study production systems. Rather, pigs from differing production systems were sampled to ensure that factors that might influence the incidence or severity of the halo condition were represented in the sample.

As hams were separated into 3-piece hams, the bottom ham was obtained and the semitendinosus was removed from the *biceps femoris*. The *biceps femoris* muscles were individually bagged, without vacuum, and placed in a single layer in boxes. Boxes then were placed inside insulated containers with ice-packs so that the muscles were not subjected to the forces of being stacked within the insulated containers. The muscles were transported to the U.S. Meat Animal Research Center and held overnight (1°C) before further processing.

Muscle processing and color measurement

Muscles were removed from the bags and allowed to bloom at least 20 min before color measurements were collected on the outside (lateral) and inside (medial) surfaces of the muscle. Instrumental color readings were taken with a Hunter Miniscan XE Plus Colorimeter (Hunter-Lab, Reston, VA) with a 25-mm port which was set to collect spectral data with Illuminant A and a 10° observer. The CIE $L*$ (lightness), $a*$ (redness), and $b*$ (yellowness) color-space values were reported as the average of duplicate readings taken on each muscle surface and were used to calculate hue angle, chroma, and overall color change as prescribed by the American Meat Science Association (2012). Care was taken to avoid heavy connective tissue on the both surfaces during color measurement. Greater $L*$, $a*$, and $b*$ values signify increased lightness, redness, and yellowness, respectively. Color intensity (also referred to as chroma or saturation index) was calculated using the following formula: $[(a*^2 + b*^2)0.5]$. Hue angle (redness) was calculated using the formula: [(arctangent $(b*/a*) \times 180/3.142$]. Overall color difference between the surfaces (referred to as ΔE) was calculated using the following formula: $[(\Delta L*^2 + \Delta a*^2 + \Delta b*^2)0.5]$, where $\Delta L*$, $\Delta a*$, and $\Delta b*$ was the difference between the inside and outside values for $L*$, $a*$, and $b*$, respectively.

Muscles were then cut into 2.54-cm-thick slices perpendicular to the long axis of the muscle (Fig. 2). Slices were numbered from the proximal to the distal end so that slice number 5 was consistently the largest portion of muscle. Thus, 47% of the hams in the present study did not have either a slice number 1 or a slice number 9. Slices were laid out in the order from proximal to distal and photographed so that color variation within each slice was visible. Then, at least 20 min following slicing, colorimeter readings were taken on slices 3, 5, and 7 of each muscle using previously described equipment and settings. Three measurements were taken on each slice from the most anterior, central, and posterior portion of the slice. Location identity was maintained on each reading.

Slices were individually identified, vacuum packaged and stored at -20°C until further analysis. The photographs of the slices of each muscle were used to select locations for myoglobin concentration and pH measurement. Moreover, photographs were utilized to identify samples for muscle fiber typing. Examination of the photographs of the slices confirmed observations that the color difference was greatest in the distal portion of the muscle. Slices 6 and 7 were selected for myoglobin concentration and pH measurement. Each slice was partially thawed enough to facilitate dissection, but not enough to allow the muscle to purge. The halo (lateral) portion of the muscle was removed from slices 6 and 7 and then pooled (Fig. 1). Tissue from the remaining (inside) portion of slices 6 and 7 was pooled as well. Dissection was conducted so that a small amount of intermediate tissue between the halo and inside portions of the muscle was discarded. After dissection, the 2 tissue pools from each muscle were minced, frozen in liquid nitrogen and pulverized to a fine powder, which was used for determination of myoglobin concentration and muscle pH.

Muscle pH was determine as prescribed by Bendall (1973). Duplicate 2.5-g samples were homogenized in 10 volumes of a 5 mM iodoacetate, 150 mM KCl solution (pH = 7.0). Homogenates were allowed to rest for a minimum of 1 h at room temperature, mixed via vortexing, and pH was measured using a Reed SD-230 handheld pH meter with a pH probe (Omega PHE 2385 pH probe, Omega Engineering INC., Stamford, CT).

Myoglobin was extracted and quantified following the method and equations described by the American Meat Science Association (2012). Briefly, duplicate 2.5-g samples were homogenized in 10 volumes of 40 mM potassium phosphate buffer (pH = 6.8). Homogenates were held, on ice, for 1 h to allow complete pigment extraction before centrifugation (15,000 × *g*) for 30 min at 4°C. Supernatant was then syringe filtered (Nalgene

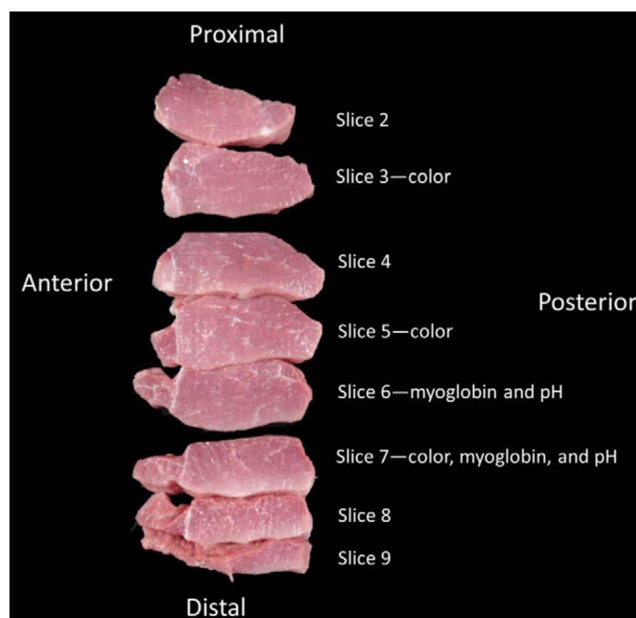

Figure 2. Picture of *biceps femoris* muscle cut into slices perpendicular to long axis of the muscle. The picture depicts orientation descriptors as they were applied in the present experiment and describes the measurements made on each slice.

0.45 μm, surfactant-free cellulose acetate membrane; Thermo Fisher Scientific, Rochester, NY) into 1.5 mL micro-centrifuge tubes. A 200 μL aliquot of the sample was transferred in triplicate to a 96 well plate and blanked against a standard solution of sodium acetate. Absorbance spectra at 525 nm and 700 nm were collected using a Spectramax plus 96-well plate reader (Molecular Devices, Sunnydale CA). Extracted myoglobin pigment concentration (mg/g meat) was calculated taking the difference between the absorbance at 525 nm and 700 nm, a millimolar extinction coefficient of 7.6 m*M*-1 cm-1, the molecular weight of myoglobin (17,000 Da), and the appropriate dilution factor.

Using the photographs of the slices, 5 muscles were selected to have the greatest portion of the muscle affected by the halo condition (severe halo). An additional 5 muscles were selected to have minimal halo. However, one of these samples was removed from fiber typing comparisons because of the degree of the condition present in the sample after thawing was greater than expected. Inside and outside portions of slice 8 of the selected muscles were transverse sectioned 10-mm thick. Sections were stained for NADH and myofibrillar ATPase activities, a simultaneous combination staining procedure, with an acid preincubation solution as described by Solomon and Dunn (1988). A minimum of 200 fibers per animal were classified as slow-twitch red oxidative Type I fibers (dark purple), fast-twitch intermediate oxidative Type

IIA fibers (purple), or fast-twitch white glycolytic Type IIB fibers (light purple), according to the classification of Ashmore and Doerr (1971).

Statistical analysis

Data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). Models comparing the inside and halo portions of the *biceps femoris* muscles tested the effect of location within the muscle and muscle was included as a random effect. Comparisons of color traits across slice location (3, 5, and 7) and location within slice (anterior, center, and posterior) were accomplished with a model including fixed effects included slice, location within slice, and their interaction and muscle as a random effect. Fiber type data were analyzed as a split-plot using a model with fixed effects of condition severity, location within muscle, and their interaction. Muscle within condition severity was included as a random effect. Least-squares means were generated for significant interactions and main effects not involved in higher order interactions. When appropriate, means were separated using the DIFF and LINES options. A predetermined probability of Type I error (α) of 0.05 was used for all determinations of statistical significance.

The PROC CORR procedure of SAS was used to generate Pearson correlation coefficients among color traits, myoglobin concentration, and muscle pH from the inside and outside portions of the muscle. These relationships were further investigated using principal component analysis using the PROC PRINCOMP procedure of SAS. The PROC STEPWISE PROCEDURE of SAS also was used to compare the relative contribution of myoglobin and muscle pH to muscle color in the inside and halo portions of the muscle with an entry criteria of a *P*-value of 0.15.

Results and Discussion

Examination of the slices of the *biceps femoris* confirmed our previous preliminary observations that the pale tissue condition, which has been referred to as the “halo” condition, was most pronounced in the distal portion of the muscle. In the present experiment, this corresponded to slice 6 and 7 of 9 total slice locations. Least-squares means for color traits, muscle pH, and myoglobin concentration for the inside (medial) and halo (lateral) portions of pork *biceps femoris* muscles are presented in Table 1. All color traits evaluated in the present experiment differed ($P < 0.001$) between the inside and halo muscle locations. The halo portion of the *biceps femoris* muscle was much lighter (more than 10 units greater for L^*; $P < 0.001$) and less red (more than 8 units lower for a^* values; $P < 0.001$ and 6 units higher for hue angle; $P < 0.001$). Color intensity (chroma) also was much higher ($P < 0.001$) in the inside portion. The magnitude of differences in color scores between the inside and halo portion of the muscle were quite large and were comparable to differences reported by (Norman et al., 2003) between pork loin chops with NPPC (1999) color scores of 1 and 2 versus pork loin chops with color scores of 5 and 6.

Differences in color between the inside and halo portion of the muscles corresponded with a large difference in myoglobin concentration ($P < 0.001$). Myoglobin concentration of the inside portion of the muscle was more than two-fold greater than the myoglobin concentration of the halo portion. Muscle pH also was lower ($P < 0.001$) in the halo portion of the muscle than in the inside portion of muscle. However, the pH values observed in the present experiment for both muscle regions were well within values that would be considered normal. Moreover, none of the muscle portions evaluated in the present experiment exhibited the pale, soft, and exudative condition.

Pearson correlation coefficients among muscle color traits, muscle pH, and myoglobin concentration in the inside and halo portions of the muscle are presented in Table 2. Generally, color traits within a given location of the muscle were correlated to a higher degree than across locations within the muscle. This may be due, in part, to the traits within locations being measured simultaneously with the same instrument reading. It is notable that across muscle locations, all

Table 1. Least-squares means for color traits, pH, and myoglobin concentration for the inside (medial) and halo (lateral) portions of pork biceps femoris muscles

Location	L^*	a^*	b^*	Hue angle	Chroma	pH	Myoglobin, mg/g
Inside	53.09	23.2	18.46	35.43	29.66	5.70	1.97
Halo	63.42	15.34	15.42	41.75	21.77	5.53	0.85
SEM	0.2	0.13	0.1	0.13	0.16	0.01	0.02
$P > F$	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 2. Pearson correlation coefficients between color traits, myoglobin content, and pH values of the inside (medial) and halo (lateral) portions of pork biceps femoris muscles

Trait	Inside L*	Halo L*	Inside a*	Halo a*	Inside b*	Halo b*	Inside hue angle	Halo hue angle	Inside chroma	Halo chroma	Inside myoglobin concentration	Halo myoglobin concentration	Inside pH
Halo L*	0.26[a]												
Inside a*	0.08	-0.11											
Halo a*	-0.16[a]	-0.73[a]	0.33[a]										
Inside b*	0.47[a]	0.01	0.85[a]	0.22[a]									
Halo b*	0.00	-0.36[a]	0.30[a]	0.84[a]	0.28[a]								
Inside hue angle	0.75[a]	0.18[a]	0.08	-0.10	0.59[a]	0.06							
Halo hue angle	0.28[a]	0.84[a]	-0.27[a]	-0.85[a]	-0.09	-0.43[a]	0.25[a]						
Inside chroma	0.26[a]	-0.06	0.97[a]	0.30	0.95[a]	0.30[a]	0.31[a]	-0.20[a]					
Halo chroma	-0.10	-0.61[a]	0.33[a]	0.98[a]	0.25	0.93[a]	-0.04	-0.72[a]	0.31[a]				
Inside myoglobin concentration	-0.27[a]	-0.36[a]	0.23[a]	0.48[a]	0.14[a]	0.42[a]	-0.09	-0.39[a]	0.20[a]	0.47[a]			
Halo myoglobin concentration	-0.11	-0.48[a]	0.14	0.48[a]	0.11	0.36[a]	0.00	-0.45[a]	0.13	0.46[a]	0.57[a]		
Inside pH	-0.45[a]	-0.17[a]	-0.58[a]	-0.15	-0.63[a]	-0.29[a]	-0.29[a]	-0.03	-0.63[a]	-0.21[a]	0.03	0.04	
Halo pH	-0.39[a]	-0.40[a]	-0.46[a]	0.06	-0.52[a]	-0.13	-0.28[a]	-0.24[a]	-0.50[a]	-0.01	0.10	0.23[a]	0.84[a]

[a]$P < 0.05$.

color traits were moderately correlated (ranging from 0.25 to 0.33; $P < 0.05$) with the same trait measured on the other portion of the muscle. Myoglobin concentration and muscle pH were both correlated to a much greater extent across muscle locations than the instrumental color traits. Interestingly, myoglobin concentration of the inside portion of the muscle was more highly correlated to color attributes measured on the halo portion of the muscle than to the same color traits measured on the inside portion of the muscle. However, myoglobin measured on the halo portion of the muscle was not related to color attributes measured on the inside portion of the muscle. Muscle pH measured in the inside portion of the muscle was correlated to almost all the color attributes measured in either portion of the muscle.

Loadings for the first 2 principal components describing the variance in color traits, myoglobin concentration, and muscle pH for the inside and halo portions of pork *biceps femoris* muscles are presented in Fig. 3. The first principal component, describing 36.9% of the variance was strongly related to the color attributes of both the inside and halo portions of the muscle. Strong, positive loadings for component 1 were associated with *a**, *b**, and chroma values of both the inside and halo portions of the *biceps femoris* muscles. Negative loadings for component 1 were associated with *L** and hue angle values for the halo portion, as well as the overall color difference between the inside and halo portions of the muscle. Neither *L** nor hue angle of the inside portion contributed strongly to component 1. Loadings indicate that the myoglobin concentrations of the inside and halo portion of the muscle were strongly related to each other and that both were strongly related to color attributes of the halo portion of the muscle.

Component 2 was most strongly associated with color attributes of the inside portion of the muscle, particularly *L**, *a**, *b**, and chroma of the inside portion. Additionally, *L** and hue angle of the halo portion of the muscle were moderately related to component 2. Loadings indicate that muscle pH was strongly related to component 2, and thus, to color of the inside portion of the muscle. Moreover, the loadings indicate a negative correlation between muscle pH and *a**, *b**, and chroma values of the inside portion of the muscle.

Results of the principal component analysis suggested differences between muscle locations in the relationships of muscle color traits to myoglobin concentration and muscle pH. To further characterize these relationships, stepwise regression was used to investigate the contribution of muscle pH and myoglobin concentration to *L** and *a** values in both portions of the muscle (Table 3). For both *L** and *a** of the inside portion of the muscle, pH entered the model first and explains approximately 20 and 35% of the variation in *L** and *a**, respectively (both at $P < 0.001$). For both traits, myoglobin concentration entered the model next and explained an additional 7% of the variation ($P < 0.001$). Conversely, in the halo portion of the muscle, myoglobin entered the model first explaining 23% of

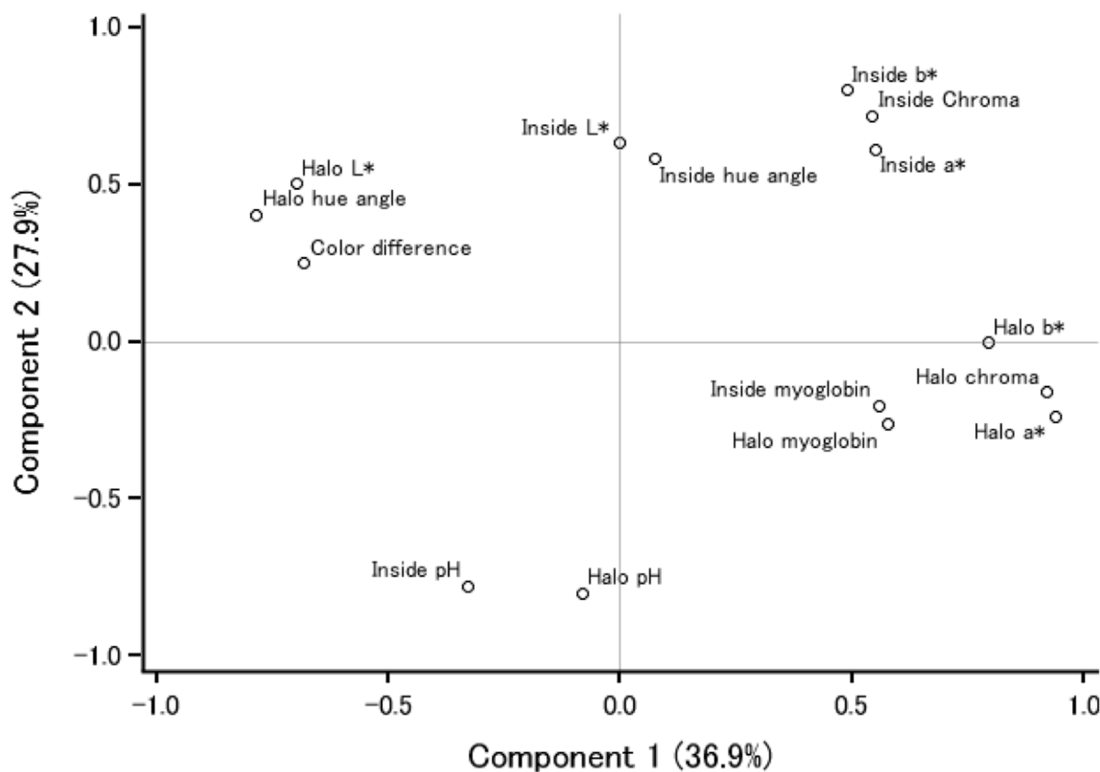

Figure 3. Loadings plot for the first 2 principal components of lean color traits, myoglobin content, and muscle pH of the inside (medial) and halo (lateral) portions of pork *biceps femoris* muscles.

Table 3. Results of PROC STEPWISE forward entry regression to predict $L*$ and $a*$ values for the inside and halo portions of pork *biceps femoris* muscles

Step	Variable entered	Partial R^2	Model R^2	C(p)	Pr > F
L for inside(medial) portion*					
1	pH	0.202	0.20	18.58	< 0.001
2	Myoglobin	0.0657	0.27	3.00	< 0.001
L for halo (lateral) portion*					
1	Myoglobin	0.2285	0.23	25.22	< 0.001
2	pH	0.0848	0.31	3.00	< 0.001
a for inside (medial) portion*					
1	pH	0.351	0.35	24.14	< 0.001
2	Myoglobin	0.0685	0.42	3.00	< 0.001
a for halo (lateral) portion*					
1	Myoglobin	0.2345	0.23	1.66	< 0.001

the variation of both $L*$ and $a*$ values. Muscle pH entered the model and explained an additional 8% of the variation in $L*$ values of the halo portion of the muscle. However, pH did not explain a sufficient amount of the variation in $a*$ values of the halo portion of the muscle to meet the criterion ($P \leq 0.15$) for entry into the model.

Collectively, the correlation, PCA, and stepwise regression results suggest that both muscle pH and myoglobin concentration contributed to lean color of both regions of the *biceps femoris* muscle. However, the relationships of myoglobin concentration and muscle pH to lean color attributes are not equal in the inside and halo portions of the muscle. Muscle pH was the primary driver of variation in muscle color in the interior of the muscle, which possessed higher levels of myoglobin. In the halo-affected portion of the muscle, where myoglobin concentration was limiting, the amount of myoglobin present was the greatest contributor to lean color variation. Importantly, myoglobin concentration was strongly correlated ($r = 0.57$) between the inside and halo locations. We speculate that a certain concentration of myoglobin is needed to reach "normal" color of *biceps femoris* muscles. Increases in myoglobin beyond that level have less impact on lean color and muscle pH becomes a greater determinant of lean color. Thus, we suggest a potential long-term solution to mitigating the halo condition is genetic selection for increased myoglobin concentration.

Newcom et al. (2004) reported a heritability estimate for myoglobin of 0.27 in a population of pigs with diverse breed type and suggested that selection for increased myoglobin could improve lean color of pork loins. Cross et al. (2018)reported a much lower genomic heritability estimate (0.09) for myoglobin concentration of pork loins. However, Cross et al. (2018) also identi-

fied highly significant QTL for myoglobin concentration in that population indicating that genetic influences on myoglobin concentration existed in their population.

The differences in myoglobin concentration between the inside and halo portions of the *biceps femoris* muscle suggested a difference in muscle metabolism. The pale band of halo tissue existed in virtually all sampled muscles (198 of 200 ham muscles sampled), but great variation existed in the severity of the condition. Thus, muscle fiber type was compared in the inside and halo portions of minimally and severely affected muscles (Table 4.). Severity and muscle location interacted to affect the percentage of muscle fibers classified as red (Type I; $P = 0.03$) and white (Type IIB; $P = 0.04$). The halo portion of severely affected muscles had a much lower percentage of Type I fibers than the inside portion of severely affected muscles ($P < 0.001$) or the inside ($P = 0.005$) or halo ($P = 0.04$) portions of minimally affected muscles. Conversely, the proportion of type IIB muscle fibers in the halo portion of severely affected muscles was much greater than the proportion of Type IIB fibers in the inside portion of severely affected muscles ($P = 0.001$) or the inside ($P = 0.002$) or halo ($P = 0.01$) portions of minimally affected muscles. The proportion of Type IIA fibers was greater ($P = 0.03$) in muscles minimally affected by the halo condition than in severely affected muscles. However, the percentage of fibers classified as Type IIA did not differ ($P = 0.23$) between locations within muscle.

Muscle fiber areas were larger for all muscle fiber types ($P = 0.04$, < 0.001, 0.02, and 0.02 for Type I, IIA, IIB, and all fibers, respectively) in muscles minimally affected by the halo condition relative to severely affected muscles regardless of location. Interestingly, fibers classified as Type IIA had greater ($P = 0.04$) fiber areas in the inside portion of the muscle than in the halo portion of the muscle. Other muscle fiber types did not differ in fiber area regarding location within the muscle ($P = 0.27$, 0.70, and 0.60 for Type I, IIB, and all fiber types, respectively).

It is notable that increased severity of the condition was associated with a shift toward white muscle fibers, particularly in the halo portion of the muscle. This is consistent with observations in myoglobin concentration and muscle pH, which tend to suggest increased glycolytic metabolism in the halo portion of the muscle. Ryu and Kim (2005) reported that increased percentage of white (Type IIb) muscle fibers in pork longissimus muscle was associated with increased glycolytic rate, and increased L^* values at 45 min and 24 h postmortem, and decreased a^* values at 45 min postmortem. England et al. (2016) reported greater myoglobin concentration in pork masseter muscle [predominately composed of slow (red) muscle fibers] than longissimus muscle, which has a greater proportion of fast (white) muscle fibers.

Table 4. Least-squares means of the percentage and muscle fiber areas of muscle fiber types of pork *biceps femoris* muscles differing in the severity of the halo condition and sampled in inside (medial) and halo (lateral) locations

Halo Severity	Type I	Type IIA	Type IIB	Overall
Muscle fiber type percentage				
Minimal	22.24	23.85	53.91	–
Severe	17.50	18.18	64.33	–
SEM	2.73	1.42	3.19	–
$P > F$	0.30	0.03	0.05	–
Inside	26.85	22.03	51.15	–
Halo	12.89	20.00	67.10	–
SEM	2.32	1.26	2.84	–
$P > F$	< 0.001	0.23	< 0.01	–
Minimal Inside	25.68 ^{z}	24.08	50.28 ^{y}	–
Minimal Halo	18.80 ^{z}	23.63	57.55 ^{y}	–
Severe Inside	28.02 ^{z}	19.98	52.02 ^{y}	–
Severe Halo	6.98 ^{y}	16.38	76.64 ^{z}	–
SEM	3.28	1.78	4.01	–
$P > F$	0.03	0.34	0.04	–
Muscle fiber area, μm^2				
Minimal	2763.5	3229.2	5461.3	4318.0
Severe	2113.9	2157.3	3800.0	3269.2
SEM	177.4	136.0	388.1	246.1
$P > F$	0.04	< 0.001	0.02	0.02
Inside	2573.5	2889.7	4688.0	3732.8
Halo	2303.9	2496.7	4573.3	3854.4
SEM	167.8	123.3	309.1	206.3
$P > F$	0.27	0.04	0.7	0.6
Minimal Inside	2881.2	3497.5	5824.1	4509.9
Minimal Halo	2645.8	2960.8	5098.4	4126.1
Severe Inside	2265.7	2281.9	3551.8	2955.6
Severe Halo	1962.0	2032.6	4048.3	3582.7
SEM	237.0	174.0	436.4	291.3
$P > F$	0.88	0.38	0.07	0.06

^{y,z}Least squares means within and effect lacking common superscripts differ ($P < 0.05$).

Cross et al. (2018) identified QTL for myoglobin concentration of pork longissimus on chromosome 14 that were associated with calcineurin regulation. Activation of calcineurin has been linked to increased expression of the myoglobin gene and slow-fiber- specific troponin-I gene (Chin et al., 1998) and myosin heavy chain isoforms (Delling et al., 2000). Moreover, inhibition of calcineurin expression in rats increased the proportion of fast muscle fibers (Chin et al., 1998). In support of this rationale, Kim et al. (2018) reported the differential proteome profiles in portions of pork *semimembranosus* muscle with differences in lean color, myoglobin content, and muscle pH. Those investigators reported greater expression of meta-

bolic proteins associated with fast-twitch muscle fibers in light portions of the muscle, and greater expression of proteins associated with slow-twitch muscle fibers in darker portions of the muscle.

Increased severity of the halo condition was associated with smaller muscle fiber areas even in the unaffected portion of the muscle. Growth promotants commonly used in the U.S. pork industry (i.e., ractopamine hydrochloride) have been reported to increase the proportion of white-type muscle fibers (Depreux et al., 2002). However, β-agonists also have been reported to increase muscle fiber areas (Wheeler and Koohmaraie, 1992; Vestergaard et al., 1994). Thus, the localized shift in muscle fiber types observed in the halo condition is not consistent with the effects of β-agonist supplementation.

Variation in lean color attributes across slice locations (proximal to distal; Fig. 2) and location within slice (anterior, center, or posterior; Fig. 2) are presented in Table 5. Interactions between slice and location within slice ($P < 0.001$) for all traits indicate that intramuscular variation in color exists. Compared to color differences associated with the halo condition, these differences were more subtle. Generally, L^* values indicate that color was lightest in the proximal portion of the muscle and became darker as the position moved more distal. The posterior portion of each slice was generally lighter than more anterior portions of the slice.

Differences in a^* and hue angle values were generally in agreement, indicating that the distal portions of the muscle were slightly more red than more proximal locations. Also for slices 3 and 5, the posterior location was generally slightly less red than the anterior locations. However, for slice 7, the posterior location was more red than the center location as evidenced by higher ($P < 0.001$) a^* values and lower ($P = 0.02$) hue angle values. The center location of slice 7 also had lower hue angle values ($P < 0.001$) but similar a^* values ($P = 0.45$) compared to the anterior location of slice 7. Differences in b^* values across muscle locations followed a pattern similar to those observed for a^* values. Differences in chroma across muscle locations followed a pattern similar to the one observed for hue angle. Stufft et al. (2017) reported posterior portions of pork semimembranosus muscles had greater L^* values and lower a^* values than the anterior portions. These results indicate that variation in color traits exist across locations in the *biceps femoris* muscle. However, these differences are relatively small in comparison to those resulting from the halo condition.

Table 5. Least-squares means of instrumental color attributes of pork *biceps femoris* muscle stratified by slice location and location with slice

Slice	Location	L^*	a*	b*	Hue angle	Chroma
3	Anterior	51.54 ^{y}	21.83 ^{w}	15.31 ^{xwv}	32.23 ^{yz}	26.68 ^{w}
3	Center	51.82 ^{y}	21.80 ^{w}	15.33 ^{vw}	32.30 ^{yz}	26.67 ^{w}
3	Posterior	52.41 ^{z}	21.28 ^{v}	14.89 ^{u}	32.17 ^{y}	25.98 ^{v}
5	Anterior	50.06 ^{w}	22.52 ^{y}	15.37 ^{x}	31.55 ^{x}	27.27 ^{xy}
5	Center	50.32 ^{wx}	22.29 ^{x}	15.29 ^{xwv}	31.67 ^{x}	27.04 ^{x}
5	Posterior	51.78 ^{y}	21.66 ^{w}	15.13 ^{v}	32.12 ^{y}	26.44 ^{w}
7	Anterior	48.97 ^{v}	22.42 ^{xy}	15.16 ^{vw}	31.33 ^{w}	27.08 ^{x}
7	Center	50.33 ^{wx}	22.50 ^{y}	15.75 ^{y}	32.19 ^{y}	27.47 ^{y}
7	Posterior	50.45 ^{x}	22.93 ^{z}	16.22 ^{z}	32.41 ^{z}	28.10 ^{z}
SEM		0.19	0.10	0.10	0.13	0.13
P > F		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

$^{u-z}$Least squares means, within a column, lacking common superscripts, differ ($P < 0.05$).

Conclusion

The results of the present experiment indicate that the condition of pale tissue on the superficial aspect of pork *biceps femoris* muscles is associated with a localized shift in muscle fiber types to white glycolytic fibers. This shift in fiber types results in drastically lower myoglobin concentration in the affected portion of the muscle. It appears that selection for myoglobin concentration or for greater oxidative (red) fiber content may mitigate this ham muscle color defect.

Literature Cited

American Meat Science Association. 2012. AMSA Meat Color Measurement Guidelines. AMSA Meat Color Measurement Guidelines. American Meat Science Association, Champaign, IL.

Ashmore, C. R., and L. Doerr. 1971. Comparative aspects of muscle fiber types in different species. Exp. Neurol. 31(3):408-418. doi:10.1016/0014-4886(71)90243-3

Bendall, J. R. 1973. Postmortem changes in muscle. Academic Press, New York. doi:10.1016/B978-0-12-119102-3.50012-4

Chin, E. R., E. N. Olson, J. A. Richardson, Q. Yang, C. Humphries, J. M. Shelton, H. Wu, W. Zhu, R. Bassel-Duby, and R. S. Williams. 1998. A calcineurin-dependent transcriptional pathway controls skeletal muscle fiber type. Genes Dev. 12(16):2499–2509. doi:10.1101/gad.12.16.2499

Cross, A., D. A. King, S. D. Shackelford, T. L. Wheeler, D. Nonneman, B. Keel, and G. A. Rohrer. 2018. Genome-wide association of myoglobin concentrations in pork loins. Meat Musc. Bio. doi:10.22175/mmb2017.08.0042

Delling, U., J. Tureckova, H. W. Lim, L. J. De Windt, P. Rotwein, and J. D. Molkentin. 2000. A calcineurin-NFATc3-dependent pathway regulates skeletal muscle differentiation and slow myosin heavy-chain expression. Mol. Cell. Biol. 20(17):6600–6611. doi:10.1128/MCB.20.17.6600-6611.2000

Depreux, F. F., A. L. Grant, D. B. Anderson, and D. E. Gerrard. 2002. Paylean alters myosin heavy chain isoform content in pig muscle. J. Anim. Sci. 80(7):1888–1894.

England, E. M., S. K. Matarneh, E. M. Oliver, A. Apaoblaza, T. L. Scheffler, H. Shi, and D. E. Gerrard. 2016. Excess glycogen does not resolve high ultimate pH of oxidative muscle. Meat Sci. 114:95–102. doi:10.1016/j.meatsci.2015.10.010

Kim, G.-D., H.-S. Yang, and J.-Y. Jeong. 2018. Intramuscular variations of proteome and muscle fiber type distribution in semimembranosus and semitendinosus muscles associated with pork quality. Food Chem. 244:143-152. doi:10.1016/j.foodchem.2017.10.046

King, D. A., and L. Pierce. 2015. Intramuscular variation in fresh ham muscle color affecting consumer acceptance of curred ham color. 68th Recip. Meat Conf. Proc., Lincoln NE

McKeith, R. O., and T. D. Pringle. 2013. Quality attributes and color characteristics in three-piece boneless hams. Meat Sci. 95(1):59–63. doi:10.1016/j.meatsci.2013.04.020

Newcom, D. W., K. J. Stalder, T. J. Baas, R. N. Goodwin, F. C. Parrish, and B. R. Wiegand. 2004. Breed differences and genetic parameters of myoglobin concentration in porcine longissimus muscle. J. Anim. Sci. 82(8):2264–2268. doi:10.2527/2004.8282264x

Norman, J. L., E. P. Berg, H. Heymann, and C. L. Lorenzen. 2003. Pork loin color relative to sensory and instrumental tenderness and consumer acceptance. Meat Sci. 65(2):927-933. doi:10.1016/S0309-1740(02)00310-8

NPPC. 1999. Official Color and Marbling Standards. National Pork Producers Council, Des Moines, IA.

Ryu, Y. C., and B. C. Kim. 2005. The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. Meat Sci. 71(2):351–357. doi:10.1016/j.meatsci.2005.04.015

Solomon, M. B., and M. C. Dunn. 1988. Simultaneous histochemical determination of three fiber types in single sections of ovine, bovine and porcine skeletal muscle. J. Anim. Sci. 66(1):255–264. doi:10.2527/jas1988.661255x

Stufft, K., J. Elgin, B. Patterson, S. K. Matarneh, R. Preisser, H. Shi, E. M. England, T. L. Scheffler, E. W. Mills, and D. E. Gerrard. 2017. Muscle characteristics only partially explain color variations in fresh hams. Meat Sci. 128(Supplement C):88–96. doi:10.1016/j.meatsci.2016.12.012

USDA. 2014. Institutional Meat Purchase Specifications Fresh Pork Series 400. https://www.ams.usda.gov/sites/default/files/media/IMPS_400_Fresh_Pork%5B1%5D.pdf (accessed 23 April 2018).

Vestergaard, M., P. Henckel, N. Oksbjerg, and K. Sejrsen. 1994. The effect of cimaterol on muscle fiber characteristics, capillary supply, and metabolic potentials of longissimus and semitendinosus muscles from young Friesian bulls. J. Anim. Sci. 72(9):2298–2306. doi:10.2527/1994.7292298x

Wheeler, T. L., and M. Koohmaraie. 1992. Effects of the beta-adrenergic agonist L644,969 on muscle protein turnover, endogenous proteinase activities, and meat tenderness in steers. J. Anim. Sci. 70(10):3035–3043. doi:10.2527/1992.70103035x