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Sire Variation in the Severity of the Ham Halo Condition

D. Andy King[1]*, Steven D. Shackelford[1], Dan Nonneman[2], Gary A. Rohrer[2], and Tommy L. Wheeler[1]

[1]Meat Safety and Quality Research Unit, Roman L. Hruska US Meat Animal Research Center, Agricultural Research Service, US Department of Agriculture, Clay Center, NE 68933-0166, USA
[2]Reproduction Research Unit, Roman L. Hruska US Meat Animal Research Center, Agricultural Research Service, US Department of Agriculture, Clay Center, NE 68933-0166, USA
*Corresponding author. Email: andy.king@usda.gov (D. Andy King)

Abstract: A study was conducted to examine genetic variation in the ham halo condition. The distal portion of the *biceps femoris* was sampled by taking cores (2.54-cm diameter) from progeny ($n = 1{,}016$) from a Duroc meat quality–focused line. Commission Internationale de l´Éclairage (CIE; "International Commission on Illumination") color-space values (L^*, a^*, and b^*) and myoglobin concentration were measured on the halo ("Halo") and inside ("Inside") portion of each core. The Halo portion of the *biceps femoris* had greater L^* and b^* and lesser a^* and myoglobin content (all $P < 0.001$) than the Inside portion. Sires with 11 or more progeny were compared. The sire × muscle-location interaction affected ($P < 0.001$), L^*, a^*, and myoglobin concentration. Sire progeny groups differed for each trait in both portions of the muscle, but differences in the Halo portion of the muscle were not mirrored in the Inside portion of the muscle. Similarly, sire group affected the magnitude of the difference in L^* ($P = 1.4 \times 10^{-4}$) and a^* ($P = 9.0 \times 10^{-6}$) between the Halo and Inside portions of the muscle and tended ($P = 0.08$) to affect myoglobin content. However, the largest sire-group differences were not necessarily seen in the sires with the highest means for these attributes. Thus, selecting for myoglobin concentration, L^*, or a^* content in the Halo portion of the *biceps femoris* muscle would be an effective strategy for reducing the severity of the ham halo condition.

Key words: color, genetics, ham halo, intramuscular variation, pork
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Introduction

Color uniformity continues to be a primary driver of cured-ham-product consumer acceptance. Previous work examining color variation of ham products primarily focused on differences across muscles (McKeith and Pringle, 2013; Stufft et al., 2017). An increase in customer complaints to ham processors was linked to a muscle-color defect characterized by a band of very pale muscle tissue in the superficial portion of ham muscles with normal lean color in the deeper portions of the muscle (King and Pierce, 2015). This has been labeled the halo condition and results in inconsistent cured color development.

The halo-affected portion of the muscle has been reported to have much higher L^* values and lower a^* values than the inside portion of the muscle (King et al., 2018). This lighter, less red color corresponds to a much lower myoglobin concentration in the affected portion. Moreover, myoglobin concentration was the primary factor influencing lean color in the halo-affected muscle tissue.

The halo condition has been observed in the vast majority of ham muscles examined However, the numeric differences in color attributes between the halo and inside portions of the *biceps femoris* varied among individual samples. Additionally, the proportion of the area affected varied as well. We speculated that increasing the myoglobin content of the

halo-affected tissue would be an effective strategy to mitigate this condition.

Swine genetics companies have expressed interest in reducing the halo condition through genetic selection, but measuring myoglobin concentration is not amenable to routine phenotyping of progeny. Currently, quantitative trait loci (QTL) have been identified for myoglobin concentration in pork *longissimus* (Cross et al., 2018), but work remains to provide a validated genetic test for myoglobin. In the meantime, selection based on the color of the halo portion or for the difference in lean color between the halo portion and the inside portion of the muscle may be an appropriate strategy for mitigating the halo condition.

The present study was conducted to determine the extent of genetic variation in lean color and myoglobin content of the halo-affected and unaffected portions of pork *biceps femoris* muscle. Specifically, the objectives of the experiment were to (1) contrast color attributes and myoglobin content of the halo ("Halo") and inside ("Inside") portions of *biceps femoris* muscle, (2) determine relationships between color attributes and myoglobin concentration in both portions of the *biceps femoris* muscle, (3) examine indices of Halo lean color and Halo lean color coupled with the difference in color between the Halo portion and the Inside portion as selection criteria, and (4) determine the sire effects on lean color and myoglobin concentration of the Halo and Inside portions of pork *biceps femoris* muscles.

Materials and Methods

Pigs used this experiment were produced, managed, and harvested in a United States Department of Agriculture–inspected processing facility by a commercial swine company. At no time were live pigs under the control of the U.S. Meat Animal Research Center. Ham muscle samples and sire information were provided to the U.S. Meat Animal Research Center by the swine company 1 to 4 wk after harvest. Thus, animal care and use approval was not obtained for this experiment.

Sample selection and handling

Pigs ($n = 1{,}246$) were produced as part of progeny tests of a meat quality–focused Duroc sire line. Pigs were harvested using conventional procedures in a large commercial processing facility with carbon-dioxide stunning and blast chilling. The samples obtained for the present experiment represent a subset of the progeny harvested in a total of 28 harvest days during the summer and fall of 2016 and spring and summer of 2017. After exiting the blast chill, carcasses were placed in a 0°C cooler. Approximately 24 h post mortem, a 2.54-cm diameter core was removed from the distal portion of the *biceps femoris* muscle. The core was taken through the skin surface at approximately the midpoint (dorsal to ventral) at the location depicted by slice I in the cross-sectional views section of the Porcine Myology website (Jones, 2000). Subcutaneous fat and connective tissue were removed from the superficial surface of the core. Instrumental color readings were taken on the superficial and deep surface of each core with a Hunter Miniscan XE Plus Colorimeter (HunterLab, Reston, VA) with a 25-mm port that was set to collect spectral data with Illuminant D65 and a 10° observer, which was calibrated as prescribed by the manufacturer. The Commission Internationale de l´Éclairage (CIE; "International Commission on Illumination") L^* (lightness), a^* (redness), and b^* (yellowness) color-space values were reported for readings taken on each muscle surface.

Cores then were cut in half longitudinally. One-half of each core was frozen and used by the swine genetics company for a concurrent research project. The remaining half was frozen and transported to the U.S. Meat Animal Research Center. Some samples ($n = 230$) arrived thawed and were excluded from myoglobin concentration determination. Myoglobin concentration was determined on tissue from the superficial (Halo) portion of the core and from the inside (Inside) portion of the core. The border of the Halo portion was difficult to distinguish from the Inside portion in the frozen core, so the most superficial 1 cm of the core was minced and considered to be the Halo sample. The Inside sample was taken from the deepest portion of the muscle.

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 filtered by syringe (Nalgene 0.45 µm, surfactant-free cellulose-acetate membrane; Thermo Fisher Scientific, Rochester, NY) into 1.5-mL microcentrifuge 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).

The extracted myoglobin pigment concentration (milligrams per gram of meat) was calculated by taking the difference between the absorbance at 525 nm and the absorbance at 700 nm, using a millimolar extinction coefficient of 7.6 mM^{-1} cm^{-1}, the molecular weight of myoglobin (17,000 Da), and the appropriate dilution factor.

Statistical analysis

Two indices were calculated to reflect the severity of the halo condition. The Halo Color Index (HCI) was calculated as follows: $HCI = ([L^*_{Halo} - 45] / 20) + ([20 - a^*_{Halo}] / 20)$. Arbitrary values were selected to be somewhat lower (45 for L^*) or higher (20 for a^*) than the most extreme values observed in the halo tissue evaluated in the present experiment. The difference from these values was divided by 20, which represented the approximate range in the observed values in the evaluated halo tissue from these arbitrary values. The HCI index represents the sum of these proportional differences. A second index, the Halo Color Difference Index (HCDI), was calculated to combine the HCI index with the sum of the proportional differences in L^* and a^* between the halo and inside portions of the muscle: $HCDI = ([L^*_{Halo} - 45] / 20) + ([20 - a^*_{Halo}] / 20) + ([L^*_{Halo} - L^*_{Inside}] / L^*_{Inside}) + ([a^*_{Halo} - a^*_{Inside}] / a^*_{Inside})$. For the purposes of evaluating the effects of these indices on CIE color-space values, samples were classified into 5 approximately equal classes for each index on the basis of the values for that index.

All data were utilized to evaluate muscle location and index effects on the CIE color-space values ($n = 1{,}246$) and myoglobin concentration ($n = 1{,}016$) by using the PROC GLIMMIX procedure in SAS software (version 9.4; SAS Institute Inc., Cary, NC). The model included the fixed effect of muscle location and index as well as their interaction and the random effects of harvest date and carcass. A Kenward-Rogers adjustment for denominator degrees of freedom was used.

Pearson and Spearman rank correlation coefficients among CIE color-space values and myoglobin concentrations within and across muscle locations, as well as the absolute value of the difference in each variable across locations, were determined using the PROC CORR procedure of SAS.

A total of 204 sires were represented in the dataset. The number of progeny per sire ranged from 1 to 29. To obtain reliable estimates of sire-progeny-group effects, progeny from sires with fewer than 11 progeny with data for myoglobin concentrations were omitted from the analysis. Thus, 25 sires were included in the sire effect analysis. Sire effects were estimated using the PROC GLIMMIX procedure of SAS. The model included fixed effects of sire, location, and their interaction. Random effects were harvest date and carcass nested within sire. Models for the difference in each variable between the halo and inside portion included the fixed effect of sire and the random effect of harvest date. A predetermined level of type I error (α) of 0.05 was used for all judgment of statistical significance.

Results and Discussion

Simple statistics for the color and myoglobin traits included in this experiment, as well as the number of progeny per sire included in the sire-group analysis, are presented in Table 1. These results indicate that lean color and myoglobin concentration were highly variable in both portions of the muscle. Least-squares means for CIE color-space values and myoglobin concentration of the Halo and Inside portions of fresh pork *biceps femoris* muscles as well as the main effects for the HCI and HCDI and their interaction with muscle location are presented in Table 2. The Halo portion of the *biceps femoris* muscles had much greater ($P < 0.001$) L^* and lesser a^* ($P < 0.001$) values than the Inside portion. The Halo portion also had greater ($P < 0.001$) b^* values than the Inside portion. These color differences coincided with a large decrease ($P < 0.001$) in myoglobin concentration in the Halo portion of the *biceps femoris* relative to the Inside portion. The characterization of the Halo condition as having much lighter and less red lean color associated with a reduced amount of myoglobin in the affected portion of the muscle is consistent with our initial reports on the halo condition (King et al., 2018).

In King et al. (2018), we suggested that strategies to mitigate the halo condition should focus on increasing myoglobin concentration. Swine genetics companies have expressed interest in using genetic selection to reduce or eliminate the halo condition. Currently, genetic markers for myoglobin concentration are not available to the industry. Moreover, measuring muscle myoglobin concentration is time consuming and requires invasive sample collection. Thus, measuring myoglobin concentration does not fit into routine progeny testing programs. As a result, the suitability of using CIE color-space data for progeny testing needs to be addressed.

Because the Halo condition is a result of both increased lightness and a lack of redness in the affected

Table 1. Descriptive statistics of color traits and myoglobin concentration used for muscle location and HCI class analysis and number of progeny for color traits and myoglobin concentration included in sire-group analysis

Descriptive statistics for full dataset used in location and HCI class analysis					
Variable	*n*	Mean	Standard deviation	Minimum	Maximum
Inside L^*	1,246	51.5	3.0	31.2	62.1
Halo L^*	1,246	62.0	3.3	50.3	71.8
Inside a^*	1,246	8.5	1.3	3.6	16.1
Halo a^*	1,246	2.9	1.2	−0.9	8.5
Inside b^*	1,246	12.0	1.0	8.4	16.1
Halo b^*	1,246	12.3	0.8	9.1	16.8
Inside myoglobin concentration	1,020	1.64	0.36	0.89	3.93
Halo myoglobin concentration	1,020	0.85	0.22	0.28	2.10
Difference in L^*[1]	1,246	10.5	4.0	0.1	26.0
Difference in a^*[2]	1,246	5.6	1.6	0.2	12.0
Difference in b^*[3]	1,246	0.9	0.7	0.0	4.2
Difference in myoglobin[4]	1,020	0.79	0.28	0.01	2.57
HCI[5]	1,246	1.7	0.2	1.0	2.4

Number of progeny per sire group		
Sire	**Color traits**	**Myoglobin concentration**
105	16	16
119	18	17
125	19	17
141	14	14
142	14	14
145	13	13
149	19	12
154	29	29
156	11	11
157	18	16
169	13	12
179	11	11
190	12	12
191	14	14
196	15	15
208	19	18
219	13	13
223	16	15
228	21	15
250	12	11
251	13	13
258	23	15
275	31	30
283	18	15
304	14	13

[1]Absolute value of difference in L^* values between Halo and Inside portion of pork biceps muscle.

[2]Absolute value of difference in a^* values between Halo and Inside portion of pork biceps muscle.

[3]Absolute value of difference in b^* values between Halo and Inside portion of pork biceps muscle.

[4]Absolute value of difference in myoglobin concentration between Halo and Inside portion of pork biceps muscle.

[5]$\text{HCI} = ([L^*_{\text{Halo}} - 45] / 20) + ([a^*_{\text{Halo}} - 20] / 20)$.

HCI = Halo Color Index.

Table 2. Least-squares means for muscle-location and Halo-selection indices on CIE color-space values and myoglobin concentration of muscle tissue from pork *biceps femoris* muscles

Muscle location	Index class	L^*	a^*	b^*	Myoglobin concentration, mg/g
Location effect[1]					
Halo		62	2.9	12.3	0.85
Inside		51.5	8.5	12.0	1.64
SEM[2]		0.12	0.06	0.05	0.03
P value		<0.001	<0.001	<0.001	<0.001
HCI class effect[3,4]					
	Low	53.9	6.6	11.8	1.37[a]
	Moderately low	55.8	6.0	12.0	1.28[b]
	Moderate	56.8	5.6	12.1	1.25[b]
	Moderately high	57.7	5.4	12.4	1.20[c]
	High	59.4	4.9	12.4	1.13[d]
SEM		0.14	0.07	0.06	0.04
P value		<0.001	<0.001	<0.001	<0.001
Location × HCI class effect					
Halo	Low	57.3[e]	4.5[d]	11.8[f]	0.98
Halo	Moderately low	60.2[d]	3.4[e]	12.0[c,d]	0.88
Halo	Moderate	62.0[c]	2.7[f]	12.3[b]	0.84
Halo	Moderately high	63.8[b]	2.2[g]	12.6[a]	0.81
Halo	High	66.7[a]	1.5[h]	12.7[a]	0.75
Inside	Low	50.5[h]	8.8[a]	11.8[e,f]	1.77
Inside	Moderately low	51.4[f]	8.6[b]	11.9[e,f]	1.68
Inside	Moderate	51.6[g]	8.5[b]	11.9[d,e]	1.66
Inside	Moderately high	51.7[f,g]	8.5[b]	12.2[b,c]	1.59
Inside	High	52.1[f]	8.2[c]	12.0[c,d]	1.51
SEM		0.17	0.08	0.07	0.04
P value		<0.001	<0.001	<0.001	0.37

[1]Halo = most superficial 1 cm of a 2.54-cm-diameter core removed from the distal portion of the *biceps femoris* muscle. Inside = tissue removed from the 2.54-cm-thick core representing the deep portion of the *biceps femoris* muscle.

[2]SEM = Standard Error of the Mean

[3]HCI = Halo Color Index; $([L^*_{Halo} - 45] /20) + ([a^*_{Halo} - 20] / 20)$.

[4]HCI classes were determined by ranking HCI values and sorting into 5 approximately equally sized groups.

[a,b,c,d,e,f,g,h]Least-squares means within a column and within an effect that lack common superscripts differ ($P < 0.05$).

CIE = Commission Internationale de l′Éclairage ("International Commission on Illumination").

tissue, both L^* and a^* should be addressed in genetic selection. Thus, the HCI was derived to weigh each of these values equally. Lower values for the HCI represent darker, more red tissue in the halo condition. The effects of classifying samples on the basis of this index and its interaction with muscle location on CIE color-space values and myoglobin concentration are presented in Table 2.

Increasing the HCI class generally resulted in decreased ($P \leq 0.01$) myoglobin concentration. However, the moderately low class for HCI did not differ ($P = 0.17$) from the moderate class for the HCI. Color-space values were affected ($P < 0.001$) by a location × HCI interaction. Increasing the HCI class resulted in increased ($P < 0.001$) L^* values in the Halo portion of the muscle. Differences in L^* across HCI classes were much smaller in the Inside portion of the muscle, and the moderate class was not different from the moderately low class ($P = 0.26$) or the moderately high class ($P = 0.55$). As Halo index class increased, a^* values of the Halo portion of the muscle decreased ($P < 0.001$). As with L^* values, a^* values generally decreased in the Inside portion of the muscle as HCI class increased, although the differences were much smaller than the differences detected in the Halo portion of the muscle. As HCI class increased, b^* values generally increased in both the Halo and Inside portions of the muscle, although the differences were much smaller than those observed regarding L^* and a^*.

Another index (HCDI) including the magnitude of the differences in L^* and a^* between the Halo and Inside portions of the muscle along with the L^* and a^*

values of the Halo portion of the muscle was also derived. It was thought that such an index might better address the visual severity of the halo condition by addressing the contrast in color between the two muscle portions. However, changes in CIE color-space values and myoglobin concentration across muscle locations and HCDI classes were nearly identical to those observed when samples were classified according to the HCI. Thus, results regarding this index are not shown.

Pearson correlation coefficients for CIE color-space values and myoglobin content of the Halo and Inside portion of pork *biceps femoris* muscles, as well as the absolute difference in each of these variables between the Halo and Inside portions of the *biceps femoris*, are presented in Table 3. Color-space values were moderately correlated ($P < 0.001$) between the Halo and Inside portions of the *biceps femoris* muscles. Myoglobin concentration in both the Halo and Inside portions of the muscle were moderately correlated ($P < 0.001$) with L^* and a^* values in both the Halo and Inside portions of the muscle. Interestingly, the magnitude of the correlations between myoglobin concentration of the Inside portion of the muscle and color-space values of the Inside portion of the muscle were only slightly greater than correlations between the Inside-portion myoglobin concentration and Halo color-space values. Similarly, moderate correlations ($P < 0.001$) existed between myoglobin concentration of the Halo portion and L^* and a^* values of the Inside portion of the *biceps femoris* muscle. The magnitude of the difference in L^* values between the Halo and Inside portions of the muscle was positively correlated ($P < 0.001$) with L^* value of the Halo portion and negatively correlated ($P < 0.001$) with L^* value of the Inside portion of the muscle. Conversely, the difference in a^* between muscle locations was negatively correlated with the a^* value of the Inside portion of the muscle and positively correlated with the a^* value of the Halo portion of the *biceps femoris*. The difference in myoglobin concentration between muscle locations was highly related ($P < 0.001$) to the concentration of myoglobin in the Inside portion of the muscle. However, the difference in myoglobin concentration was not correlated ($P > 0.05$) with the

Table 3. Pearson correlation coefficients for CIE color-space values and myoglobin concentration of Halo and Inside portions of pork *biceps femoris* muscles

Variable	Inside L^*	Halo L^*	Inside a^*	Halo a^*	Inside b^*	Halo b^*	Inside myoglobin	Halo myoglobin concentration	Difference in L^*[1]	Difference in a^*[2]	Difference in b^*[3]	Difference in myoglobin concentration
Halo *L**	0.20[a]											
Inside *a**	−0.72[a]	−0.12[a]										
Halo *a**	−0.10[a]	−0.76[a]	0.20[a]									
Inside *b**	0.28[a]	0.13[a]	0.26[a]	0.04								
Halo *b**	0.10[a]	0.50[a]	0.09[a]	−0.13[a]	0.27[a]							
Inside myoglobin concentration	−0.32[a]	−0.30[a]	0.27[a]	0.22[a]	−0.16[a]	−0.11[a]						
Halo myoglobin concentration	−0.21[a]	−0.47[a]	0.21[a]	0.45[a]	−0.06	−0.15[a]	0.63[a]					
Difference in *L[1]**	−0.59[a]	0.68[a]	0.44[a]	−0.56[a]	−0.10[a]	0.33[a]	−0.02	−0.24[a]				
Difference in *a[1]**	−0.51[a]	0.49[a]	0.66[a]	−0.61[a]	0.18[a]	0.17[a]	0.04	−0.19[a]	0.79[a]			
Difference in *b[1]**	−0.01	0.11[a]	−0.02	−0.02	−0.12[a]	0.20[a]	0.09[a]	0.05	0.10[a]	0.00		
Difference in myoglobin[1]	−0.25[a]	−0.03	0.19[a]	−0.06[b]	−0.16[a]	−0.03	0.81[a]	0.05	0.16[a]	0.20[a]	0.08[b]	
HCI[5]	0.18[a]	0.98[a]	−0.15	−0.87[a]	0.09[a]	0.42[a]	−0.29[a]	−0.49[a]	0.68[a]	0.55[a]	0.09[a]	−0.01

HCDI = Halo Color Difference Index; $([L^*_{Halo} - 45] / 20) + ([a^*_{Halo} - 20] / 20) + (\text{abs}[L^*_{Inside} - L^*_{Halo}] / L^*_{Inside}) + (\text{abs}[a^*_{Inside} - a^*_{Halo}] / a^*_{Inside})$.

[1]Absolute value of difference in L^* values between Halo and Inside portion of pork biceps muscle.

[2]Absolute value of difference in a^* values between Halo and Inside portion of pork biceps muscle.

[3]Absolute value of difference in b^* values between Halo and Inside portion of pork biceps muscle.

[4]Absolute value of difference in myoglobin concentration between Halo and Inside portion of pork biceps muscle.

[5]HCI = Halo Color Index; $([L^*_{Halo} - 45] / 20) + ([a^*_{Halo} - 20] / 20)$.

[a]$P < 0.001$.

[b]$P < 0.05$.

CIE = Commission Internationale de l´Éclairage ("International Commission on Illumination").

myoglobin concentration of the Halo portion of the muscle. Additionally, the difference in myoglobin concentration between muscle locations was negatively related ($P < 0.001$) to L^* and b^* values of the Inside portion of the *biceps femoris* and positively related to ($P < 0.001$) a^* values of the inside portion of the *biceps femoris*. However, the difference in myoglobin between muscle locations was only weakly related ($P < 0.05$) to a^* values.

HCI attempts to quantify the color in the halo portion of the muscle. Values of this index had more correlation with color-space values and myoglobin concentration of the Halo portion of the muscle than the Inside portion of the muscle. Increased HCI values were strongly related ($P < 0.001$) to increased L^* values and decreased a^* values in the Halo portion of the muscle. Values for this index were negatively related ($P < 0.001$) with the myoglobin concentrations of both portions of the muscle. HCI values were also positively correlated ($P < 0.001$) with Halo b^* values as well as with the difference in L^* and a^* values between muscle locations. The HCDI, which included the same variables as the HCI with the addition of the percentage difference in L^* and a^* values of the Inside and Halo portions of the muscle, was very highly correlated with values of the HCI. Spearman rank correlations among color traits and myoglobin concentrations in both portions of the muscle are presented in Table 4. These relationships are very similar to those identified by Pearson correlation coefficients.

Correlation results from the present experiment are consistent with our previous report on the relationships among color traits and myoglobin concentration within and across halo locations in *biceps femoris* muscles (King et al., 2018). In that report, we speculated that a minimum concentration of myoglobin is required for normal lean color, which was not present in the Halo condition. Thus, we suggested that genetic selection for myoglobin content in *biceps femoris* muscles might mitigate the halo condition. Correlation results from the present experiment suggest that

Table 4. Spearman correlation coefficients for CIE color-space values and myoglobin concentration of Halo and Inside portions of pork *biceps femoris* muscles

Variable	Inside L^*	Halo L^*	Inside a^*	Halo a^*	Inside b^*	Halo b^*	Inside myoglobin	Halo myoglobin concentration	Difference in L^*[1]	Difference in a^*[2]	Difference in b^*[3]	Difference in myoglobin concentration
Halo *L**	0.18[a]											
Inside *a**	−0.66[a]	−0.12[a]										
Halo *a**	−0.07[b]	−0.75[a]	0.18[a]									
Inside *b**	0.32[a]	0.14[a]	0.23[a]	0.01								
Halo *b**	0.10[a]	0.49[a]	0.06	−0.14[a]	0.26[a]							
Inside myoglobin concentration	−0.36[a]	−0.37[a]	0.32	0.28[a]	−0.19[a]	−0.16[a]						
Halo myoglobin concentration	−0.21[a]	−0.50[a]	0.23	0.47[a]	−0.07[b]	−0.14[a]	0.59[a]					
Difference in *L[1]**	−0.52[a]	0.70[a]	0.36	−0.58[a]	−0.09[b]	0.35[a]	−0.04	−0.27[a]				
Difference in *a[2]**	−0.45[a]	0.50[a]	0.60	−0.63[a]	0.17[a]	0.17[a]	0.02	−0.22[a]	0.76[a]			
Difference in *b[3]**	−0.02	0.12[a]	−0.02	−0.04	−0.18[a]	0.19[a]	0.07[b]	0.04	0.11[a]	0.02		
Difference in myoglobin[4]	−0.29[a]	−0.06[b]	0.22	−0.02	−0.18[a]	−0.07[b]	0.76[a]	−0.01	0.17[a]	0.20[a]	0.05	
HCI[5]	0.16[a]	0.98[a]	−0.14	−0.86[a]	0.11[a]	0.42[a]	−0.36[a]	−0.51[a]	0.70[a]	0.56[a]	0.11[a]	−0.04

[1]Absolute value of difference in L^* values between Halo and Inside portion of pork biceps muscle.

[2]Absolute value of difference in a^* values between Halo and Inside portion of pork biceps muscle.

[3]Absolute value of difference in b^* values between Halo and Inside portion of pork biceps muscle.

[4]Absolute value of difference in myoglobin concentration between Halo and Inside portion of pork biceps muscle.

[5]HCI = Halo Color Index; $([L^*_{Halo} - 45] / 20) + ([a^*_{Halo} - 20] / 20)$.

[a]$P < 0.001$.

[b]$P < 0.05$.

CIE = Commission Internationale de l´Éclairage ("International Commission on Illumination").

any phenotyping and selection for myoglobin concentration should focus on the halo portion of the muscle.

One objective of the present experiment was to evaluate genetic variation in color traits and myoglobin content in the halo and inside portions of *biceps femoris* muscles. Least-squares means for sire-progeny-group × muscle-location interactions on *L** values of pork *biceps femoris* muscles are presented in Figure 1. Substantial sire-progeny-group differences existed in *L** values within muscle locations. In general, sire groups with greater *L** values in the Halo portion also had greater *L** values in the Inside portion of the *biceps femoris* muscle. However, differences across sire groups in *L** values of the Halo portion did not directly correspond to sire-group effect differences in *L** values of the Inside portion of the *biceps femoris*. This was also apparent when differences in *L** between the Halo and Inside portions of the *biceps*

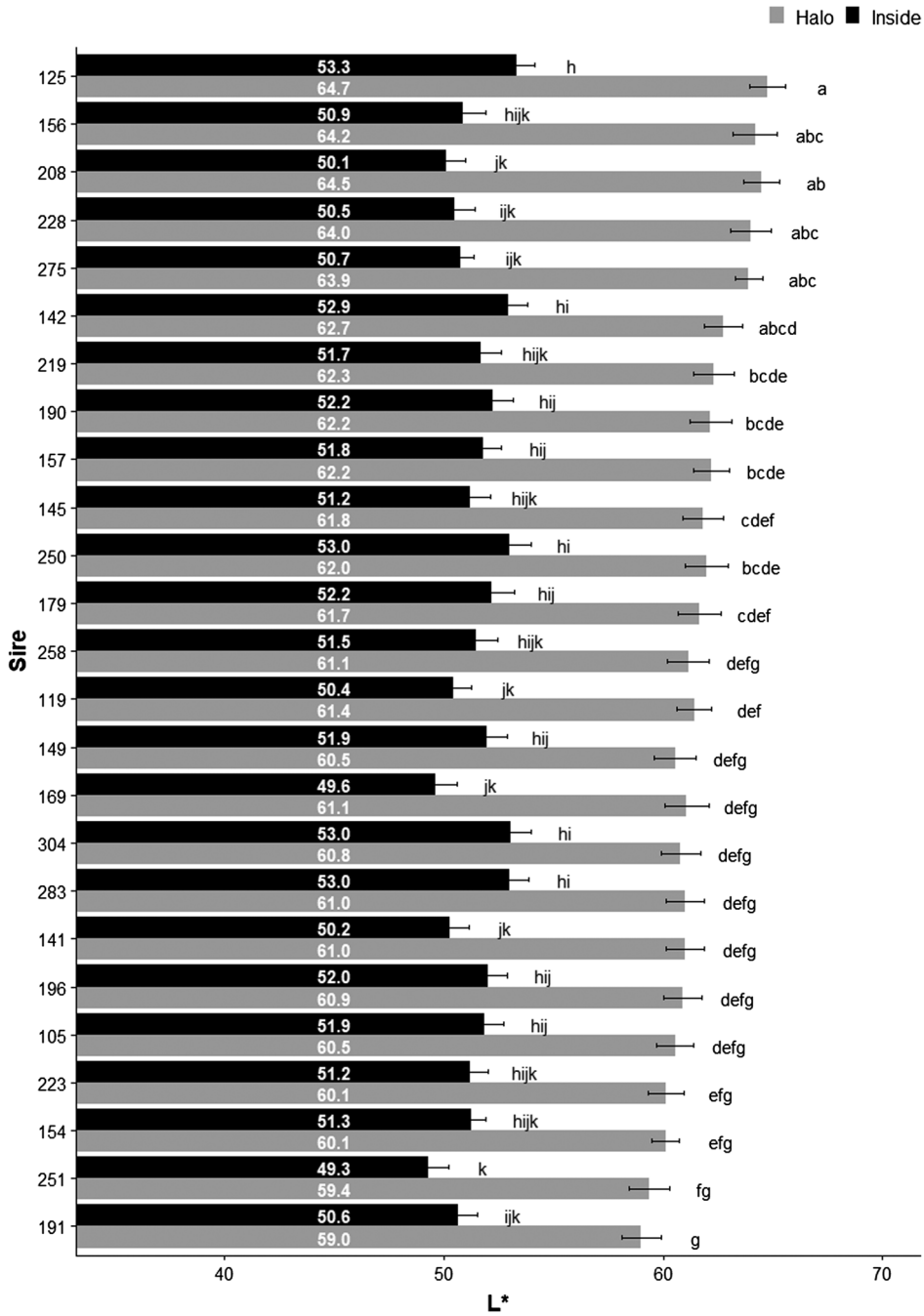

Figure 1. Least-squares sire-progeny-group × location interaction ($P = 1.4 \times 10^{-8}$) means of *L** values of pork *biceps femoris* muscles. Least-squares means lacking a common letter differ ($P < 0.05$).

femoris muscle were calculated. Sire-progeny-group least-squares means for differences in L^* between the Halo and Inside portions of the muscle are presented in Figure 2. The magnitude of the difference between muscle locations ranged from 13.4 to 8.0. Generally, sire progeny groups with the highest L^* values in the Halo portion of the muscle tended to have larger differences between muscle locations. Thus, genetic selection for darker lean color of the Halo portion of the muscle should reduce Halo-condition severity. However, it must be noted that several sire groups with moderate L^* values relative to the other sires included in this experiment also had large differences in L^* across muscle locations.

Least-squares means for the interactions of sire-progeny-group and muscle-location effects on a^* values are presented in Figure 3. In both locations, a^* values differed across sire groups, indicating that

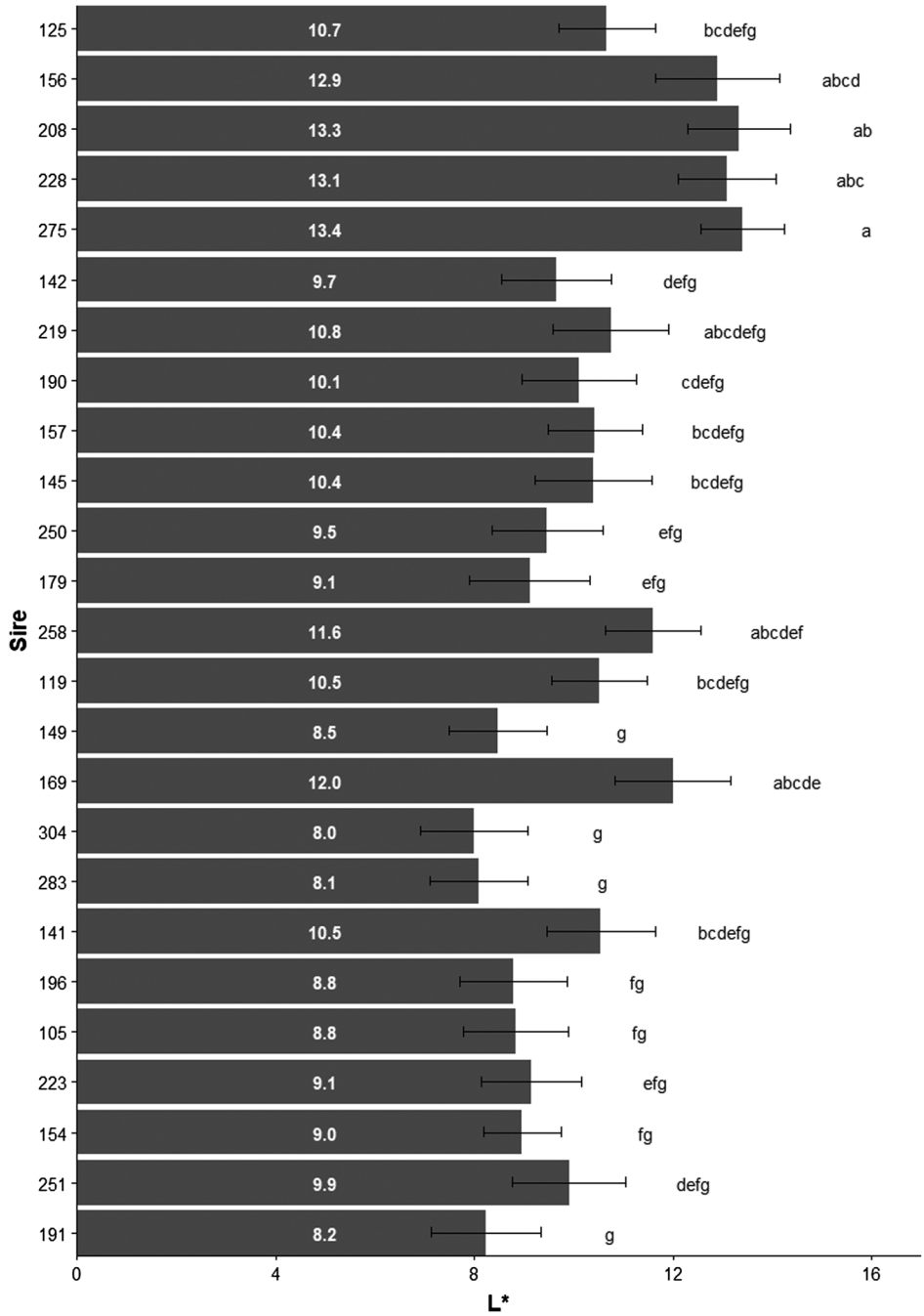

Figure 2 Least-squares means for sire-progeny-group ($P = 1.4 \times 10^{-4}$) effect on the difference in L^* values between the halo and inside portions of the *biceps femoris* muscle. Least-squares means lacking a common letter differ ($P < 0.05$).

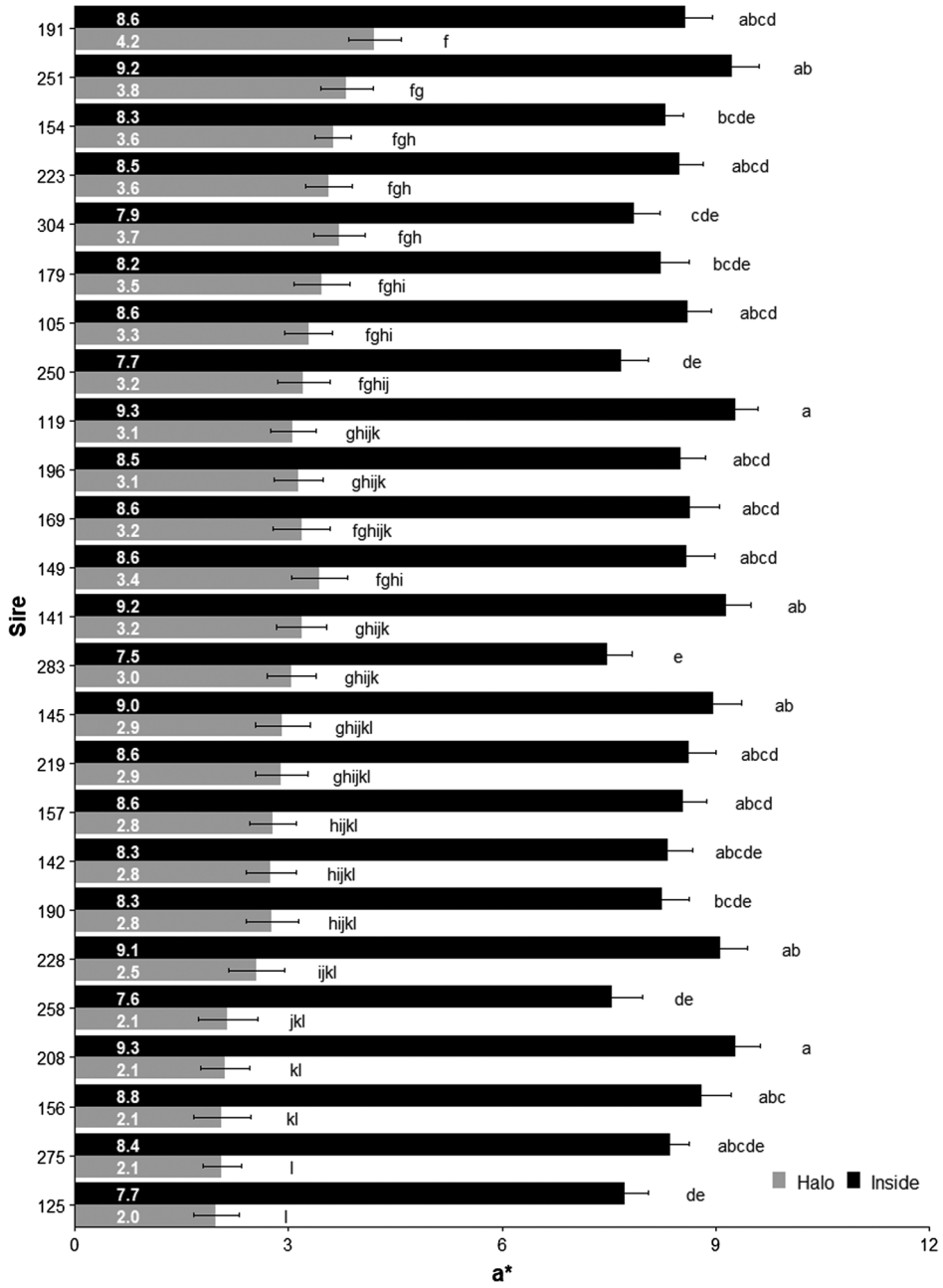

Figure 3. Least-squares sire-progeny-group × location interaction ($P = 1.0 \times 10^{-9}$) means of a^* values of pork *biceps femoris* muscles. Least-squares means lacking a common letter differ ($P < 0.05$).

genetic selection could be used to increase redness of pork *biceps femoris* muscle, particularly in the Halo portion of the muscle. As with L^* values, differences in a^* values of the Inside portion of the *biceps femoris* did not mirror differences detected in a^* values of the Halo portion. Moreover, the magnitude of the difference in a^* between the Inside and Halo portions of the *biceps femoris* were generally larger in sires with lower a^* values in the Halo portion of the muscle (Figure 4). Sire progeny group did not have any effect on b^* values ($P = 0.15$) or the difference in b^* values between the Halo and Inside portions of the muscle ($P = 0.62$).

Least-squares means for sire-progeny-group myoglobin content are presented in Figure 5. Sire effects for myoglobin content differed in both the Halo and Inside portions of the *biceps femoris* muscle. The sire progeny group with the greatest myoglobin content in the Halo portion of the *biceps femoris* muscle had a mean myoglobin content that was 1.8-fold greater than the mean myoglobin concentration of the sire progeny group with the lowest myoglobin concentration in the Halo

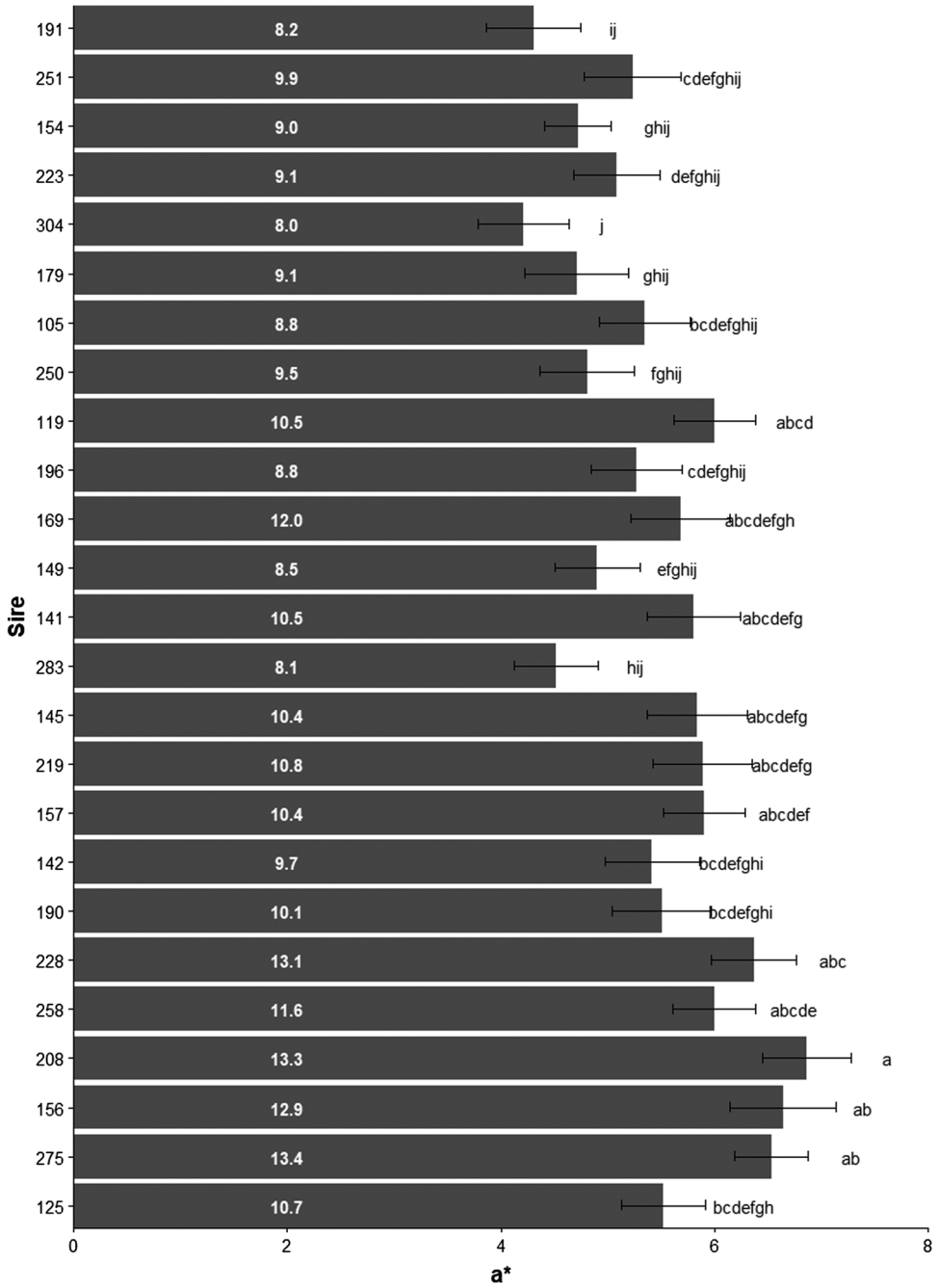

Figure 4. Least-squares means for sire-progeny-group ($P = 9.0 \times 10^{-6}$) effect on the difference in a^* values between the halo and inside portions of the *biceps femoris* muscle. Least-squares means lacking a common letter differ ($P < 0.05$).

portion. Sire progeny groups differed in myoglobin content in both portions of the *biceps femoris* muscles; however, differences across sire progeny groups in the Inside portion of the muscle did not necessarily mirror sire-progeny-group differences in myoglobin content of the Halo portion. Thus, selection for myoglobin concentration in the inside portion of the muscle may not be the most effective approach to mitigate the halo condition. This is supported by the differences in myoglobin content between the Halo and Inside portions of the muscle across sire progeny groups (Figure 6). The magnitude of the difference in myoglobin concentrations between the Halo and Inside portions of the *biceps femoris* tended ($P = 0.08$) to differ across sire progeny groups. The magnitude of the differences did not directly correspond to the concentration of myoglobin present in the muscle.

Our previous characterization of the halo condition identified lighter and less red lean color in the superficial portion of the *biceps femoris* muscle compared

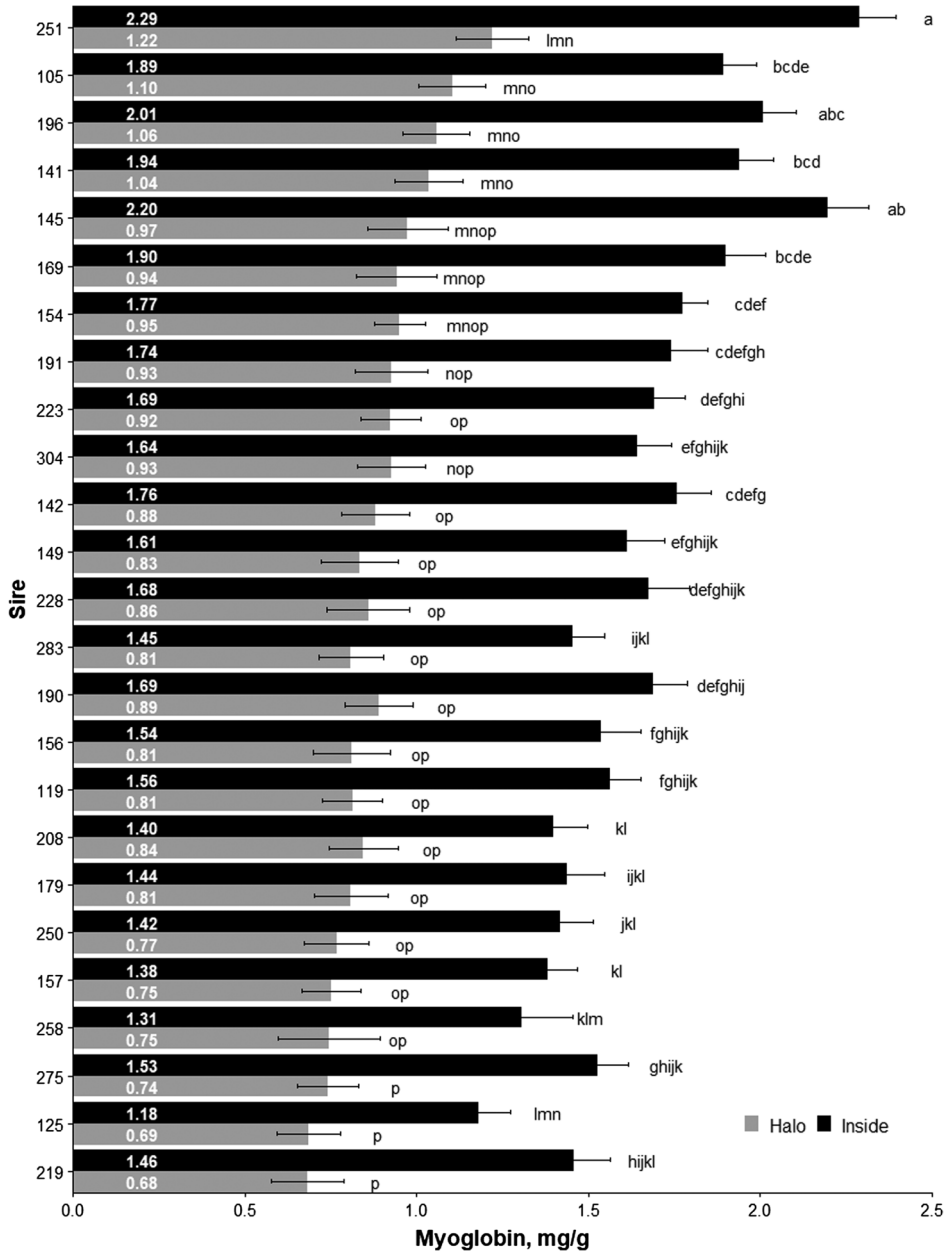

Figure 5. Least-squares sire-progeny-group × location interaction ($P = 3.5 \times 10^{-12}$) means of myoglobin concentration (milligrams per gram of wet tissue) in pork *biceps femoris* muscles. Least-squares means lacking a common letter differ ($P < 0.05$).

with the deep, inside portion of the muscle (King et al., 2018). This color difference was associated with much lower (approximately half of the myoglobin concentration) myoglobin content in the superficial portion of the *biceps femoris* compared with the deep portion. Regression analysis indicated that, in that experiment, myoglobin concentration was the primary driver of lean color of the Halo portion of the muscle. Thus, we speculated that increasing the myoglobin concentration of the Halo portion of the muscle would mitigate the Halo condition and suggested genetic selection for myoglobin to reduce the incidence and severity of the Halo condition. Results of the present experiment suggest that sufficient genetic variation exists in the halo condition for this approach to be successful. This is supported by the heritability estimates (0.27) by Newcom et al. (2004) of *longissimus* myoglobin concentrations in a population of pigs with diverse breed types. Cross et al. (2018) reported lower genomic heritability (0.09) for *longissimus* myoglobin content but also identified QTL for myoglobin in their sample of pork loins obtained from various industry sources.

A QTL identified by Cross et al. (2018), on chromosome 14, is located near the calmodulin-dependent

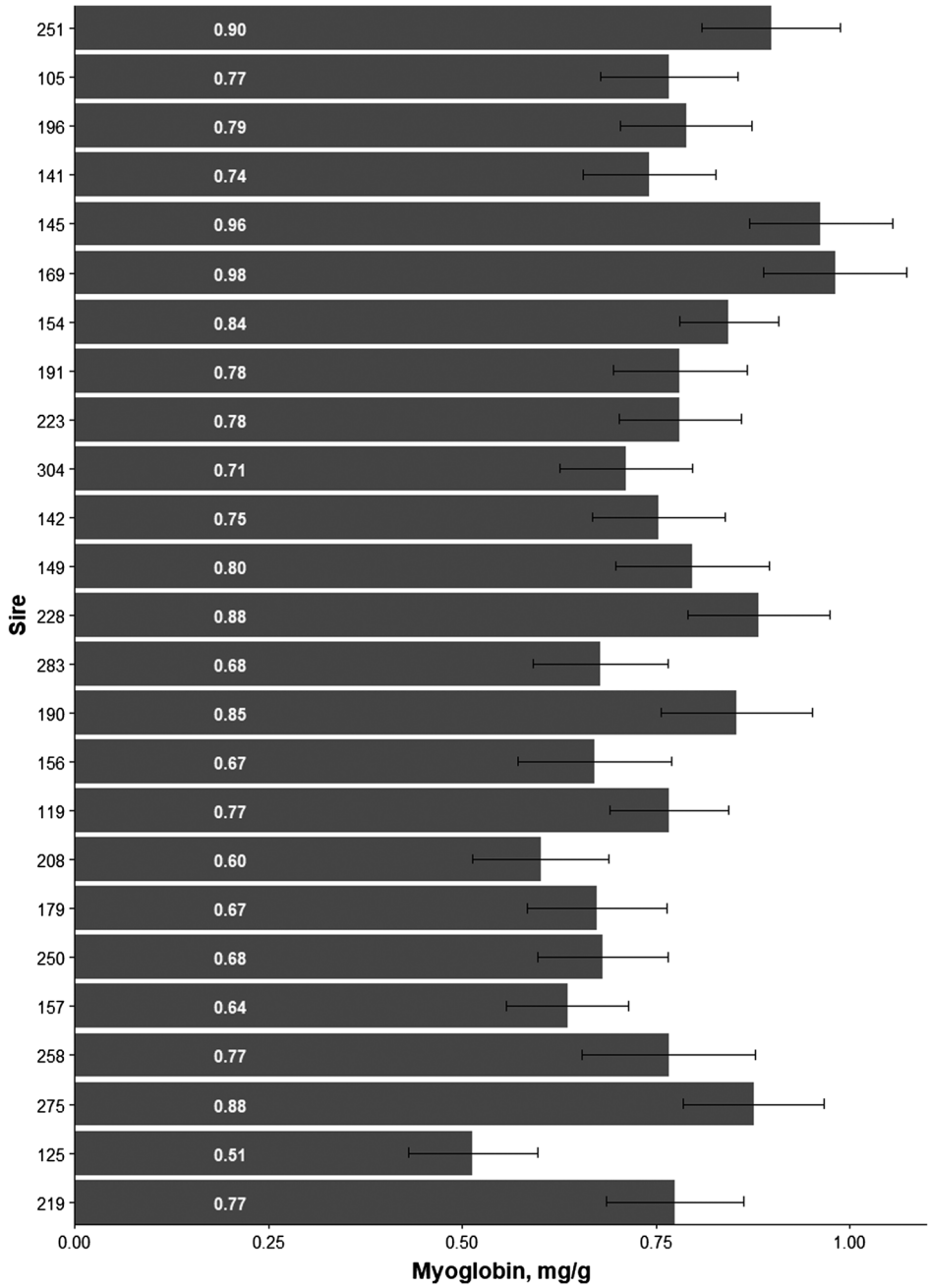

Figure 6. Least-squares means for sire-progeny-group ($P = 0.08$) effect on the difference in myoglobin concentration (milligrams per gram of wet tissue) between the halo and inside portions of the *biceps femoris* muscle.

calcineurin A gene (*PPP3CB*). Calcineurin activation has been linked to increased expression of the myoglobin gene as well as to the slow-fiber–specific troponin-I gene (Chin et al., 1998) and myosin heavy-chain isoforms (Delling et al., 2000). The myoglobin reduction associated with the halo condition is associated with a decrease in Type I fibers and an concomitant increase in Type IIB fibers (King et al., 2018). Thus, selection for the favorable forms of the calcineurin gene may be an appropriate strategy for mitigation of the halo condition. Further efforts to identify and validate predictive markers for myoglobin content in this and other genes is needed to facilitate this selection.

Swine genetics companies have indicated an interest in using genetic selection to reduce or eliminate the halo condition. However, as noted above, validated genetic markers are not currently available for muscle myoglobin concentration. We calculated the HCI to quantify the halo portion of the muscles' L^* and a^* values relative to the range observed in the present experiment. Differences in this index across sire progeny groups are presented in Figure 7. As noted for the L^*

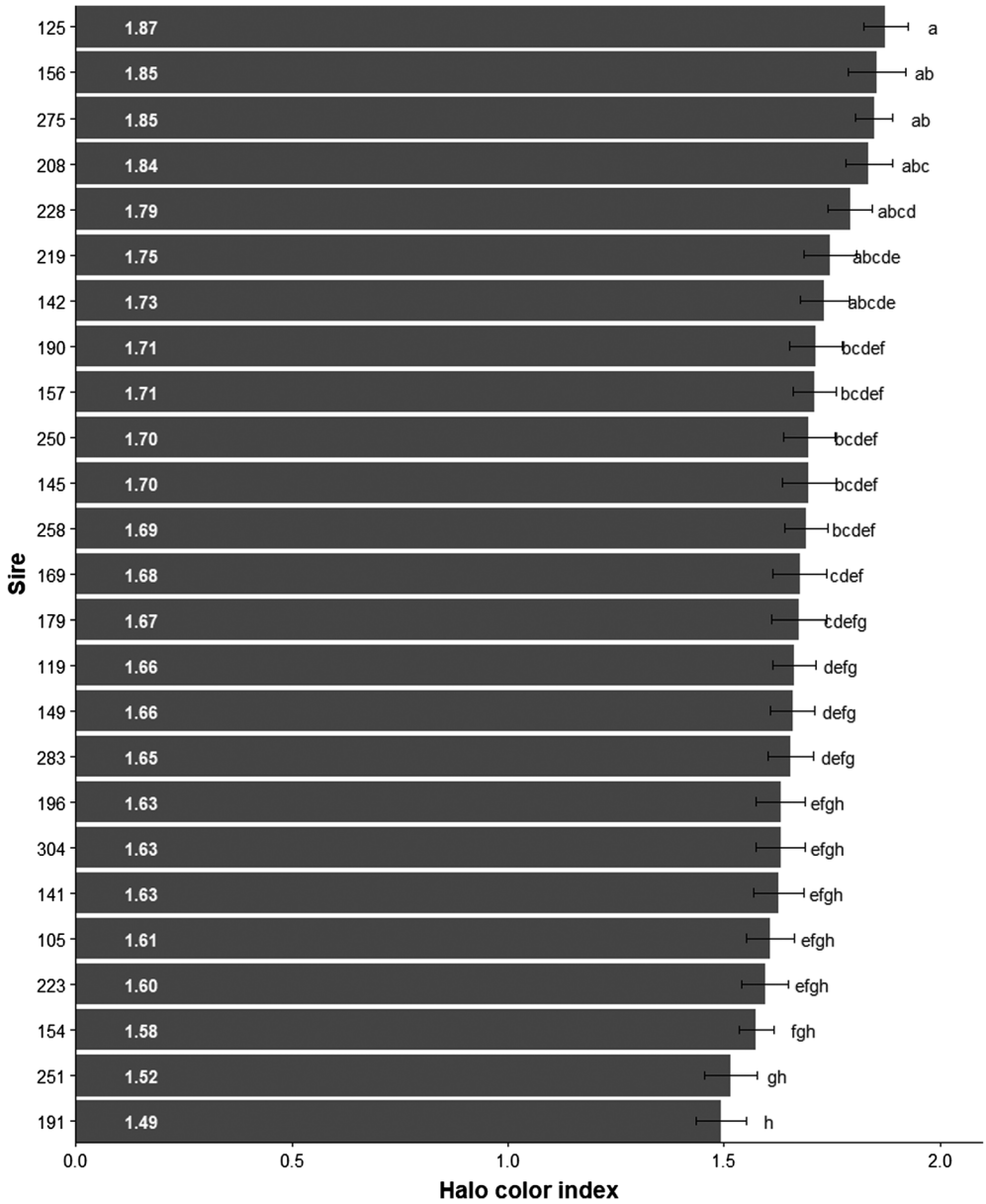

Figure 7. Least-squares means for sire-group effects ($P = 1.5 \times 10^{-5}$) on the Halo Color Index. Halo Color Index = ([$L^*_{halo} - 45$] / 20) + ([$a^*_{halo} - 20$] / 20). Least-squares means lacking a common letter differ ($P < 0.05$).

and a^* values and myoglobin concentration, differences in the HCI existed across sires ($P = 1.6 \times 10^{-6}$). An additional index, the HCDI, was calculated to account for the magnitude of the difference in color between the Halo and Inside portions of the muscle as well as for the color of the Halo portion of the muscle itself. This index also differed ($P = 2.5 \times 10^{-6}$; data not shown) across sire progeny groups. The absolute ranking of sires differed slightly between the two indexes and by myoglobin concentration. However, statistical differences among sire groups were generally consistent across the two indexes and myoglobin concentration. Thus, genetic selection based on the color attributes of the Halo portion of the muscle would be as effective in mitigating the halo condition as selection for myoglobin. The two indices investigated in the present experiment were very similar in their relationships to other color traits. Thus, it appears that inclusion of the difference in L^* and a^* values in the HCDI did not add appreciably to the discrimination among sires regarding the halo condition. It must be noted that these indices were arbitrarily derived to address the question of whether lean color of the halo portion of the muscle would be an effective selection criterion to mitigate the halo condition. Other calculations may account for a greater portion of genetic variation and should be considered.

Conclusions

The halo condition is indicated by a band of very pale muscle tissue in the superficial portion of fresh pork *biceps femoris* muscle. This pale tissue is further characterized by a drastic reduction (48%) in myoglobin concentration in this portion of the muscle. Differences detected among sires in L^*, a^*, and myoglobin content of the halo-affected portion of the *biceps femoris* muscles indicate that sufficient genetic variation exists to successfully reduce the severity of the halo condition by applying selection to either lean color or myoglobin concentration of the Halo portion of the muscle.

Acknowledgments

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 US Department of Agriculture. The authors are grateful to Patty Beska, Kristen Ostdiek, and Casey Trambly 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. The USDA is an equal-opportunity provider and employer.

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