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Contribution of genetic influences to animal-to-animal variation in myoglobin content and beef lean color stability[1]

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ABSTRACT: Longissimus thoracis steaks from steers (n = 464) with 0 to 50% inheritance of Angus, Charolais, Gelbvieh, Hereford, Limousin, Red Angus, and Simmental were evaluated during 6 d of display to assess genetic contributions to color stability. Color space values [CIE L* (lightness), a* (redness), b* (yellowness)], chroma, color change (ΔE), and surface metmyoglobin (K/S 572/525) were determined on d 0 and 6 of display. Myoglobin concentration was highly heritable (0.85), but ultimate pH was weakly heritable (0.06). Day 0 L* values were moderately heritable (0.24). Variation in metmyoglobin, L*, and ΔE on d 6 was moderately explained by genetic factors (41, 40, and 29%, respectively). Change during display was moderately heritable for a* (0.31), b* (0.23), chroma (0.35), and surface metmyoglobin (0.29). At the start of display, Angus steaks had greater ($P < 0.05$) L* values than those from all breeds except Charolais. On d 6, Angus steaks had greater ($P < 0.05$) L* (50.0) values than Gelbvieh, Hereford, and Simmental steaks (46.1, 44.0, and 44.5, respectively). Day 0 values for a*, b*, chroma, and ΔE were not affected by breed ($P > 0.05$). On d 6, a* values were greater ($P < 0.05$) for Charolais and Limousin steaks (31.1 and 30.5) than Angus, Hereford, and Red Angus steaks (27.4, 27.7, and 26.3, respectively). Thus, a* changed less ($P < 0.05$) in Charolais and Limousin steaks (1.8 and 2.6, respectively) vs. steaks from other breeds. Day 6 b* values were greater ($P < 0.05$) in Charolais (24.5) and Limousin steaks (24.0) vs. Gelbvieh (22.2), Hereford (21.9), and Red Angus steaks (21.4). Thus, b* values changed less ($P < 0.05$) in Charolais and Limousin steaks (1.5 and 1.7, respectively) than in Angus, Gelbvieh, Hereford, and Red Angus steaks (4.3, 3.8, 4.4, and 5.1, respectively). After 6 d of display, Charolais and Limousin steaks had greater chroma ($P < 0.05$; 39.5 and 38.8, respectively) compared with Angus, Hereford, and Red Angus steaks (35.4, 35.3, and 33.9, respectively). Less ($P < 0.05$) change in chroma occurred for Charolais and Limousin (2.1 and 2.8, respectively) than in Angus, Gelbvieh, Hereford, and Red Angus steaks (7.1, 6.6, 7.4, and 9.0, respectively). Myoglobin concentration was less for Charolais and Limousin ($P < 0.05$; 2.77 and 2.72, respectively) compared with Gelbvieh, Red Angus, and Simmental steaks (3.62, 3.43, and 3.71, respectively). Breeds did not differ in pH ($P > 0.05$). These data suggest Charolais- and Limousin-carcasses produced steaks with greater lean color stability than Angus, Hereford, and Red Angus carcasses. Furthermore, these findings suggest that genetics contribute substantially to animal-to-animal variation in lean color, particularly in maintaining color.

Key words: beef, breed, color stability, genetics, heritability

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INTRODUCTION

Case-ready packaging has increased retailer demands for beef color-life. Anecdotal evidence from industry sources suggests that some carcasses produce cuts with insufficient color life for case-ready product lines. Previous investigators concluded that animal effects are a smaller source of variation in color stability compared

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with effects such as muscle or storage temperature (Hood, 1980; Renerre and Labas, 1987), and few investigations have addressed animal variation in color stability.

Thus, color stability research has focused on differences due to ante- or postmortem management (Ledward, 1985; Lawrence et al., 2004; Mancini et al., 2008) and across muscles (Talmant and Monin, 1986; McKenna et al., 2005). It is understood that environmental factors such as chilling rate, storage temperature, and microbial load have profound effects on color stability (Seyfert et al., 2005, 2006). Clearly, direct effects of such factors would be expected to be larger than variation in the same muscle across animals (Mancini and Hunt, 2005). However, inherent muscle metabolic characteristics such as pigment concentration, mitochondrial oxygen consumption, and reducing capacity through enzymatic and nonenzymatic mechanisms have been implicated in regulating color stability (Faustman and Cassens, 1990; Bekhit and Faustman, 2005; Mancini and Hunt, 2005). Given the evidence of the influence of genetic regulation on growth rate, muscularity, and leanness (Casas et al., 2003, 2005; White et al., 2007), it seems likely that genetic influences may have some role in influencing metabolic characteristics contributing to variation in color stability.

Genetic contributions to color stability have not been adequately characterized, though biological type has been reported to affect color life (Faustman and Cassens, 1991; Lanari and Cassens, 1991). The present experiment was conducted to characterize animal-to-animal variation and heritability of color stability traits and to determine breed effects on these traits in a population of the most prevalent breeds in the US beef population.

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the US Meat Animal Research Center (**USMARC**) Animal Care and Use Committee.

Animals

Semen was sampled from the 7 most prominent US beef breeds as determined by registration numbers (22 Angus, 21 Red Angus, 21 Hereford, 20 Limousin, 22 Charolais, 23 Gelbvieh, and 20 Simmental sires) and used in matings with Angus, Hereford, and composite MARCIII (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, 1/4 Pinzgauer) cows to create F_1 cows in 1999, 2000, and 2001 as described by Wheeler et al. (2005). The F_1 generation of cows included some straightbred Angus and Hereford cows. In 2001, F_1 bulls were produced from semen of these same 7 breeds (4 sires per breed) mated to Hereford and Angus cows. Resulting bulls and cows were multi-sire mated to produce progeny with 0, 25, or 50% inheritance of each AI sire breed included in the project. Parentage was verified using SNP markers. Steer progeny (n = 464) born in 2006 and 2007 were calf-fed the same corn and corn-silage-based diet and serially slaughtered at a commercial processing plant. Breed representations were approximately equal across slaughter groups. Within 45 min postmortem, carcasses were exposed to 4 zones of electrical stimulation (1 s on, 1 s off; 27 V, 33 V, 38 V, and 45 V, each for 3 to 5 s) as the carcasses were transferred from the slaughter floor to the chilling cooler.

Sample Handling and Preparation

At approximately 36 h postmortem, carcasses were ribbed at the 12th- to 13th-rib interface and presented for grading before fabrication. Wholesale ribs were obtained from 1 side of each animal and transported to the USMARC meat laboratory. Wholesale ribs were dissected as part of a concurrent experiment. Beef ribeye rolls, lip off (similar to Institutional Meat Purchase Specification #112; NAMP, 2003) were obtained after dissection. The most caudal section (18 cm) of the ribeye roll was removed and used in a concurrent project. The remaining portion was vacuum packaged and stored (1°C) until 18 d postmortem. Then a 1-cm slice was removed from the caudal end of the ribeye roll section, and then one 2.54-cm-thick steak was cut (approximately 7th rib) for simulated retail display. A second steak (1.27 cm thick) was cut for determination of pH and myoglobin concentration. Steaks were placed on polystyrene trays with soaker pads and overwrapped with oxygen permeable polyvinylchloride film (Crystal Clear PVC Wrapping Film; Koch Supplies, Kansas City, MO; oxygen transmission rate = 15,500 to 16,275 cm^3 O_2/m^2/24 h at 23°C). Steaks were placed under continuous fluorescent lighting (color temperature = 3,500 K; color rendering index = 86; 32 W; T8 Ecolux bulb, model number F32T8/SPX35 GE; GE Lighting, Cleveland, OH) for 6 d. Light intensity at the meat surface was approximately 2,000 lx. Display was conducted in a refrigerated room (1°C), and no temperature fluctuations associated with defrost cycles were encountered.

Steaks were allowed to bloom for at least 2 h before color measurement began. Instrumental color readings were taken on the longissimus thoracis muscle on d 0 and 6 of display using a Hunter Miniscan XE Plus colorimeter (HunterLab, Reston, VA) with a 25-mm port. The colorimeter 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 steak. Greater L*, a*, and b* values are indicative of increased lightness, redness, and yellowness, respectively. Chroma (color intensity) was calculated as $[(a^{*2} + b^{*2})^{0.5}]$. Overall color change ($\Delta E$) was calculated as $[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}]$, where ΔL^*, Δa^*, and Δb^* are the difference between d 0 and 6 values of L*, a*, and b*, respectively. The accumulation of surface metmyoglobin was monitored using the ratio of the reflectance at 572 and 525 nm after K/S

transformation as described by Hunt et al. (1991). A smaller ratio indicates more metmyoglobin, which gives the meat a brownish color. Change in color variables was calculated as the difference between the measurements made on d 0 and those made on d 6.

pH and Myoglobin Concentration

Steaks reserved for determination of ultimate pH and myoglobin concentration were trimmed free of external fat and epimyseal connective tissue, diced, and pulverized in liquid nitrogen to produce a homogenous powder. Muscle pH was determined as prescribed by Bendall (1973). Duplicate 2.5-g samples were homogenized in 10 vol of a 5 m*M* iodoacetate, 150 m*M* 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 semi-micro combination electrode (Corning Inc., Corning, NY) attached to a Corning 125 pH meter.

Myoglobin was extracted and quantified following the method described by Warriss (1979) as modified by Hunt et al. (1999). Briefly, duplicate 2.5-g samples were homogenized in 10 vol of 40 m*M* 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 (1.5 mL) was syringe filtered (Nalgene 0.45 μm, surfactant-free cellulose acetate membrane; Thermo Fisher Scientific, Rochester, NY) into a 4-mL cuvette with 1 mL of 40 m*M* phosphate buffer and 0.5 mL of sodium hydrosulfite (10 mg/mL) in phosphate buffer. Absorbance spectra from (400 to 700 nm) were obtained using a DU 640 spectrophotometer (Beckman Coulter Inc., Fullerton, CA). Once samples were verified to be in the reduced state (absorbance peak within 2 nm of 433), extracted pigment concentration was calculated using the absorbance at 433 nm, a molar extinction coefficient of 114,000 $M^{-1}\cdot cm^{-1}$, the molecular weight of myoglobin (16,800), and the appropriate dilution factor.

Statistical Analysis

Data were analyzed using an animal model with genetic groups for breed of origin (Westell et al., 1988). The Angus and Hereford cows at USMARC were considered separate genetic groups from the AI sires sampled from those breeds. Therefore, estimates of breed differences that are reported reflect only the AI sires that were sampled. The model included random effects of animal (accounting for genetic relationships), residual, fixed effects of slaughter group, and fixed covariates for % heterosis, and the fractional percentage of each breed (as a genetic group). All data were analyzed using MTDFREML (Boldman et al., 1995). Heritability was estimated first and then held constant to obtain SE and hypothesis tests for breed differences. A predetermined level of 0.05 was used in all judgments of statistical significance.

Although the cattle in the experiment were crossbreds, they varied considerably in the breeds represented in each animal. Each of the breeds was represented as a fraction of multiple crossbreeds, making the breed effects estimable. The breed effects reported were differences in the genetic group estimates. In essence, the observation of each animal was allocated to each of the breeds composing that animal in proportion to the contribution of that breed to the animal (0 to 50%). Thus, the breed effects reported represent the differences that would be expected among purebreds of the respective breeds.

RESULTS

Heritability Estimates

Simple statistics of the traits measured in this study (Table 1) indicate considerable variation in initial lean color and lean color stability during simulated retail display. However, lean color was more variable relative to its mean when measured on d 6 than on d 0 as evidenced by the CV. Similarly, in general, the greatest animal-to-animal variation was observed in the change in these variables between d 0 and 6, suggesting that more animal-to-animal variation was associated with the ability of the muscle to resist color change than in initial color.

Heritability estimates for lean color attributes measured at the initiation and conclusion of simulated retail display are presented in Table 2. Myoglobin concentration was very heritable with 85% of the variation in myoglobin content being explained by genetic factors. Of the lean color variables, only L* values were moderately heritable when measured at the initiation of retail display. Initial a*, b*, chroma, and surface metmyoglobin values were not heritable. Lean color variables were more dependent on genetic factors after 6 d of simulated retail display. Muscle lightness (L*), surface metmyoglobin, and overall color change (ΔE) were moderately heritable (0.20 to 0.41) on d 6. Heritability estimates for a*, b*, and chroma indicated that a very small portion of the variation in these traits on 6 d of retail display could be explained by genetic influences. However, the change in a*, b*, chroma, and surface metmyoglobin content during display were lowly to moderately heritable. Genetic factors did not explain variation in ultimate muscle pH. Estimates of heterosis were not significant for any of the traits evaluated in this study.

Breed Effects

Breed effects are summarized as least squares means in Table 3. At the beginning of display, Angus inheritance resulted in greater ($P < 0.05$) L* values than

Table 1. Simple statistics across all animals for initial and final values of lean color variables and change in lean color variables measured on longissimus thoracis steaks in simulated retail display for 6 d

Variable	Mean	SD	Minimum	Maximum	CV
d 0 L*	48.6	3.03	38.8	58.5	6.24
d 6 L*	47.1	3.16	33.6	56.3	6.70
ΔL*[1]	1.4	1.94	−7.7	8.5	137.68
d 0 a*	33.7	1.56	28.8	38.4	4.63
d 6 a*	26.6	2.63	16.6	34.8	9.87
Δa*[1]	7.1	2.79	−1.6	15.7	39.18
d 0 b*	26.5	1.93	21.9	32.1	7.30
d 6 b*	21.4	1.83	14.6	28.2	8.56
Δb*[1]	5.1	2.43	−2.8	12.0	47.90
d 0 Chroma	42.9	2.37	36.9	50.1	5.53
d 6 Chroma	34.2	3.10	22.5	44.6	9.08
ΔChroma[1]	8.7	3.52	−2.9	18.1	40.42
d 0 K/S 572/525	1.45	0.05	1.33	1.62	3.12
d 6 K/S 572/525	1.24	0.08	0.92	1.62	6.43
Δ K/S 572/525[1]	0.21	0.08	−0.08	0.55	40.12
ΔE[2]	9.2	3.32	0.3	18.1	36.10

[1]Change in color variables calculated as the difference between values measured on d 0 and 6 of display.
[2]Overall color change $[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}]$ calculated using the change in instrumental color space variables between d 0 and 6 of display.

Gelbvieh, Hereford, Limousin, Red Angus, and Simmental inheritance. Charolais inheritance resulted in intermediate L* values on d 0. After 6 d of retail display, steaks from Angus-influenced animals still had greater ($P < 0.05$) L* values than steaks from Gelbvieh-, Hereford-, and Simmental-influenced animals. However, the change in L* values during display was not affected by breed type.

In contrast, initial values for a*, b*, and chroma were not affected by breed on d 0, but these traits on d 6 and their change during display differed across breeds. On d 6, steaks from Charolais- and Limousin-influenced steer carcasses had greater ($P < 0.05$) a* values than those from carcasses of Angus-, Red Angus-, and Hereford-influenced steers. Gelbvieh and Simmental inheritance produced steaks with intermediate a* values on d 6. Consequently, the change in a* during display was much smaller ($P < 0.05$) for steaks from Charolais- and Limousin-influenced carcasses than for those from all other breeds included in the study.

Similarly, Gelbvieh, Hereford, and Red Angus inheritance produced longissimus thoracis steaks with smaller ($P < 0.05$) b* values on d 6 of retail display than those from carcasses of Charolais- and Limousin-influenced steers. Consequently, Limousin and Charolais inheritance produced longissimus thoracis steaks with less change in yellowness than Angus, Red Angus, Gelbvieh, and Hereford inheritance during simulated retail display. Steaks from carcasses of Charolais- and Limousin-influenced steers had greater ($P < 0.05$) lean color intensity (chroma) values on d 6 of display than steaks from Angus, Hereford, and Red Angus steer carcasses, with steaks from Gelbvieh and Simmental steer carcasses being intermediate. Results for change in color intensity during the 6 d of retail display followed the same pattern. Overall color change (ΔE) was much smaller ($P < 0.05$) in steaks from Charolais- and Limousin-influenced carcasses than those from Angus, Gelbvieh, Hereford, and Red Angus carcasses, with color change in steaks from Simmental-influenced carcasses being intermediate. Myoglobin concentrations were least in Limousin-influenced carcasses and greatest in Gelbveih-, Red Angus-, and Simmental- influenced carcasses, and intermediate in Charolais-, Angus-, Red Angus-, and Hereford-influenced carcasses.

Table 2. Heritability estimates (SE) of lean color at the initiation and conclusion of retail display and the change in these

Trait	d 0	d 6	Change[1]
L*	0.24 (0.13)	0.40 (0.16)	0.00 (0.07)
a*	0.06 (0.09)	0.14 (0.12)	0.31 (0.15)
b*	0.00 (0.05)	0.13 (0.12)	0.23 (0.13)
Chroma	0.00 (0.07)	0.13 (0.12)	0.35 (0.15)
K/S 572:K/S 525	0.00 (0.08)	0.41 (0.18)	0.29 (0.15)
ΔE[2]	—	0.29 (0.14)	—
Myoglobin concentration	0.85 (0.18)	—	—
pH	0.06 (0.10)	—	—

[1]Change in color variables calculated as the difference between values measured on d 0 and 6 of display.
[2]Overall color change $[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}]$ calculated using the change in instrumental color space variables between d 0 and 6 of display.

Table 3. Least squares means (SE) for breed effects on lean color attributes measured at the initiation or conclusion of retail display and for the change in color attributes during retail display

Item	Angus	Charolais	Gelbvieh	Hereford	Limousin	Red Angus	Simmental	$P > F$
L*								
d 0	52.8^{d}	49.7^{cd}	48.0^{c}	47.3^{c}	49.2^{c}	48.6^{c}	47.0^{c}	0.02
	(1.72)	(1.81)	(1.85)	(1.89)	(1.72)	(0.75)	(1.80)	
d 6	50.1^{d}	46.9^{bcd}	46.1^{bc}	44.0^{b}	47.8^{cd}	46.8^{bcd}	44.5^{b}	0.03
	(1.88)	(1.95)	(1.99)	(2.05)	(1.87)	(0.79)	(1.97)	
Change[1]	2.5	3.0	1.9	3.1	1.3	1.8	2.4	0.34
	(1.02)	(1.13)	(1.16)	(1.11)	(1.04)	(0.50)	(1.05)	
a*								
d 0	32.5	32.6	33.6	33.2	32.3	33.7	33.1	0.29
	(0.75)	(0.82)	(0.84)	(0.83)	(0.77)	(0.35)	(0.78)	
d 6	27.4^{bc}	31.1^{d}	28.4^{bcd}	27.7^{bc}	30.5^{d}	26.3^{b}	29.7^{cd}	0.02
	(1.41)	(1.51)	(1.55)	(1.56)	(1.43)	(0.64)	(1.48)	
Change[1]	5.7^{cd}	1.8^{b}	5.4^{cd}	6.0^{cd}	2.4^{b}	7.4^{d}	4.1^{bc}	0.02
	(1.66)	(1.74)	(1.77)	(1.81)	(1.65)	(0.71)	(1.73)	
b*								
d 0	26.2	26.0	25.9	26.1	25.4	26.5	25.8	0.55
	(0.69)	(0.77)	(0.79)	(0.76)	(0.71)	(0.34)	(0.71)	
d 6	22.3^{bcd}	24.5^{d}	22.2^{b}	21.9^{b}	24.0^{cd}	21.4^{b}	23.2^{bcd}	0.03
	(0.98)	(1.05)	(1.08)	(1.08)	(0.99)	(0.45)	(1.03)	
Change[1]	4.3^{d}	1.5^{c}	3.8^{d}	4.4^{d}	1.7^{c}	5.1^{d}	3.0^{cd}	0.02
	(1.15)	(1.22)	(1.24)	(1.26)	(1.16)	(0.51)	(1.21)	
Chroma								
d 0	41.7	41.7	42.4	42.2	41.0	42.9	41.8	0.45
	(0.97)	(1.07)	(1.10)	(1.06)	(0.99)	(0.47)	(1.00)	
d 6	35.4^{ab}	39.5^{d}	36.1^{abc}	35.2^{ab}	38.8^{cd}	33.9^{a}	37.7^{bcd}	0.02
	(1.66)	(1.78)	(1.83)	(1.83)	(1.68)	(0.75)	(1.74)	
Change[1]	7.1^{d}	2.1^{c}	6.6^{d}	7.4^{d}	2.8^{c}	9.0^{d}	5.0^{cd}	0.01
	(1.98)	(2.08)	(2.12)	(2.17)	(1.98)	(0.86)	(0.05)	
K/S 572/525								
d 0	1.44	1.43	1.42	1.42	1.44	1.44	1.43	0.89
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.01)	(0.02)	
d 6	1.26	1.34	1.26	1.25	1.33	1.23	1.29	0.12
	(0.05)	(0.05)	(0.05)	(0.05)	(0.05)	(0.02)	(0.05)	
Change[1]	0.17	0.10	0.15	0.18	0.10	0.21	0.13	0.14
	(0.04)	(0.05)	(0.05)	(0.05)	(0.04)	(0.02)	(0.05)	
ΔE^{2}	7.7^{d}	3.0^{c}	7.3^{d}	8.1^{d}	3.8^{c}	9.3^{d}	6.2^{cd}	0.02
	(1.93)	(2.01)	(2.05)	(2.11)	(1.93)	(0.82)	(2.02)	
Myoglobin, mg/mL	3.05^{cd}	2.77^{bc}	3.62^{d}	3.34^{cd}	2.72^{b}	3.43^{d}	3.71^{d}	0.01
	(0.36)	(0.37)	(0.37)	(0.39)	(0.36)	(0.15)	(0.38)	
pH	5.50	5.57	5.54	5.58	5.56	5.56	5.57	0.11
	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.01)	(0.03)	

[a–d]Least squares means within a row lacking common superscripts differ ($P < 0.05$).

[1]Change in color variables calculated as the difference between values measured on d 0 and 6 of display.

[2]Overall color change $[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}]$ calculated using the change in instrumental color space variables between d 0 and 6 of display.

Muscle pH was not affected ($P > 0.05$) by breed. Similarly, breeds did not differ with regard to surface metmyoglobin at the initiation of the display period. Breed effects were not statistically significant ($P < 0.05$) for final K/S 572/525 values or the change in these values during the 6 d of display. However, final values for K/S 572/525 and the change in this variable tended ($P = 0.12$ and 0.14, respectively) to be affected by breed. In agreement with other color variables, Charolais- and Limousin-influenced steaks tended to have the greatest K/S 572/525 ratios (least surface metmyoglobin accumulation) on d 6. Consequently the change in K/S 572/525 ratio tended to be least for these breeds. Thus, accumulation of surface metmyoglobin was numerically less in steaks from carcasses of Charolais and Limousin influence compared with those influenced by the other breeds evaluated in the study.

DISCUSSION

To our knowledge, this experiment represents the first attempt in characterizing the influence of genetic inheritance on lean color and lean color stability in a large population of cattle developed to represent the US beef population. The amount of animal-to-animal variation in the change in lean color observed in this experiment was large enough to support anecdotal evidence from the industry that substantial variation exists across animals with regard to color stability. Further-

more, it appears that the animal-to-animal differences are primarily displayed in the ability of the muscle to maintain color rather than in initial color.

Display conditions used in the present study were at a colder temperature (1°C) than is typically used in retail display, and no temperature fluctuations associated with defrost cycles were encountered. Additionally, the display period was limited to 6 d in an attempt to measure steaks when animal-to-animal variation would still be evident (i.e., not at a point when all steaks were completely discolored). However, 6 d may not have been long enough for differences in metmyoglobin formation to fully develop in the relatively color-stable longissimus thoracis, particularly at reduced temperatures. Thus, variation and breed effects in K/S 572/525 ratios are likely underestimated.

Heritability estimates for lean color attributes at the conclusion of display and the change in these variables during display suggest that significant opportunity exists to improve color stability through genetic selection. Large SE associated with the heritability estimates of color variables at the end of display and the change in these variables during display indicate that additional work is needed to further define the extent to which lean color stability is genetically regulated. However, these estimates clearly demonstrate the existence of inherent animal-to-animal differences for lean color stability in the US beef population. Furthermore, this work provides evidence that genetic factors play a greater role in maintaining lean color than in determining initial color values.

Heritability estimates for initial CIE color space values of pork LM reported by Newcom et al. (2004; 0.98 to 0.52) were much greater than those reported for beef in the present study. However, Newcom et al. (2004) estimated heritability of soluble myoglobin ($h^2 = 0.27$) to be much less than the estimate calculated in the present study. Previous work in beef has reported that animal effects explain a relatively small portion of the variance in lean color stability compared with muscle effects (Hood, 1980; Renerre and Labas, 1987) and storage temperature (Hood, 1980).

Breed differences in color variables and the change in those variables during 6 d of display were remarkably consistent with one another. In general, Charolais and Limousin inheritance resulted in increased color stability compared with the other breeds investigated, whereas Angus, Red Angus, and Hereford inheritance was associated with decreased color stability compared with other breeds included in the experiment. Simmental and Gelbvieh inheritance appeared to result in intermediate color stability characteristics, though these breeds appeared to be more like the breeds with more labile color attributes. Wheeler et al. (2005) reported breed comparisons for carcass and palatability traits of F_1 steer progeny of the population that produced the dams of the animals used in the current experiment. Rankings of these breeds with regard to color stability indicating traits are generally consistent with the ranking of these breeds with regard to carcass yield and inversely related to the rankings of these breeds with regard to marbling score. Slowed chilling of very large muscles would likely have deleterious effects on color stability (Sammel et al., 2002), but metabolic differences that contribute to increased muscle and reduced fatness in these breeds may contribute to increased color life.

In a detailed review pertaining to the biological basis of lean color stability, Faustman and Cassens (1990) identified oxygen consumption, reducing capacity, and lipid oxidation as endogenous characteristics that contribute to variation in color-life of fresh meat. These factors influence the ability of the muscle to maintain color via their effects on oxygenation, oxidation, and reduction reactions involving myoglobin (Mancini and Hunt, 2005). Furthermore, variation in reducing activity and oxygen consumption may be a function of the ability of the muscle to conserve or regenerate NADH postmortem, or both (Hunt and Mancini, 2009). Thus, genetic regulation of metabolism likely would influence animal-to-animal variation in each of these factors.

Faustman and Cassens (1991) reported that beef LM and gluteus medius steaks from Holstein steers were more color labile vs. those from crossbred beef steers. Those investigators suggested that selection for milk production may have made muscle metabolism in Holstein steers more oxidative than in crossbred steers, resulting in darker and more labile lean color. Lanari and Cassens (1991) tested this hypothesis and found LM and gluteus medius muscles from Holstein carcasses to have less mitochondrial protein than the same muscles from crossbred beef steers, but muscles from Holstein carcasses also had greater oxygen consumption rate and metmyoglobin reducing activity, and were more color labile than muscles from crossbred beef carcasses.

In the present experiment, animals from breeds with the most stable lean color generally had decreased myoglobin concentrations compared with animals from the other breeds. With the exception of Angus, breeds with more labile lean color generally had darker initial lean color (smaller numerical L* values) than more color-stable breeds. This may indicate that LM from animals of these breeds had greater oxidative metabolism than those with more stable lean color. This premise is supported by the findings of Cuvelier et al. (2006), which reported that Angus bulls had greater cytochrome c oxidase activity, less lactate dehydrogenase activity, and smaller L* values than Limousin and Belgian Blue bulls. Ozawa et al. (2000) reported that Japanese Black steers from multiple closed herds differed with regard to LM fiber type distribution and fiber size. Moreover, lean color was positively correlated with β- and α-red muscle fiber diameter. Thus, it is evident that differences in muscle metabolism are genetically influenced, though environmental effects also would play a large role in determining these characteristics. May et al. (1977) reported that Limousin × Angus crossbred steers had a greater proportion of α-white muscle fibers

in the LM than Hereford × Angus and Simmental × Angus crossbred steers. Johnston et al. (1975) reported that LM from Charolais steers had larger fiber areas of all types, and α-white fibers in particular, when compared with LM from Angus steers. These findings also suggest that differences exist in LM muscle metabolism among the breeds used in this study.

Color stability differences across muscles are generally attributed to increased oxidative metabolism in less stable muscles (O'Keefe and Hood, 1982; Faustman and Cassens, 1991; Lanari and Cassens, 1991). Muscles possessing greater oxidative metabolism would have a greater concentration of mitochondria and mitochondrial enzymes that compete with myoglobin for available oxygen in postmortem muscle. Thus, a greater proportion of myoglobin is in the deoxygenated form, which is more susceptible to oxidation than oxymyoglobin. Moreover, the surface layer of cherry-red oxymyoglobin is thinner in steaks from muscles with greater oxidative metabolism.

Muscles with increased oxidative metabolism also would have greater metmyoglobin reductase activity (Echevarne et al., 1990) because this enzyme is associated with the electron transport chain. However, the role of metmyoglobin reductase activity in maintaining myoglobin in the reduced state has been debated in the literature. Numerous investigators have reported little relationship between metmyoglobin reductase activity and metmyoglobin formation (O'Keefe and Hood, 1982; Echevarne et al., 1990; Lanari and Cassens, 1991). Others have indicated metmyoglobin reducing activity is important in inhibiting discoloration (Ledward, 1985; Bekhit et al., 2003; Mancini et al., 2008).

Echevarne et al. (1990) suggested that NADH, which is required for metmyoglobin reduction via enzymatic and nonenzymatic processes, may be the limiting factor in maintaining reduced myoglobin. In support of this hypothesis, injection enhancement of beef steaks with solutions containing lactate has been reported to increase color-life (Lawrence et al., 2004; Kim et al., 2006). Kim et al. (2006) demonstrated that lactate was converted to pyruvate via lactate dehydrogenase, generating NADH. Watts et al. (1966) suggested that this reaction may occur normally in postmortem muscle.

Giardina et al. (1996) reported that increased lactate concentration decreased affinity of myoglobin for oxygen. Mancini and Ranianathan (2008) found increased content of lactate (100 to 200 m*M*) stabilized horse myoglobin and suggested that lactate had a direct effect in stabilizing myoglobin in the reduced form. Perhaps those animals from breeds demonstrating greater color stability had greater ability to produce NADH or greater lactate concentrations, which enabled greater myoglobin stability in those muscles. Utilizing animals from breeds differing in color stability characteristics in future work may be useful in elucidating the causative mechanisms regulating animal variation in color stability.

The present experiment suggests that animal-to-animal variation in color stability is, to some extent, genetically regulated. Furthermore, genetic effects had greater influence on the maintenance of lean color than on initial color; some breeds of cattle produce steaks with greater color life than other breeds, perhaps because their muscles generate greater amounts of key metabolic intermediates, resulting in more NADH being available for metmyoglobin reduction. Steaks from carcasses of animals from these breeds would result in fewer losses by retailers and would likely be more suited to case-ready programs than steaks from less stable breeds.

LITERATURE CITED

Bekhit, A. D., and C. Faustman. 2005. Metmyoglobin reducing activity. Meat Sci. 71:407–439.

Bekhit, A. D., G. H. Geesink, M. A. Ilian, J. D. Morton, R. Sedcole, and R. Bickerstaffe. 2003. Particulate myoglobin reducing activity and its relationship with meat color. J. Agric. Food Chem. 51:6026–6035.

Bendall, J. R. 1973. Postmortem changes in muscle. Pages 243–309 in Structure and Function of Muscle. Vol. 2. G. H. Bourne, ed. Academic Press, New York, NY.

Boldman, K. G., L. A. Kriese, L. D. Van Vleck, C. P. Van Tassel, and S. D. Kachman. 1995. A manual for the use of MTDFREML. A set of programs to obtain estimates of variance and covariances (Draft). USDA, ARS, Lincoln, NE.

Casas, E., S. D. Shackelford, J. W. Keele, M. Koohmaraie, T. P. L. Smith, and R. T. Stone. 2003. Detection of quantitative trait loci for growth and carcass composition in cattle. J. Anim. Sci. 81:2976–2983.

Casas, E., S. N. White, D. G. Riley, T. P. L. Smith, R. A. Brenneman, T. A. Olson, D. D. Johnson, S. W. Coleman, G. L. Bennett, and C. C. Jr. Chase. 2005. Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in *Bos indicus* cattle. J. Anim. Sci. 83:13–19.

Cuvelier, C., A. Clingquart, J. F. Hocquette, J. F. Cabaraux, I. Dufrasne, L. Istasse, and J. L. Hornick. 2006. Comparison of composition and quality traits of meat from young finishing bulls from Belgian Blue, Limousin, and Aberdeen Angus breeds. Meat Sci. 74:522–531.

Echevarne, C., M. Renerre, and R. Labas. 1990. Metmyoglobin reductase activity in bovine muscles. Meat Sci. 27:161–172.

Faustman, C., and R. G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: A review. J. Muscle Foods 1:217–243.

Faustman, C., and R. G. Cassens. 1991. The effect of cattle breed and muscle type on discoloration and various biochemical parameters in fresh beef. J. Anim. Sci. 69:184–193.

Giardina, B., P. Ascenzi, M. E. Clementi, G. De Sanctis, M. Rizzi, and M. Coletta. 1996. Functional modulation by lactate of myoglobin. A monomeric allosteric hemoprotein. J. Biol. Chem. 271:16999–17001.

Hood, D. E. 1980. Factors affecting the rate of metmyoglobin accumulation in pre-packaged beef. Meat Sci. 4:247–265.

Hunt, M. C., J. C. Acton, R. C. Benedict, C. R. Calkins, D. P. Cornforth, L. E. Jeremiah, D. G. Olson, C. P. Salm, J. W. Savell, and S. D. Shivas. 1991. Guidelines for meat color evaluation. Appendix to Proc. Recip. Meat Conf. 44:1–17.

Hunt, M. C. and R. A. Mancini. 2009. Colour stability of fresh meat. Proc. Int. Congr. Meat Sci. Technol. 55:PS8.03.

Hunt, M. C., O. Sørheim, and E. Slinde. 1999. Color and heat denaturation of myoglobin forms in ground beef. J. Food Sci. 64:847–851.

Johnston, D. M., D. F. Stewart, W. G. Moody, J. Boling, and J. D. Kemp. 1975. Effect of breed and time on feed on the size and distribution of beef muscle fiber types. J. Anim. Sci. 40:613–620.

Kim, Y. H., M. C. Hunt, R. A. Mancini, M. Seyfert, T. M. Loughin, D. H. Kropf, and S. Smith. 2006. Mechanism for lactate-color stabilization in injection-enhanced beef. J. Agric. Food Chem. 54:7856–7862.

Lanari, M. C., and R. G. Cassens. 1991. Mitochondrial activity and beef muscle color stability. J. Food Sci. 56:1476–1479.

Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2004. Effects of enhancing beef *longissimus* with phosphate plus salt, or calcium lactate plus non-phosphate water binders plus rosemary extract. Meat Sci. 67:129–137.

Ledward, D. A. 1985. Post-slaughter influence on the formation of metmyoglobin in beef muscles. Meat Sci. 15:149–171.

Mancini, R. A., and M. C. Hunt. 2005. Current research in meat color: 51st International Congress of Meat Sci. and Technology (ICoMST). Meat Sci. 71:100–121.

Mancini, R. A., and R. Ranianathan. 2008. Sodium lactate influences myoglobin redox stability in vitro. Meat Sci. 78:529–532.

Mancini, R. A., M. Seyfert, and M. C. Hunt. 2008. Effects of data expression, sample location, and oxygen partial pressure on initial nitric oxide metmyoglobin formation and metmyoglobin-reducing-activity measurement in beef muscle. Meat Sci. 79:244–251.

May, M. L., M. E. Dikeman, and R. Schalles. 1977. *Longissimus* muscle histological characteristics of Simmental × Angus, Hereford × Angus, and Limousin × Angus crossbred steers as related to carcass composition and meat palatability traits. J. Anim. Sci. 44:571–580.

McKenna, D. R., P. D. Mies, B. E. Baird, K. D. Pfeiffer, J. W. Ellebracht, and J. W. Savell. 2005. Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. Meat Sci. 70:665–682.

NAMP. 2003. Meat Buyers Guide. Natl. Assoc. Meat Purveyors, Reston, VA.

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. 82:2264–2268.

O'Keefe, M., and D. E. Hood. 1982. Biochemical factors influencing metmyoglobin formation on beef from muscles of differing colour stability. Meat Sci. 7:209–228.

Ozawa, S., T. Mitsuhashi, M. Mitsumoto, S. Matsumoto, N. Itoh, K. Itagaki, Y. Kohno, and T. Dohgo. 2000. The characteristics of muscle fiber types of *longissimus thoracis* muscle their influences on the quantity and quality of meat from Japanese Black steers. Meat Sci. 54:65–70.

Renerre, M., and R. Labas. 1987. Biochemical factors influencing metmyoglobin formation in beef muscles. Meat Sci. 19:151–165.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister, C. L. Kastner, and D. E. Johnson. 2002. Influence of chemical characteristics of beef inside and outside semimembranosus on color traits. J. Food Sci. 67:1323–1330.

Seyfert, M., M. C. Hunt, R. A. Mancini, K. A. Hachmeister, D. H. Kropf, J. A. Unruh, and T. M. Loughin. 2005. Beef knuckle hot boning and modified atmosphere packaging influence properties of injection-enhanced beef round muscles. J. Anim. Sci. 83:686–693.

Seyfert, M., R. A. Mancini, M. C. Hunt, J. Tang, C. Faustman, and M. Garcia. 2006. Color stability, reducing activity, and cytochrome c oxidase activity of five bovine muscles. J. Agric. Food Chem. 54:8919–8925.

Talmant, A., and G. Monin. 1986. Activities of metabolic and contractile enzymes in 18 bovine muscles. Meat Sci. 18:23–40.

Warriss, P. D. 1979. The extraction of haem pigments from fresh meat. J. Food Technol. 14:75–80.

Watts, B. M., J. Kendrick, M. W. Zipser, B. K. Hutchins, and B. Saleh. 1966. Enzymatic reducing pathways in meat. J. Food Sci. 31:855–861.

Westell, R. A., R. L. Quaas, and L. D. Van Vleck. 1988. Genetic groups in an animal model. J. Dairy Sci. 71:1310–1318.

Wheeler, T. L., L. V. Cundiff, S. D. Shackelford, and M. Koohmaraie. 2005. Characterization of biological types of cattle (Cycle VII): Carcass, yield, and longissimus palatability traits. J. Anim. Sci. 83:196–207.

White, S. N., E. Casas, M. F. Allan, J. W. Keele, W. M. Snelling, T. L. Wheeler, S. D. Shackelford, M. Koohmaraie, and T. P. L. Smith. 2007. Evaluation in beef cattle of six deoxyribonucleic acid markers developed for dairy traits reveals an osteopontin polymorphism associated with postweaning growth. J. Anim. Sci. 85:1–10.