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# Effect of time of measurement on the relationship between metmyoglobin reducing activity and oxygen consumption to instrumental measures of beef longissimus color stability ☆

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ABSTRACT

Contributions of initial and retained levels of oxygen consumption and reducing capacity to animal variation in color stability were evaluated. Instrumental color values were determined on longissimus steaks (n = 257) during 6 d of display. Oxygen consumption (OC), nitric oxide metmyoglobin reduction (NORA), initial metmyoglobin formation (IMF), and post-reduction metmyoglobin (PRM) were measured on d 0 and 6. During display, color variables, OC and reducing ability decreased ($P<0.05$). Color stable steaks had greater ($P<0.05$) reducing ability on d 0 and 6 and lower ($P<0.05$) OC on d 0 than unstable steaks. Color change was correlated to OC, NORA, and PRM on d 0 ($r = 0.19$, $-0.44$ and 0.45, respectively) and to NORA and PRM on d 6 ($r = -0.50$ and 0.52, respectively). These data suggest that initial capacity for OC and reducing ability, combined with retained reducing ability contribute to animal variation in color stability.

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## 1. Introduction

Anecdotal evidence from the industry suggests that substantial animal-to-animal variation in color stability exists, with some carcasses producing cuts with color-life that is insufficient for use in case-ready product lines. King et al. (2010) indicated animal variation in lean color stability exists, and is to some extent, genetically regulated. Greater understanding of the mechanisms responsible for animal variation in color stability would aid in designing strategies to improve lean color stability. Metmyoglobin reducing activity and oxygen consumption have been implicated as important determinants of color stability (Faustman & Cassens, 1990; Bekhit & Faustman, 2005; Mancini & Hunt, 2005). Many studies characterizing the relationships between these factors and color change during retail display report correlations calculated using data from multiple muscles varying greatly in metabolism. These relationships may not be representative of those within single muscles among animals.

Furthermore, metmyoglobin reducing activity is generally measured at multiple points during display and correlations across days are reported. It is evident that oxygen consumption and metmyoglobin reducing activity contribute to color stability and that changes in these traits correspond to degradation of lean color. However, it is not clear whether the variation observed in these traits during display is due to variation in the capacity for reducing activity and oxygen consumption initially present in the muscle or due to variation in the capacity maintained or regenerated throughout display or a combination. The present experiment was designed to determine whether animal-to-animal variation in lean color stability is better explained by oxygen consumption and metmyoglobin reducing activity when measured at the initiation or conclusion of simulated retail display.

## 2. Materials and methods

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

### 2.1. Animals

Semen was sampled from the seven most prominent U.S. beef breeds as determined by registration numbers (22 Angus, 21 Red Angus, 21 Hereford, 20 Limousin, 22 Charolais, 23 Gelbvieh, and 20 Simmental) and used in matings with Angus, Hereford, and composite MARCIII (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, 1/4 Pinazgauer) cows to create $F_1$ cows in 1999, 2000, and 2001 as described by Wheeler, Cundiff, Shackelford, and Koohmaraie (2005). 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

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product to the exclusion of other products that may also be suitable.

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multi-sire mated to produce progeny with 0 to 50% inheritance of each of the sampled sire breeds. Male progeny (n = 257) born in 2006 were castrated within 24 h of birth. Steers were weaned at approximately 165 d of age, calf-fed a corn and corn-silage based diet, and serially slaughtered in four groups at a commercial processing plant at approximately 15 months of age. 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, each and 45 V for 3 to 5 s) as the carcasses were transferred from the slaughter floor to the chilling cooler.

### 2.2. Sample handling and preparation

At 36 h postmortem, carcasses were ribbed at the 12th–13th rib interface and presented for grading prior to fabrication. Descriptive statistics for the carcass traits of the animals used in this experiment are presented in Table 1. Wholesale ribs were obtained from one side of each animal and transported to the U.S. Meat Animal Research Center (USMARC) meat laboratory, where wholesale ribs were dissected as part of a concurrent experiment. Beef ribeye roll, lip off (similar to IMPS # 112; NAMP, 2003) were obtained after dissection, and the most caudal section (18 cm) was removed and used in a concurrent project. The remaining portion was vacuum packaged and stored (1 °C) until 18 d postmortem. After aging, the ribeye sections were opened, and a 1.27-cm thick slice was removed for pH and myoglobin concentration determination. Then, two 2.54-cm thick steaks were cut. The first steak was placed in simulated retail display; the second was used to measure oxygen consumption and metmyoglobin reducing activity. Steaks were oriented so that the top of each steak (which was exposed to light) represented the interface between the two steaks, thereby minimizing location differences.

Steaks used for simulated retail display were placed on plastic trays with soaker pads and overwrapped with oxygen permeable polyvinylchloride film (Crystal Clear PVC Wrapping Film; Koch Supplies, Kansas City, MO; Oxygen transmission rate = 15,500–16,275 $cm^3$ $O_2/m^2$/24 h at 23 °C). Steaks were placed under continuous fluorescent lighting (Color temperature = 3500 K; CRI = 86; 32 W; T8 Ecolux bulb, model number F32T8/SPX35 GE; GE Lighting, Cleveland, OH). 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 occurred.

After overwrapping, 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 each day of display (d 0 through 6) 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. CIE L* (lightness), a* (redness), and b* (yellowness) values were reported as the average of duplicate readings taken on each steak. Hue angle was calculated as [ATAN(b*/a*)*180/3.142]. Chroma (also referred to as saturation index and color intensity) was calculated as: $[(a^{*2} + b^{*2})^{0.5}]$. Overall color change (ΔE) 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. Percentage reflectance from 400 to 700 nm were also collected to estimate the accumulation of surface metmyoglobin using the ratio of the reflectance at 572 and 525 nm after K/S transformation as described by Hunt et al. (1991). Lower $K/S_{572}/K/S_{525}$ ratios indicate greater metmyoglobin levels. Change in color parameters was calculated as the difference between the measurements made on d 0 and those made on d 6.

From the second steak, a 2.54 cm × 2.54 cm × steak thickness (ca 2.54 cm) cube was removed from the center portion of each steak taking care to avoid connective tissue and or large pieces of marbling, which was used immediately for oxygen consumption and nitric oxide metmyoglobin reducing activity determination. The cube was cut so that the original steak thickness (2.54 cm) was divided in half. The top portion which had previously been exposed to light was designated for metmyoglobin reducing activity measurement. The bottom portion, which had never been exposed, was used for oxygen consumption measurement. At the conclusion of 6 d retail display, the display steak was sampled in the same manner used to measure the remaining oxygen consumption and metmyoglobin reducing activity.

**Table 1**
Descriptive statistics for carcass traits fro steers sampled for this experiment.

| Label | Mean | Standard deviation | Minimum | Maximum | C.V. |
|---|---|---|---|---|---|
| Hot carcass weight, kg | 358.8 | 33.5 | 275.0 | 469.6 | 9.3 |
| Adjusted fat thickness, cm | 1.18 | 0.52 | 0.25 | 2.79 | 44.44 |
| Longissimus muscle area, $cm^2$ | 78.97 | 7.97 | 57.14 | 102.84 | 10.09 |
| Kidney, pelvic, and heart fat, % | 2.05 | 0.75 | 0.00 | 4.00 | 36.41 |
| Yield grade | 3.15 | 0.83 | 1.15 | 5.58 | 26.4 |
| Lean maturity[a] | 141.9 | 13.4 | 120.0 | 190.0 | 9.5 |
| Skeletal maturity[a] | 156.7 | 13.6 | 130.0 | 190.0 | 8.7 |
| Overall maturity[a] | 149.1 | 10.0 | 125.0 | 180.0 | 6.7 |
| Marblling score[b] | 508.6 | 70.1 | 370.0 | 790.0 | 13.8 |

[a] 100 = $A^{00}$; 200 = $B^{00}$.
[b] 100 = Devoid$^{00}$; 200 = Practically devoid$^{00}$; 300 = Traces$^{00}$; 400 = Slight$^{00}$; 500 = Small$^{00}$; 600 = Modest$^{00}$; 700 = Moderate$^{00}$; 800 = Slightly Abundant$^{00}$.

### 2.3. pH and myoglobin concentration

Steaks reserved for determination of 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 volumes of a 5 mM iodoacetate, 150 mM KCl solution (pH = 7.0; temperature = 20 °C). Homogenates were allowed to rest for a minimum of 1 h at approximately 20 °C, 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, Sørheim and Slinde (1999). 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 (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 40 mM phosphate buffer and 0.5 ml sodium hydrosulfite (10 mg/ml). Absorbance spectra from 400 to 700 nm were scanned on each sample 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 nm), extracted pigment concentration was calculated using the absorbance at 433 nm, a molar extinction coefficient of 114,000 $M^{-1}$ $cm^{-1}$, the molecular weight of myoglobin (16,800), and the appropriate dilution factor.

### 2.4. Oxygen consumption

The top surface of the bottom half of the cube removed from each steak, which had previously been the interior of the steak, was used for measuring oxygen consumption (OC). The newly exposed portion of the steak was allowed to oxygenate, with no packaging, for 30 min at 1 °C. The sample then was vacuum packaged and scanned immediately with a Hunter Miniscan colorimeter with the settings previously described with the modification that the colorimeter was calibrated through the oxygen impermeable film of a vacuum bag. The vacuum package was incubated for 30 min in a 30 °C water bath and

scanned with the colorimeter to collect spectral data. The proportion of oxymyoglobin was calculated on the oxygenated and deoxygenated samples as described by Hunt et al. (1991). Oxygen consumption was reported as the percentage of oxymyoglobin prior to incubation minus the percentage of oxymyoglobin after incubation.

### 2.5. Nitric oxide metmyoglobin reducing activity

Metmyoglobin reducing activity was measured on the top half of the cube removed from the steak on the surface that had been previously exposed to light in a manner as described by Sammel, Hunt, Kropf, Hachmeister and Johnson (2002). The sample was oxidized in 50 mL of a 0.3% sodium nitrite solution for 30 min at approximately 20 °C. The sample then was removed from the solution, blotted, and vacuum packaged. Immediately following packaging the sample was scanned in duplicate with the Hunter Miniscan colorimeter with previously described settings. The sample was then allowed to reduce at room temperature (approximately 20 °C) for 2 h, when the sample was rescanned in duplicate. Surface metmyoglobin was quantified using the equations defined by Hunt et al. (1991). The proportion of surface metmyoglobin initially recorded immediately after oxidation with nitrite was reported as initial metmyoglobin formation (IMF). Metmyoglobin reducing activity was reported as the absolute difference in surface metmyoglobin between the initial (oxidized) readings and the final (reduced) readings. Similarly, the proportion of surface metmyoglobin recorded after the 2 h reduction period is reported as post-reduction metmyoglobin (PRM).

### 2.6. Statistical analysis

Simple statistics, including the mean, standard error, minimum, maximum, and coefficient of variation, were generated for each trait on d 0 and 6 and the change during display using the PROC MEANS procedure of SAS. These statistics also were determined for the change in each trait during display.

Oxygen consumption, nitric oxide metmyoglobin reducing activity traits, and display data were analyzed as a repeated measures design using the PROC MIXED procedure of SAS with an autoregressive covariance structure. The model tested for the main effect of display day. Slaughter group was included as a random effect. Animal was the subject of the repeated measures. Least-squares means were generated and separated with the DIFF option. Additionally, data were stratified according to overall color change (ΔE). Within each slaughter group, animals were classified with regard to color stability. Animals producing steaks with Day 6 ΔE values below the mean were considered to be color stable, while those with values above the mean were considered to have unstable color. Color variables, OC and metmyoglobin reducing activity variables were analyzed using a repeated measures model as described above with the addition of stability classification as a fixed effect. The stability class × day interaction was highly significant ($P<0.001$) for all traits, so simple effects of stability class were tested on each day of display.

Preliminary analyses indicated that initial color and color change differed across slaughter groups. To avoid drawing erroneous conclusions based on simple correlations that were influenced by slaughter group differences, residual values were generated for each variable using a model in PROC MIXED that included slaughter group as a fixed effect. The PROC CORR procedure of SAS was then used to generate residual correlations.

## 3. Results

Least-squares means for CIE L*, a* and b* values of the longissimus thoracis steaks across the 6 d of simulated retail display are presented in Table 2. Color change in these steaks was typical of meat color deterioration during retail display. Lightness (L*) values increased ($P<0.05$) slightly between d 0 and d 1 and then decreased ($P<0.05$) between d 1 and d 2 to a point that was equivalent to the values observed at the initiation of display before declining ($P<0.05$) again between d 3 and 4.

Redness (a*) values decreased ($P<0.05$) progressively during each day of retail display. Similarly, b* (yellowness) and chroma (color intensity) declined ($P<0.05$) with each increasing day of display, and $K/S_{572}/K/S_{525}$ values decreased ($P<0.05$) progressively on each day of simulated retail display, indicating that surface metmyoglobin concentration increased with each day of display. However, hue angle ($P<0.05$) decreased slightly between d 0 and 1 and then increased between d 1 and 4 to a point that was similar ($P>0.05$) to the hue angle measured on d 0. Thus, the color change of these steaks across the 6 d of simulated retail display can be characterized by a loss of color intensity rather than a change in hue. The low temperature (1 °C) and lack of defrost cycles during display most likely slowed the deterioration of color observed in these steaks.

Simple statistics for color parameters measured on d 0 or 6 of display and the change in these parameters are presented in Table 3. Substantial variation existed in myoglobin concentration. Ultimate muscle pH values were all well within the range expected for normal beef color. In general, instrumental color traits had higher coefficients of variation on d 6 than on d 0. The greatest coefficients of variation were associated with the change in color traits during display. This is particularly apparent with regard to hue angle, which had a mean near 0. On average, hue angle changed very little in these steaks; though the C.V. for hue angle indicates that substantial variation existed across animals in the change in hue angle during display. The minimum and maximum values for hue angle indicate that some animals increased in hue angle (hue became less red and more yellow) while hue angle decreased in others (hue became more red). In comparison to nitric oxide metmyoglobin reducing activity (NORA) and post-reduction metmyoglobin (PRM), very little variation existed in initial metmyoglobin (IMF) values. In contrast to most traits, variation in PRM and b* was greater on d 0 than on d 6.

Capacity for oxygen consumption (OC) and reducing activity decreased ($P<0.05$) during the 6 d of display (Table 4). The decline in OC between d 0 and d 6 was relatively small compared to the loss of nitric oxide metmyoglobin reducing activity (NORA). Nitric oxide metmyoglobin reducing activity on d 0 was more than 3 times greater

**Table 2**
Least-squares means for color variables of longissimus thoracis steaks during 6 d of simulated retail display.

| Variable | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | SEM | P<F |
|---|---|---|---|---|---|---|---|---|---|
| L* | 49.7b | 50.2a | 49.7b | 49.5b | 48.7c | 48.3c | 48.5c | 0.23 | <0.001 |
| a* | 34.0a | 32.7b | 30.6c | 29.9d | 28.7e | 27.4f | 26.7 g | 0.46 | <0.001 |
| b* | 26.8a | 24.9b | 23.5c | 23.1d | 22.2e | 21.5f | 21.0 g | 0.30 | <0.001 |
| Chroma | 43.3a | 41.1b | 38.6c | 37.8d | 36.3e | 34.9f | 34.0 g | 0.55 | <0.001 |
| $K/S_{572}/K/S_{525}$[a] | 1.42a | 1.37b | 1.33c | 1.32d | 1.30e | 1.28f | 1.24 g | 0.01 | <0.001 |
| Hue angle | 38.2a | 37.3 cd | 37.0d | 37.6bc | 37.8abc | 38.0ab | 38.3a | 0.22 | <0.001 |

Least-squares means, within a row, with differing letters (a–f) differ ($P<0.05$).
[a] Ratio of reflectance at 572 nm to 525 nm after K/S transformation, lower values indicate greater metmyoglobin content.

**Table 3**
Simple statistics for color parameters, oxygen consumption, and metmyoglobin reducing activity on d 0 and d 6 and the change in those variables during display in beef longissimus thoracis steaks in simulated retail display for 6 d.

| Variable | Mean | Standard deviation | Minimum | Maximum | Coefficient of variation |
|---|---|---|---|---|---|
| Myoglobin concentration, (mg/g) | 3.84 | 0.52 | 2.63 | 5.26 | 13.67 |
| pH | 5.59 | 0.05 | 5.41 | 5.74 | 0.95 |
| Day 0 L* | 49.7 | 2.64 | 42.7 | 56.2 | 5.32 |
| Day 6 L* | 48.5 | 2.53 | 41.2 | 55.5 | 5.22 |
| ΔL* | 1.4 | 1.29 | 0.0 | 6.7 | 90.31 |
| Day 0 a* | 34.0 | 1.6 | 30.5 | 38.4 | 4.69 |
| Day 6 a* | 26.7 | 2.1 | 19.1 | 31.7 | 7.92 |
| Δ a* | 7.3 | 2.2 | 1.9 | 14.3 | 29.99 |
| Day 0 b* | 26.8 | 2.2 | 22.8 | 32.1 | 8.11 |
| Day 6 b* | 21.1 | 1.2 | 15.8 | 23.9 | 5.75 |
| Δb* | 5.8 | 2.01 | 1.4 | 12.0 | 34.79 |
| Day 0 Chroma | 43.4 | 2.53 | 38.2 | 50.1 | 5.85 |
| Day 6 Chroma | 34.0 | 2.4 | 24.7 | 39.7 | 6.90 |
| ΔChroma | 9.4 | 2.7 | 2.6 | 16.9 | 29.16 |
| Day 0 Hue angle | 38.21 | 1.17 | 36.18 | 40.91 | 3.07 |
| Day 6 Hue angle | 38.31 | 1.08 | 36.14 | 41.88 | 2.82 |
| ΔHueangle | -0.10 | 1.65 | -4.43 | 4.04 | -1634.68 |
| Day 0 $K/S_{572}/K/S_{525}$[a] | 1.43 | 0.04 | 1.33 | 1.53 | 2.47 |
| Day 6 $K/S_{572}/K/S_{525}$ [a] | 1.24 | 0.06 | 1.00 | 1.40 | 5.07 |
| $\Delta K/S_{572}/K/S_{525}$[a] | 0.18 | 0.06 | 0.00 | 0.46 | 33.63 |
| ΔE[b] | 9.7 | 2.7 | 3.2 | 17.1 | 28.06 |
| Day 0 OC[c], (%) | 57.11 | 8.89 | 25.04 | 81.40 | 15.56 |
| Day 6 OC[c], (%) | 52.37 | 8.95 | 15.51 | 74.92 | 17.09 |
| Day 0 NORA[d], (%) | 28.25 | 10.35 | 8.28 | 56.69 | 36.62 |
| Day 6 NORA[d], (%) | 8.50 | 9.60 | 0.00 | 49.28 | 112.86 |
| Day 0 IMF[e] (%) | 68.82 | 1.98 | 63.66 | 75.38 | 2.88 |
| Day 6 IMF[e], (%) | 70.81 | 2.50 | 64.33 | 79.89 | 3.53 |
| Day 0 PRM[f], (%) | 40.56 | 11.33 | 11.91 | 62.53 | 27.92 |
| Day 6 PRM[f], (%) | 62.94 | 9.91 | 25.61 | 79.31 | 15.74 |

[a] Ratio of reflectance at 572 nm to 525 nm after K/S transformation, lower values indicate greater metmyoglobin content.
[b] Overall color change = $[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}]$.
[c] Oxygen consumption.
[d] Nitric oxide metmyoglobin reducing activity; Initial met myoglobin formed – post-reduction metmyoglobin.
[e] Initial metmyoglobin formed during 30 min incubation with 0.3% sodium nitrite.
[f] Post-reduction metmyoglobin; nitric oxide met myoglobin remaining after 2 h in vacuum package.

than the level on d 6. Day 6 steaks had slightly greater ($P<0.05$) surface metmyoglobin formation upon exposure to 0.3% sodium nitrite (IMF) and a greater ($P<0.05$) proportion of the surface myoglobin that remained in the oxidized state (PRM) after the steaks had been placed in reducing conditions (vacuum packaged at room temperature) for 2 h than d 0 steaks. This difference in IMF between days of display was small in magnitude in comparison with the differences detected in NORA and PRM.

When the steaks were classified into color stability groups according to overall color change (ΔE), ultimate pH did not differ between classes (Table 5). Myoglobin concentration was higher ($P<0.05$) in steaks classified as unstable compared to those classified as stable. Lightness (L*) did not differ between groups on any day of display. Redness (a*), however, was greater ($P<0.05$) on d 0 for steaks classified as unstable. However, redness declined much more rapidly in steaks classified as unstable so that there was no difference on d 1 and the unstable steaks had lower a* values on d 2 through 6. After d 2, the difference in redness grew progressively larger as time of display increased. A similar trend was noted between steaks classified as stable and unstable with regard to b* and chroma values. Hue angle did not differ between stability classes on d 0, 1, 3, or 4. However, hue angle was lower ($P<0.05$) in steaks classified as stable than in steaks classified as unstable. Steaks classified as stable tended ($P=0.09$) to have lower hue angle values than those classified as unstable on d 5. $K/S_{572}/K/S_{525}$ values did not differ between stability classes on d 0 or 1, but these ratios indicated a greater ($P<0.05$) accumulation of surface metmyoglobin on steaks classified as unstable on d 2 through the conclusion of display.

Steaks classified as stable had lower ($P<0.05$) OC than those classified as unstable on d 0, but this difference was smaller and not statistically significant on d 6 (Table 6). Steaks classified as stable had higher ($P<0.05$) NORA values than those classified as unstable on both d 0 and d 6. It is notable that on d 6, steaks classified as stable retained approximately one-third of their d 0 NORA values, but those classified as unstable retained approximately one-sixth of their d 0 NORA values. Initial metmyoglobin formation was slightly less ($P<0.05$) in steaks classified as stable than those with unstable lean color on d 0, but the groups did not differ in IMF on d 6. Steaks with stable lean color had lower ($P<0.05$) PRM values on both d 0 and d 6.

Preliminary analyses indicated that slaughter group was a source of variation for all traits included in this study. Because these differences were thought to be the result of environmental conditions beyond our control (such as chilling time and temperature and carcass spacing), partial correlation coefficients were generated after kill group effects were removed to evaluate the relationships between color change, OC, and reducing activity measured at the initiation or conclusion of display. Partial regression correlation coefficients between OC and measures of metmyoglobin reducing activity measured on d 0 and 6 of display are presented in Table 7. Oxygen consumption measured on d 0 was only related to d 6 OC, and day 6 OC had a weak ($P<0.05$), inverse relationship to d 0 IMF. No correlation existed between OC measured on d 0 or 6 and NORA or PRM measured on either day. Regardless of the timing of measurement, NORA was highly correlated to IMF and PRM measured on d 0 and PRM measured on d 6. This was not unexpected because both measurements are utilized in calculating NORA. On d 0, the relationship between PRM content and NORA was much stronger than the relationship between IMF and NORA. This strong relationship also existed on d 6, when IMF was not correlated to overall NORA. This suggests that the majority in the variation in NORA was attributable to variation in the amount of reduction that occurred after the oxidized sample was placed in reducing conditions rather than variation in the amount of oxidation that occurred initially. On d 0, IMF was strongly related ($P<0.05$) to d 0 and d 6 PRM, but d 6 IMF was not correlated to any other measure of reducing activity taken on either day.

Oxygen consumption measured on d 0 of display was modestly related to color stability indicating variables (Table 8). Increased OC measured on d 0 of display was associated with less ($P<0.05$) redness (lower a* value and increased hue angle), less color intensity (lower chroma values) and lower ($P>0.05$) $K/S_{572}/K/S_{525}$ ratios (more surface metmyoglobin) on d 6 of display. Furthermore, increased OC at the beginning of display was associated with greater ($P<0.05$) change in a* and overall color. Thus, greater OC at the onset of display was detrimental to color stability. Oxygen consumption measured on d 6 of display was not correlated to color stability indicating traits.

**Table 4**
Least-squares means for oxygen consumption and measures of metmyoglobin reducing activity of beef longissimus thoracis steaks on d 0 and 6 of simulated retail display.

| Variable | Day 0 | Day 6 | SEM | P>F |
|---|---|---|---|---|
| Oxygen consumption, (%) | 57.7 | 52.3 | 2.0 | <0.001 |
| Nitric oxide metmyoglobin reducing activity[a], (%) | 28.2 | 8.5 | 3.5 | <0.001 |
| Initial metmyoglobin formation[b], (%) | 68.8 | 70.8 | 0.3 | <0.001 |
| Post-reduction metmyoglobin[c], (%) | 40.6 | 63.0 | 3.5 | <0.001 |

[a] Nitric oxide metmyoglobin reducing activity; Initial met myoglobin formed – post-reduction metmyoglobin.
[b] Initial metmyoglobin formed during 30 min incubation with 0.3% sodium nitrite.
[c] Post-reduction metmyoglobin; nitric oxide met myoglobin remaining after 2 h in vacuum package.

**Table 5**
Least-squares means for color variables of longissimus thoracis steaks classified as having stable or unstable lean color during 6 d of simulated retail display.

| Variable | Stability class | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 |
|---|---|---|---|---|---|---|---|---|
| pH | Stable[a] | 5.58 | | | | | | |
| | Unstable[b] | 5.59 | | | | | | |
| | SEM | 0.01 | | | | | | |
| | P>F | 0.57 | | | | | | |
| Myolgobin (mg/g) | Stable[a] | 3.74 | | | | | | |
| | Unstable[b] | 3.96 | | | | | | |
| | SEM | 0.07 | | | | | | |
| | P>F | <0.001 | | | | | | |
| L* | Stable[a] | 49.5 | 50.0 | 49.4 | 49.5 | 48.7 | 48.0 | 48.6 |
| | Unstable[b] | 50.0 | 50.5 | 50.0 | 49.4 | 48.7 | 48.6 | 48.4 |
| | SEM | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| | P>F | 0.24 | 0.18 | 0.12 | 0.82 | 0.93 | 0.11 | 0.61 |
| a* | Stable[a] | 33.7 | 32.8 | 30.8 | 30.4 | 29.5 | 28.2 | 27.8 |
| | Unstable[b] | 34.5 | 32.6 | 30.3 | 29.3 | 27.9 | 26.6 | 25.4 |
| | SEM | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 |
| | P>F | <0.001 | 0.58 | 0.02 | <0.001 | <0.001 | <0.001 | <0.001 |
| b* | Stable[a] | 26.5 | 24.9 | 23.6 | 23.3 | 22.7 | 21.8 | 21.6 |
| | Unstable[b] | 27.2 | 24.9 | 23.4 | 22.8 | 21.8 | 21.1 | 20.4 |
| | SEM | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| | P>F | <0.001 | 0.84 | 0.27 | <0.001 | <0.001 | <0.001 | <0.001 |
| Chroma | Stable[a] | 42.8 | 41.1 | 38.8 | 38.3 | 37.2 | 35.7 | 35.2 |
| | Unstable[b] | 44.0 | 41.0 | 38.3 | 37.1 | 35.4 | 33.9 | 32.6 |
| | SEM | 0.54 | 0.54 | 0.54 | 0.54 | 0.54 | 0.54 | 0.54 |
| | P>F | <0.001 | 0.67 | 0.06 | <0.001 | <0.001 | <0.001 | <0.001 |
| Hue angle | Stable[a] | 38.18 | 37.24 | 36.51 | 37.45 | 37.57 | 37.68 | 37.92 |
| | Unstable[b] | 38.25 | 37.31 | 37.63 | 37.84 | 37.97 | 38.37 | 38.79 |
| | SEM | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 |
| | P>F | 0.86 | 0.87 | 0.01 | 0.35 | 0.35 | 0.09 | 0.04 |
| $K/S_{572}/K/S_{525}$[c] | Stable[a] | 1.43 | 1.38 | 1.34 | 1.33 | 1.32 | 1.30 | 1.27 |
| | Unstable[b] | 1.43 | 1.37 | 1.32 | 1.31 | 1.28 | 1.25 | 1.21 |
| | SEM | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | P>F | 0.68 | 0.31 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |

[a] Within a slaughter group, steaks with overall color change (ΔE) values below the mean were classified as stable.
[b] Within a slaughter group, steaks with overall color change (ΔE) values above the mean were classified as unstable.
[c] Ratio of reflectance at 572 nm to 525 nm after K/S transformation, lower values indicate greater metmyoglobin content.

Nitric oxide metmyoglobin reducing activity was moderately related to all measures of color and color stability when measured on d 0 or d 6 of display (Table 8). Increased NORA was associated with higher ($P<0.05$) a* and chroma values and less ($P<0.05$) surface metmyoglobin on d 6 of display. Consequently, NORA was inversely related ($P<0.05$) with hue angle and measures of color change during display. Initial metmyoglobin formation measured on d 0 of display was related to color and color stability, though this variable was less predictive than NORA or PRM. Further, the relationship between IMF and color change variables was stronger when measured on d 0 than on d 6. Relationships were detected between PRM measured at the initiation and conclusion of display and color stability traits were similar to those detected for NORA. The contribution of NORA and PRM to color stability were slightly stronger when those measurements were taken on d 6 of display than when taken on d 0 of display though this difference was relatively small.

**Table 6**
Least-squares means for oxygen consumption and measures of metmyoglobin reducing activity for longissimus thoracis steaks classified as having stable and unstable color-life during 6 d of display.

| Variable | Stability class | Day 0 | Day 6 |
|---|---|---|---|
| Oxygen consumption, (%) | Stable[a] | 56.41 | 51.62 |
| | Unstable[b] | 59.13 | 53.27 |
| | SEM | 2.10 | 2.10 |
| | P>F | 0.02 | 0.11 |
| Nitric oxide metmyoglobin reducing activity[c], (%) | Stable[a] | 31.05 | 10.81 |
| | Unstable[b] | 24.74 | 4.21 |
| | SEM | 3.60 | 3.60 |
| | P>F | <0.001 | <0.001 |
| Initial metmyoglobin formation[d], (%) | Stable[a] | 68.44 | 70.68 |
| | Unstable[b] | 69.25 | 70.94 |
| | SEM | 0.37 | 0.37 |
| | P>F | <0.001 | 0.34 |
| Post-reduction metmyoglobin[e], (%) | Stable[a] | 37.40 | 59.86 |
| | Unstable[b] | 44.51 | 66.73 |
| | SEM | 3.50 | 3.50 |
| | P>F | <0.001 | <0.001 |

[a] Within a slaughter group, steaks with overall color change (ΔE) values below the mean were classified as stable.
[b] Within a slaughter group, steaks with overall color change (ΔE) values above the mean were classified as unstable.
[c] Initial metmyoglobin formed − post-reduction metmyoglobin.
[d] Initial metmyoglobin formed during 30 min incubation with 0.3% sodium nitrite.
[e] Nitric oxide met myoglobin remaining after 2 h in vacuum package.

**Table 7**
Partial regression correlation coefficients between measures of oxygen consumption and metmyoglobin reducing capacity of longissimus thoracis steaks measured on d 0 or 6 of display.

| Variable | Day 6 OC[a] | Day 0 NORA[b] | Day 6 NORA[b] | Day 0 IMF[c] | Day 6 IMF[c] | Day 0 PRM[d] | Day 6 PRM[d] |
|---|---|---|---|---|---|---|---|
| Day 0 OC[a] | 0.16* | −0.05 | 0.03 | 0.00 | 0.00 | 0.05 | −0.03 |
| Day 6 OC[a] | | 0.01 | 0.08 | −0.13* | −0.01 | −0.03 | −0.08 |
| Day 0 NORA[b] | | | 0.57*** | 0.44*** | −0.11 | 0.99*** | 0.59*** |
| Day 6 NORA[b] | | | | 0.41*** | 0.05 | 0.59*** | 0.98*** |
| Day 0 IMF[c] | | | | | 0.05 | 0.58*** | 0.42*** |
| Day 6 IMF[c] | | | | | | 0.11 | 0.15 |
| Day 0 PRM[d] | | | | | | | 0.61*** |

*$P<0.05$; **$P<0.01$; ***$P<0.001$.
[a] Oxygen consumption.
[b] Nitric oxide metmyoglobin reducing activity; initial met myoglobin formed − post-reduction metmyoglobin.
[c] Initial metmyoglobin formed during 30 min incubation with 0.3% sodium nitrite.
[d] Post-reduction metmyoglobin; nitric oxide met myoglobin remaining after 2 h in vacuum package.

**Table 8**
Partial regression correlation coefficients for the relationship between oxygen consumption and metmyoglobin reducing activity measurements and changes in color parameters of beef longissimus steaks when measured at the initiation or conclusion of 6 d of simulated retail display.

| | Oxygen consumption | | Nitric oxide metmyoglobin reducing activity[a] | | Initial metmyoglobin formation[b] | | Post-reduction metmyoglobin[c] | |
|---|---|---|---|---|---|---|---|---|
| Variable | Day 0 | Day 6 | Day 0 | Day 6 | Day 0 | Day 6 | Day 0 | Day 6 |
| Day 6 a* | −0.25*** | −0.11 | 0.27*** | 0.35*** | −0.17** | −0.15* | −0.27*** | −0.27*** |
| Day 6 $K/S_{572}/K/S_{525}$[d] | −0.20** | −0.12 | 0.38*** | 0.39*** | −0.22*** | −0.13* | −0.38*** | −0.41*** |
| Day 6 Chroma | −0.24*** | −0.12 | 0.22*** | 0.29*** | −0.10 | −0.14* | −0.22*** | −0.32*** |
| Day 6 Hue angle | 0.19** | 0.02 | −0.38*** | −0.40*** | 0.41*** | 0.13* | 0.42*** | 0.44*** |
| $\Delta E^e$ | 0.19** | 0.12 | −0.44*** | −0.50*** | 0.23** | 0.11 | 0.45*** | 0.52*** |
| Δa* | 0.17* | 0.12 | −0.43*** | −0.50*** | 0.23*** | 0.13* | 0.43*** | 0.52*** |

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.
[a] Nitric oxide metmyoglobin reducing activity; Initial met myoglobin formed − post-reduction metmyoglobin.
[b] Initial metmyoglobin formed during 30 min incubation with 0.3% sodium nitrite.
[c] Post-reduction metmyoglobin; nitric oxide met myoglobin remaining after 2 h in vacuum package.
[d] Ratio of reflectance at 572 nm to 525 nm after K/S transformation, lower values indicate greater metmyoglobin content.

## 4. Discussion

We were interested in identifying the optimum timing for a single measurement of oxygen consumption and metmyoglobin reducing activity when evaluating inherent animal-to-animal variation in color stability of a given muscle. In the literature, these traits are often measured on multiple days during display and then compared with color variables measured on those days. From such experiments, it is evident that differences in these traits coincide with degradation of lean color. However, it is not clear whether variation in these traits is due to differences in initial capacity or differences in maintaining capacity. Such information would aid in designing strategies to reduce animal variation in color-life. Thus, we addressed the question of whether color variation at the end of display and color change during display was attributable to differences in initial capacity for oxygen consumption and reducing activity, or to differences in the amount of that capacity remaining or that had been regenerated at the end of display. Previous reports indicate that both OC (Ledward, 1985; Sammel et al., 2002; McKenna et al., 2005) and metmyoglobin reducing activity (Sammel, Hunt, Kropf, Hachmeister, & Johnson, 2002;Sammel, Hunt, Kropf, Hachmeister, Kastner, et al., 2002; McKenna et al., 2005; Seyfert et al., 2006) decrease as time in retail display is extended. This is supported by the results of the present experiment.

In the present experiment, oxygen consumption was negatively correlated to color stability data when measured on d 0 of display but was not significantly related to any of the color or color change variables when measured on d 6. Increased oxygen consumption decreases color stability because less oxygen is available to bind with myoglobin, creating oxidative conditions that favor metmyoglobin formation (Ledward, 1985; O'Keefe & Hood, 1982; McKenna et al., 2005). Conversely, Sammel, Hunt, Kropf, Hachmeister and Johnson (2002) suggested that very low oxygen consumption levels were deleterious to color stability because mitochondrial respiration was needed to regenerate NADH to be used as a cofactor in metmyoglobin reduction. If this is true, oxygen consumption and metmyoglobin reducing ability would be correlated to some extent. In the present experiment, very few relationships were detected between OC and measures of reducing activity. In fact, longissimus thoracis steaks classified as having stable lean color had both lower OC and greater reducing activity than those with less stable lean color.

Using data from 19 beef muscles, McKenna et al. (2005), reported moderate relationships between OC and measures of myoglobin reduction (r ranged from 0.35 to 0.55). This is likely due to muscles with more oxidative metabolism having greater concentrations of mitochondria which are the site of enzymes responsible for both oxygen scavenging and metmyoglobin reduction. However, McKenna et al. (2005) also reported that some muscles with similar OC levels had very different capacities for metmyoglobin reduction and surmised that relationships between OC, reducing ability, and color stability are dependent on the relative levels of oxygen consumption and reducing activity i.e. more stable muscle have sufficient reducing ability to mitigate oxygen consumption effects. This may be because some muscles lack the substrates necessary to replenish the NADH necessary for the enzymes to be active. Echevarne, Renerre, and Labas (1990) suggested that the NADH pool, not metmyoglobin reductase activity, was the limiting factor dictating color-life. In support of this notion, it has been suggested that specific reductase assays with excess NADH effectively differentiate muscles differing in color stability, but not steaks of a common muscle with different levels of discoloration (Sammel, Hunt, Kropf, Hachmeister and Johnson, 2002; McKenna et al., 2005). King et al. (2010) suggested that genetic influences in color stability are mediated through metabolic differences resulting in increased availability or regeneration of NADH. These reports coupled with the findings of the present experiment suggest that animal-to-animal variation in metmyoglobin reducing ability within a muscle is not simply a function of mitochondrial enzyme concentration, and is also influenced by the metabolic characteristics of the muscle.

Results presented by Ledward (1985) and Cheah and Ledward (1997) suggest that oxygen consumption plays a significant role in metmyoglobin formation initially, but as oxygen consumption decreases, reducing activity becomes the predominant factor in maintaining stability. In the present experiment, all of the measures of metmyoglobin reducing activity were correlated to color stability data on d 0 and 6. Correlations between NORA and PRM and color stability data were slightly stronger on d 6 than they were on d 0. Furthermore, steaks with stable lean color retained a greater proportion of their ability to reduce nitric oxide metmyoglobin after 6 d of display than those with more labile lean color. Thus, it appears that initial levels of reducing capacity are important in determining color stability, but variation in the ability to maintain or regenerate reducing ability is also important in regulating color stability. The increased relationships between reducing ability and color stability detected at the end of display may be due to variation in the muscle's ability to replenish the NADH pool needed to facilitate continued reduction.

On d 6, IMF was weakly correlated to d 6 redness and surface metmyoglobin values and to the change in both redness and color intensity. We hypothesize that the incubation with sodium nitrite in this experiment resulted in near-maximal metmyoglobin formation (i.e. longer incubation under these conditions would produce minimal further myoglobin oxidation). Thus, it appears that as retail display progresses, steaks became less resistant to myoglobin oxidation and the remaining resistance was less indicative of overall stability, but variation remains in the ability of muscles to reduce the oxidized

pigment and this variation is strongly related to color stability. The strong relationships between measures of reducing ability on d 0 and color stability indicate that differences in initial reducing capacity account for most of the contribution of reducing activity to color stability. However, some further contribution is likely due to variation in the ability of muscle to maintain reducing capacity, possibly through the presence of substrates necessary to maintain NADH levels. Kim et al. (2006) reported that lactate enhancement of beef products extended color-life by increasing NADH when lactate was converted to pyruvate by lactate dehydrogenase.

McKenna et al. (2005) reported that resistance to metmyoglobin formation in a 1% oxygen environment during the first step of the aerobic reducing activity assay was more strongly correlated to color change during 5 d of display in steaks from 19 beef muscles than the amount of metmyoglobin that was reduced when the samples were returned to reducing conditions. Based on those results, Mancini, Seyfert and Hunt (2008) investigated the relationship of NORA, the IMF, and PRM to color change during display and found that IMF was most strongly related to color stability data in longissimus lumborum steaks. This also appeared to be true in psoas major and semimembranosus steaks, though those correlations were non-significant. Furthermore, those investigators reported that NORA values were not significantly correlated to color stability in any of the muscles evaluated. This is in contrast to the findings of the present experiment where NORA and PRM values were more strongly correlated to color stability than IMF values, particularly when measured on d 6 of display.

Explanation for the discrepancy between the present experiment and Mancini et al. (2008) may lie in a slight difference in methodology used in measuring nitric oxide metmyoglobin reducing activity. In the present experiment, samples were incubated in 0.3% sodium nitrite for 30 min at approximately 20 °C before IMF was measured. Mancini et al. (2008) incubated samples for 20 min before making this measurement. The additional time likely allowed more complete oxidation of myoglobin in the present experiment. In the present experiment, much less variation was noted for IMF values than for NORA or PRM values, and the difference between IMF values taken on d 0 versus d 6 was much smaller in magnitude than the corresponding differences found for NORA and PRM. This may suggest that the oxidizing conditions used in this experiment (0.3% sodium nitrite at 20 °C for 30 min) were sufficient to maximally oxidize the myoglobin in these samples. Thus, the variation associated with the IMF was minimized and the variation in PRM, and consequently NORA was maximized. This suggests that investigators should carefully choose the appropriate incubation times when using nitric oxide metmyoglobin reducing activity in experiments so that variation in the trait of interest (either NORA or IMF) is optimized.

## 5. Conclusions

Oxygen consumption was related to variation in lean color stability of longissimus steaks when measured at the beginning of display, but not when measured at the end of display. Thus, this trait should be measured at the initiation of display. Metmyoglobin reducing activity was strongly related to color stability when measured at the initiation of display and this relationship was slightly increased at the end of display, indicating that color stability is influenced by variation in both initial levels and maintenance of reducing ability. The greatest amount of information would be obtained by measuring reducing activity at the conclusion of display if only one measurement is to be made. However, measurements of reducing activity made at both the beginning and end of display may allow inferences regarding the mechanisms associated with both sources of variation. All measures of reducing activity explained variation in lean color stability, but investigators should tailor measurement conditions so that the measure of choice best reflects the variation present in experimental samples.

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