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Mechanism for Lactate-Color Stabilization in Injection-Enhanced Beef

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In two experiments, the relationship between metmyoglobin (MMb) reduction and lactate to pyruvate conversion with concomitant production of reduced nicotinamide adenine dinucleotide (NADH) via lactic dehydrogenase (LDH) was investigated. In experiment 1, nonenzymatic reduction of horse MMb occurred in a lactate−LDH−NAD system. Exclusion of NAD^+, L-lactic acid, or LDH resulted in minimal MMb reduction. Increasing NAD^+ and L-lactic acid concentrations increased reduction. In experiment 2, beef strip loins (longissimus lumborum muscle) were injected with combinations of potassium lactate, sodium tripolyphosphate, sodium chloride, and/or sodium acetate. Steaks were packaged in high-oxygen (80% oxygen/20% carbon dioxide) modified-atmosphere packaging and stored for 2−9 days and then placed in a fluorescent-lighted, open-top display case for 5 days at 1 °C. Enhancing loins with 2.5% potassium lactate significantly increased LDH activity, NADH concentration, MMb-reducing activity, and subsequent color stability during display. These research results support the hypothesis that enhancing beef with lactate replenishes NADH via increased LDH activity, ultimately resulting in greater meat color stability.

KEYWORDS: Beef; color; injection enhancement; potassium lactate; LDH activity; metmyoglobin-reducing activity; NADH

INTRODUCTION

The addition of non-meat ingredients to a variety of meat products is common practice in the value-added meat industry. Injection enhancement of beef with solutions containing various lactates improves juiciness, tenderness, flavor, shelf life, and final product yields (*1*). Lactate enhancement also results in raw and cooked beef color stabilization (*1*−*5*) and raw beef darkening (*4*, *5*). The mechanism by which lactate enhancement improves meat color stability is unknown.

Surface discoloration resulting from metmyoglobin (MMb) formation affects consumers' purchasing decisions. The oxidized form of myoglobin can be converted to deoxymyoglobin (DMb) through muscle metmyoglobin-reducing activity (MRA), which can then be reoxygenated to form oxymyoglobin (OMb). Ledward (*6*) suggested that MRA is the most important intrinsic factor controlling the rate of MMb accumulation in beef. It is well-established that MMb reduction occurs through both enzymatic and nonenzymatic reducing systems and that reduced nicotinamide adenine dinucleotide (NADH) is the ultimate reducing substrate for both pathways (*7*).

The location of post-mortem NADH available for MMb reduction is unclear. Watts et al. (*8*) hypothesized that because postrigor meat contains both lactate and lactic dehydrogenase (LDH), hydrogen transfer from lactate to nicotinamide adenine dinucleotide (NAD^+) and subsequent NADH production by LDH could be coupled with MMb reduction in the presence of electron carriers such as enzymes, quinones, or methylene blue. Lactate−LDH is the most likely system responsible for MMb reduction before nitrosylmyoglobin formation in cured bacon (*9*).

We hypothesize that lactate's ability to minimize discoloration in injection-enhanced beef products is related to the replenishment of NADH via LDH activity. Therefore, the objectives of this study were to (1) examine the interaction of lactate and LDH to reduce MMb in vitro, (2) determine the effects of lactate on color stability in injection-enhanced beef, and (3) determine the influence of lactate enhancement on LDH enzyme activity, NADH concentration, and metmyoglobin-reducing activity in injection-enhanced strip loins.

MATERIALS AND METHODS

Experiment 1: Lactate−LDH System in Nonenzymatic Reduction of Horse MMb. *Reagents.* Horse heart metmyoglobin, NAD^+, EDTA (disodium), methylene blue, L/D-lactic acid, flavin mononucle-

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Table 1. Nonenzymatic Reduction of Horse Metmyoglobin with Lactate–LDH System at 22 °C and pH 8.0 (n = 3)

solution components[a]							
FMN (2.0 mM)	MB (0.1 mM)	NAD+ (6.5 mM)	L-lactic acid (200 mM)	LDH[b] (0.1 mL)	oxalate (200 mM)	D-lactic acid (200 mM)	activity (nmol/min)
+	+	+	+	+	−	−	0.69 ± 0.004
−	+	+	+	+	−	−	0.52 ± 0.012
+	−	+	+	+	−	−	0.17 ± 0.003
+	+	−	+	+	−	−	0.02 ± 0.004
+	+	+	−	+	−	−	0.03 ± 0.003
+	+	+	+	−	−	−	0.02 ± 0.000
+	+	+	+	+	+	−	0.40 ± 0.015
+	+	+	−	+	−	+	0.05 ± 0.002

[a] Substances present (+) or absent (–) in mixtures. FMN, flavin mononucleotide; MB, methylene blue; NAD^+, oxidized nicotinamide dinucleotide; LDH, lactic dehydrogenase. Assays were conducted in an aerobic environment with 0.5 mM equine MMb in 30 mM phosphate buffer. Total assay volume = 1.0 mL. Composed of 0.3 mL of equine MMb and combinations of 0.1 mL of FMN, MB, NAD^+, L-lactic acid, oxalate, and D-lactic acid with deionized distilled water to bring to final volume. [b] 202.8 units/mL.

otide (FMN), and L-lactic dehydrogenase (LDH, from bovine muscle, 600 units/mg of protein) were obtained from Sigma (St. Louis, MO).

Nonenzymatic Reduction of Horse MMb. The roles of lactate and LDH in nonenzymatic reduction of horse MMb were assessed by adding reactants in various combinations to 10 mm path length polystyrene cuvettes with 1.0 mL final reaction volume under aerobic conditions. The standard reaction mixtures at pH 8.0 contained one or more of the following (**Table 1**): 0.3 mL of 0.5 mM equine MMb in 30 mM phosphate buffer, 0.1 mL of citrate buffer (50, 80, 100, or 150 mM), 0.1 mL of distilled water, 0.1 mL of 2.0 mM FMN, 0.1 mL of 6.5 mM NAD^+, 0.1 mL of 200 mM L-lactic acid with 400 mM Tris (pH 8.0), 0.1 mL of 0.1 mM methylene blue, and distilled deionized water to make the total reaction volume of the assay 1.0 mL. The reaction was initiated by adding 0.1 mL of LDH to the mixture (202.8 units/mL: 1 unit will reduce 1.0 μmol/min of pyruvate to L-lactate). The effects of 6.5 mM NAD^+ and 200 mM L-lactic acid concentration and assay pH on the rate of MMb reduction were determined. The final assay pH (5.7, 6.6, 7.5, or 8.0) was varied by altering the concentration of the citrate buffer (150, 100, 80, or 50 mM, respectively). Furthermore, 0.1 mL of 200 mM oxalate or 0.1 mL of 200 mM D-lactate (replacing L-lactic acid) was added to the mixture to investigate potential inhibiting effects on LDH in the MMb-reducing system.

To measure MMb reduction, absorbance at 580 nm was recorded every 2 s for 5 min in a spectrophotometer (UV-2010; Hitachi Instruments, Inc., San Jose, CA). Nonenzymatic reducing activity was calculated as nanomoles of MMb reduced (equal to nanomoles of OMb formed) per minute during the initial linear phase of the assay, using a difference in molar absorptivity of 12000 mol^{-1} cm^{-1} at 580 nm (the wavelength at which the difference in absorption between MMb and OMb is maximal). Activity is expressed as the mean of triplicate samples.

Experiment 2: Effects of Lactate on Color Stability, LDH Activity, NADH Concentration, and MRA in Injection-Enhanced Beef. *Raw Materials and Processing.* Ten USDA Select (A-maturity, USDA yield grade 2−3) beef strip loins were obtained from a commercial meat facility 2 days post-mortem. On day 8 post-mortem, each loin was divided into four equal-length sections (40 total sections), and one of five treatments was assigned randomly to each of the loin sections by using an incomplete block design, resulting in eight treatment replications. All meat processing was performed at 4 °C. Each loin section was pumped to a target of 10% of uninjected weight with one of five enhancement solutions (**Table 2**) containing water, potassium lactate (PURASAL HiPure P, 60% potassium lactate/40% water; PURAC America, Inc., Lincolnshire, IL), sodium chloride (Cargill, Minneapolis, MN), sodium tripolyphosphate (ColourSure; Rhodia Food, Cranbury, NJ), sodium acetate (Verdugt, Tiel, The Netherlands), and/or rosemary extract (NatureGuard B; Newly Weds Foods, Chicago, IL). Loin sections were weighed individually before and after injection with a multineedle injector (model N30, Wolftec, Inc., Werther, Germany) to calculate actual injection levels (10.3%). Each enhanced loin section was cut into three 2.54 cm thick steaks for packaging; one steak was for analysis on day 2, one for day 9 after storage, and one for day 14 after 5 day of display and 9 days of storage.

Table 2. Composition (Percent) of Experimental Injection Enhancement Solutions for Beef Strip Loins

	treatment				
ingredient	nonenhanced control (NEC)	no-lactate control (NLC)	low lactate (L1.5)	high lactate (L2.5)	lactate + acetate (LACE)
potassium lactate	0	0	1.5	2.5	1.5
sodium chloride	0	0.3	0.3	0.3	0.3
sodium tripoly-phosphate	0	0.3	0.3	0.3	0.3
sodium acetate	0	0	0	0	0.1
rosemary extract	0	0.058	0.058	0.058	0.058

Packaging. Individual steaks were placed in preformed trays with soaker pads (polypropylene, 0.1 cm^3 of oxygen/tray/24 h at 22.7 °C/0% relative humidity, 2.0 g of water vapor/64516 cm^2/24 h at 37.8 °C/100% relative humidity; Sealed Air Corp., Duncan, SC); packages were then evacuated, flushed with a high-oxygen modified atmosphere (80% oxygen/20% carbon dioxide; Certified Standard; Airgas Specialty Gases, Los Angeles, CA), and sealed with a shrinkable barrier sealing film (MAP-Shield AF; 1.5-mil high-barrier nylon/ethylene vinyl alcohol/metallocence polyethylene, 0.02 cm^3 of oxygen/645.16 cm^2/24 h at 10 °C and 80% relative humidity, 0.92 g of water vapor/645.16 cm^2/24 h at 37.8 °C and 100% relative humidity; Honeywell, Morristown, NJ) by using a Ross Inpack Jr. (model S3180, Ross Industries, Inc., Midland, VA). This gas atmosphere is widely used within the meat industry for the packaging of case-ready meats. Maintenance of this atmosphere during distribution, storage, and retail display is imperative to maintain meat quality.

Modified-atmosphere packages (MAP) were stored in the dark at 2 °C for either 2 or 9 days and subsequently were used for either initial (day 2), start of display (day 9), or display (days 9−14) analyses. These various storage and display times represent several hypothetical retail storage and display scenarios after processing and before purchasing.

pH. A sample from each longissimus lumborum muscle on days 2 and 14 was frozen in liquid nitrogen and pulverized in a Waring table-top blender (Dynamics Corp. of America, New Hartford, CT). Ten grams of tissue was mixed with 100 mL of distilled water for 1 min, and pH values were measured with a standardized combination pH electrode attached to a pH-meter (Accumet 50; Fischer Scientific, Fair Lawn, NJ).

Display Conditions. Steaks were displayed at 1 °C from day 9 to day 14 under 2150 ± 100 lx of fluorescent light (34 W, Ultralume 30, 3000 K; Phillips, Bloomfield, NJ) in open-top display cases (DMF8; Tyler Refrigeration Corp., Niles, MI), which defrosted at 12-h intervals. Display-case temperature was monitored at the meat level with temperature loggers (RD-TEMP-XT; Omega Engineering, Inc., Stamford, CT).

Instrumental Color. Instrumental color for the longissimus lumborum muscle was evaluated on days 2, 9, and 14 during display, immediately upon removal from MAP by using a HunterLab MiniScan XE Plus

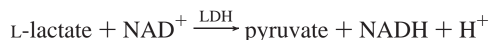
spectrophotometer (model 45/0 LAV, 2.54 cm diameter aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA). The spectrophotometer was calibrated using black and white reference standards provided by the manufacturer. Values for CIE L^*, a^*, and b^* (Illuminant A) were measured and used to calculate chroma [$(a^{*2} + b^{*2})^{1/2}$]. Steaks were scanned at three different locations, and values were averaged for statistical analyses.

Visual Color. Six trained panelists, all of whom had normal color vision and had passed the Farnsworth−Munsell 100-hue test (*10*), evaluated longissimus lumborum discoloration and darkening on day 2 and from day 9 to day 14 during display. Discoloration (percentage of MMb on the meat's surface) was evaluated on the following scale to the nearest 1.0: 1 = no discoloration (0%), 2 = slight discoloration (1−19%), 3 = small discoloration (20−39%), 4 = modest discoloration (40−59%), 5 = moderate discoloration (60−79%), 6 = extensive discoloration (80−99%), 7 = total discoloration (100%). Panelists evaluated muscle darkening to the nearest 0.5 point on the following scale: 1 = no darkening, 3 = slightly dark, 5 = moderately dark, and 7 = very dark. The average of all panelists' scores was used for statistical analyses.

NADH Concentration. The NADH was extracted (alkaline conditions; *11*) from longissimus lumborum muscle tissue from steaks assigned to analysis on days 2, 9, and 14. Then, a modified assay of McCormick and Lemuel (*12*) was used to determine NADH concentration by measuring dichlorophenolindophenol (DCPIP) reduction by muscle extracts indicated by absorbance at 600 nm (UV-2010; Hitachi Instruments, Inc., San Jose, CA). The NADH concentration (micrograms per milliliter) was calculated from a standard curve using known NADH concentrations.

MRA. The method of Watts et al. (*8*) was used to determine MRA of longissimus lumborum muscle tissue from steaks assigned to analysis on days 2, 9, and 14. A 3 × 2 × 1.27 cm sample of muscle tissue with no visible fat or connective tissue, not previously directly exposed to oxygen within the package, was removed from the interior (subsurface) of the steaks. Samples were oxidized in 50 mL of 0.3% sodium nitrite at 22 °C for 20 min with occasional stirring to form nitric oxide metmyoglobin. After exposure to the sodium nitrite solution, the samples were blotted to remove excess solution, vacuum packaged, and scanned immediately to record percentage of reflectance from 400 to 700 nm by using a Hunter LabScan 2000 (1.27 cm diameter aperture; Hunter Associates Laboratory, Inc.) to determine the initial percentage of surface metmyoglobin. Samples then were stored at 30 °C for 2 h to induce reduction of metmyoglobin to deoxymyoglobin and rescanned to determine the final percentage of surface metmyoglobin. Surface MMb was calculated as a percentage by using K/S ratios and equations that convert K/S ratios to percent metmyoglobin (*13*). The MRA was calculated as [(initial % of surface metmyoglobin − final % of surface metmyoglobin) ÷ initial % of surface metmyoglobin] × 100.

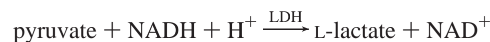
LDH Activity 1 (Lactate to Pyruvate). LDH activity was measured for longissimus lumborum muscle tissue from steaks assigned to analysis on days 2, 9, and 14 according to the UV method of Wahlefeld (*14*), which monitors the reduction of NAD^+ in the following reaction:



$$\text{L-lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{pyruvate} + NADH + H^+$$

Chopped muscle tissue (2.0 g) that did not contain any visible fat or connective tissue was homogenized in 8 mL of 0.01 M sodium phosphate buffer (pH 7.5) for 30 s or until the muscle tissue was disrupted completely. The homogenate was centrifuged at 13823g for 30 min at 4 °C. Aliquots of supernatant (2 mL) were diluted with 0.01 M phosphate buffer to yield a 200:1 sample dilution. The diluted supernatant (0.1 mL) was added to the glass cuvette with 0.1 mL of NAD and 2.4 mL of Tris/L-lactate (pH 9.3). Activity of LDH was measured in duplicate by the continuous increase in absorbance at 339 nm after 30 s and repeat readings every 30 s for 2.5 min. Increased absorbance (increased NADH) between 30 and 150 s was used to calculate LDH activity. Activity was calculated as the change in absorbance ($\Delta A/\Delta T$) × 4.21 × 10^3. Units of LDH activity were expressed as micromoles per minute per gram of sample.

LDH Activity 2 (Pyruvate to Lactate). Activity of LDH also was measured from longissimus lumborum muscle tissue from steaks assigned to analysis on days 2, 9, and 14 according to the method of Vassault (*15*), which monitors the concentration of NADH oxidized in the following reaction:



$$\text{pyruvate} + NADH + H^+ \xrightarrow{\text{LDH}} \text{L-lactate} + NAD^+$$

Chopped muscle tissue (2.0 g) that did not contain any visible fat or connective tissue was homogenized in 8 mL of a 0.01 M sodium phosphate buffer (pH 7.5) for 30 s or until the muscle tissue was disrupted completely. The homogenate was centrifuged at 13823g for 30 min at 4 °C. Aliquots of supernatant (2 mL) were diluted with 0.01 M phosphate buffer to yield a 200:1 sample dilution. The diluted supernatant (0.05 mL) was added to the glass cuvette with 2.5 mL of Tris/NaCl/NADH (pH 7.2) and 0.5 mL of Tris/NaCl/pyruvate (pH 7.2). Activity of LDH was measured in duplicate by the continuous decrease in absorbance at 339 nm after 30 s and repeat readings every 30 s for 2.5 min. Decreased absorbance (decreased NADH) between 30 and 150 s was used to calculate LDH activity. Activity was calculated as the change in absorbance ($\Delta A/\Delta T$) × 9.68 × 10^3. Units of LDH activity were expressed as micromoles per minute per gram of sample.

Statistical Analysis. Experiment 1 was a completely randomized design replicated three times. Least-square means (LSMeans) and standard errors (SE) were used to determine the effects of lactate and lactic dehydrogenase on in vitro metmyoglobin reduction.

The design for experiment 2 was a split plot. In the whole plot (incomplete randomized block), 10 loins served as blocks, and each of the four sections within a loin was an experimental unit to which injection treatments were applied randomly. This resulted in eight replications of the five injection enhancement solution treatments [(10 loins × 4 sections) ÷ 5 treatments]. In the subplot, each of the three steaks from a loin section was considered to be an experimental unit to which display time (2, 9, or 14 days) was assigned randomly.

Data were analyzed by using the Mixed Procedure of SAS (*16*). Type-3 tests of fixed effects for injection treatment, display time, and their interaction were used to evaluate F test significance. LSMeans were separated (F test, $p < 0.05$) by using least significant differences generated by the Diff option. Random error terms included loin for the incomplete-block portion and loin × injection treatment for the whole-plot error A. Residual unspecified error B was evaluated by using loin × injection treatment × display time. To account for the split plot, the Kenward Rogers adjustment was used to determine denominator degree of freedom.

RESULTS AND DISCUSSION

Experiment 1: Lactate−LDH System in Nonenzymatic Reduction of Horse MMb. Nonenzymatic MMb reduction occurred effectively in the lactate−LDH system with NAD^+, but exclusion of NAD^+, L-lactic acid, or LDH reduced or eliminated MMb reduction (**Table 1**). Addition of oxalate, a known LDH inhibitor (*14*), to the reaction mixture decreased reducing activity. Replacing L-lactic acid with D-lactic acid in the assay mixtures decreased MMb reduction, probably due to the selective interaction of LDH with L-lactic acid (*17*, *18*).

Nonenzymatic reduction in the assay mixtures of pH 8.0 increased with added NAD^+ in the reaction mixture (2.5 mM NAD^+ = 0.187 nmol of reduced MMb/min; 4.5 mM NAD^+ = 0.664; 6.5 mM NAD^+ = 0.693; and 8.5 mM NAD^+ = 0.710; SE = 0.0035). Increased MMb reduction by NAD^+ addition to ground beef samples by 23−51% over the control has been reported (*19*). Concentration of NAD^+ is directly related to meat-color stability because NAD^+, a source of oxidized substrate, decreases rapidly in post-mortem muscle (*20*). Greater amounts of L-lactic acid also increased MMb reduction (150 mM L-lactic acid = 0.576 nmol of MMb reduced/min; 200 mM = 0.693; 250 mM = 0.723; and 300 mM = 0.814; SE = 0.0035). Alkaline conditions were more conducive to reduction within the lactate−LDH system (pH 5.7 = 0.092 nmol of MMb reduced/min; pH 6.6 = 0.179; pH 7.5 = 0.344; and pH 8.0 =

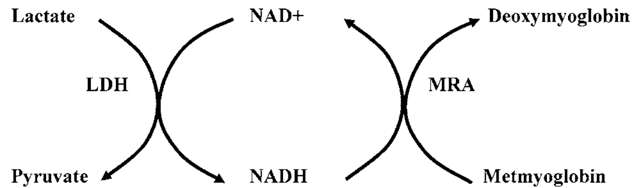


Figure 1. Proposed scheme of lactate–lactic dehydrogenase (LDH) system for generating NADH for metmyoglobin-reducing activity (MRA).

Table 3. LSMeans for Treatment Solution and Injection-Enhanced Longissimus Lumborum Muscle pH at Day 2 (Initial) and Day 14 (End of Display)

	treatment[a]					
trait	NEC	NLC	L1.5	L2.5	LACE	SE[b]
solution pH		6.4	6.5	6.5	6.5	
meat pH, day 2	6.0 by	6.0 by	5.9 by	5.9 cy	5.9 by	0.03–0.04
meat pH, day 14	5.8 cz	5.9 by	5.9 by	5.9 by	5.9 by	0.03–0.04

[a] NEC, nonenhanced control; NLC, no-lactate control; L1.5, 1.5% potassium lactate; L2.5, 2.5% potassium lactate; LACE, 1.5% potassium lactate, 0.1% sodium acetate. All enhanced treatments contained 0.3% NaCl, 0.3% phosphate, and 0.058% rosemary extract. Means within a row with different letters (b, c) are different ($p < 0.05$). Means within a column with different letters (y, z) are different ($p < 0.05$). [b] Standard error.

0.693; SE = 0.002). Although minimal, the nonenzymatic reduction through the lactate−LDH system still occurred at a pH of 5.7, which is near post-mortem muscle pH. We speculate, therefore, that the lactate−LDH system in post-mortem muscle can generate NADH by the reduction of NAD^+ and that a NADH-dependent reducing system, either enzymatic or non-enzymatic, can reduce MMb (**Figure 1**).

Experiment 2: Effects of Lactate on Color Stability, LDH Activity, NADH Concentration, and MRA in Injection-Enhanced Beef. *pH.* The pH of the enhancement solutions ranged from 6.4 to 6.5 (**Table 3**). All enhanced meat samples had a greater ($p < 0.05$) pH than that of the nonenhanced control at the end of display, which was likely due to the phosphate and/or the lactate.

Visual and Instrumental Darkening. A treatment by time interaction ($p < 0.05$) occurred for visual darkening (**Table 4**). All treatments darkened (visual darkening scores increased; $p < 0.05$) from days 2 to 14. The L1.5 treatment was the only treatment not to darken ($p > 0.05$) from days 9 to 14. Steaks containing lactate (L1.5, L2.5, and LACE) were darker ($p < 0.05$) during storage and display than the nonenhanced control (NEC) and no-lactate control (NLC), except at day 14 for the LACE treatment ($p > 0.05$). Thus, acetate may help to counteract some of the darkening effects of lactate. Meat enhanced with solutions containing potassium lactate has a darker lean color (*4*, *5*), but this darkening is not linked to an increase in meat pH when potassium lactate (*4*, *21*) or sodium lactate (*3*) is used. Why lactate darkens meat color is unclear. There were no significant differences for visual darkening among lactate treatments with no acetate (L1.5 or L2.5) on day 14.

Corresponding to visual darkening, L^* (lightness) values were affected ($p < 0.05$) by both time and treatment. The L^* values for all treatments decreased ($p < 0.05$) during storage and display (**Table 4**). Lactate-enhanced steaks were darker (lower L^* values, $p < 0.05$) than steaks without lactate during storage and display. NEC steaks were lightest (greatest L^* values, $p < 0.05$) at day 14. These differences confirm the visual darkening scores.

Color Stability. An interaction between treatment and time ($p < 0.05$) occurred for instrumental and visual discoloration (**Table 4**). There were no significant differences among treatments for visual discoloration at day 2 or on the first day of display (day 9). At the end of display, NEC steaks were most discolored ($p < 0.05$), whereas steaks enhanced with lactate 2.5% (L2.5) were less discolored ($p < 0.05$) at day 14 than were NLC and L1.5 steaks.

Instrumental measures of color stability generally support the visual discoloration observations. All treatments containing

Table 4. LSMeans ($n = 8$) for Instrumental and Visual Color of Injection-Enhanced Beef Longissimus Lumborum Steaks at Days 2 (Initial), 9 (Start of Display), and 14 (End of Display)

		treatments[a]					
trait	day	NEC	NLC	L1.5	L2.5	LACE	SE[b]
L^*	2	45.0 fx	43.5 gx	41.8 hx	39.7 hx	41.4 hix	0.57–0.66
	9	44.4 fx	42.0 gy	40.2 hy	39.2 hix	39.8 hy	
	14	42.2 fy	40.0 gz	39.5 gy	38.0 hy	38.4 ghz	
a^*	2	31.5 fx	29.8 fgx	29.9 fgx	27.9 gx	29.1 fgx	1.28–1.52
	9	28.3 fy	28.6 fx	27.8 fx	26.6 fxy	26.9 fx	
	14	13.6 hz	22.3 fgy	24.1 fgy	24.9 fy	24.2 fgy	
b^*	2	23.1 fx	21.1 gx	20.8 gx	18.5 hx	20.1 gx	0.79–0.90
	9	20.9 fy	20.3 fy	18.9 fgy	17.5 gx	17.9 gy	
	14	14.9 gz	16.4 fz	16.4 fz	15.5 fy	16.2 fxz	
chroma[c]	2	39.1 fx	36.6 fgx	36.4 fgx	33.5 gx	35.2 gx	1.61–2.01
	9	35.0 fgy	35.9 fx	33.9 fgx	31.1 gxy	32.6 fgx	
	14	20.4 fz	27.8 ghy	29.2 ghy	29.6 gy	29.0 ghy	
visual darkening[d]	2	1.7 fx	2.7 gx	3.7 hx	4.1 hx	3.9 hx	0.25–0.30
	9	2.1 fx	2.6 fx	3.4 gx	3.9 ghx	3.3 gy	
	14	3.7 fy	3.6 fy	4.4 gy	4.6 gy	3.7 fxy	
visual discoloration[e]	2	1.0 fx	1.0 fx	1.0 fx	1.0 fx	1.0 fx	0.33
	9	1.0 fx	1.0 fx	1.0 fx	1.0 fx	1.0 fx	
	14	5.1 fy	2.8 gy	2.8 gy	2.2 hz	2.4 ghy	

[a] NEC, nonenhanced control; NLC, no-lactate control; L1.5, 1.5% potassium lactate; L2.5, 2.5% potassium lactate; LACE, 1.5% potassium lactate, 0.1% sodium acetate. All enhanced treatments contained 0.3% NaCl, 0.3% phosphate, and 0.058% rosemary extract. Means within a row with different letters (f–i) are different ($p < 0.05$). Means in a column within a trait with different letters (x–z) are different ($p < 0.05$). [b] Standard error. [c] $(a^{*2} + b^{*2})^{1/2}$. [d] Darkening: 1 = no darkening, 3 = slightly dark, 5 = moderately dark. [e] Discoloration (percent of surface metmyoglobin): 1 = no discoloration (0%), 2 = slight discoloration (1–19%), 3 = small discoloration (20–39%), 4 = modest discoloration (40–59%), 5 = moderate discoloration (60–79%).

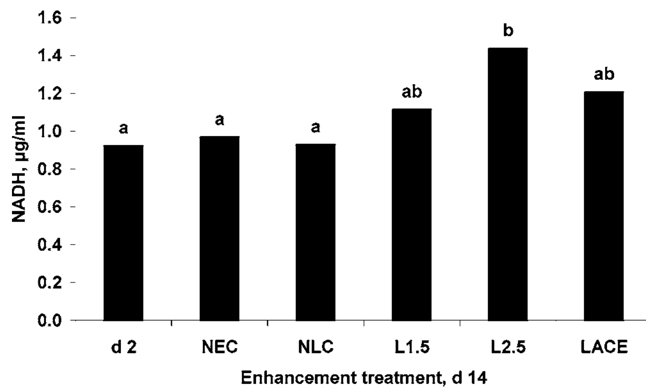


Figure 2. LSMeans ($n = 8$) for NADH concentration in injection-enhanced beef longissimus lumborum steaks on day 2 (initial) and day 14 (end of display): NEC, nonenhanced control; NLC, no lactate control; L1.5, 1.5% potassium lactate; L2.5, 2.5% potassium lactate; LACE, 1.5% potassium lactate, 0.1% sodium acetate. All enhanced treatments contained 0.3% NaCl, 0.3% phosphate, and 0.058% rosemary extract. Mean NADH concentration on day 2 for all treatments; treatments did not differ ($p >$ 0.05). Means with different letters (a, b) are different ($p < 0.05$).

lactate had similar a^* and chroma values ($p > 0.05$) during storage from days 2 to 9. As display time progressed, steaks enhanced with lactate had higher a^* values (redness) and chroma ($p < 0.05$) than did NEC steaks at the end of display (**Table 4**). All treatments decreased in a^* and chroma from days 2 to 14 ($p < 0.05$), but the L2.5 treatment was the only treatment that had stable ($p > 0.05$) a^* and chroma during display (days 9−14), indicating that it had the greatest color stability. Although all treatments except L2.5 decreased in a^* and chroma during display, the LACE and L1.5 treatments had a smaller total a^* decrease than the NLC treatment. Increased redness in injection-enhanced beef due to lactate addition has been reported (*1*, *4*, *5*).

Beef with ultimate pH >5.8 had better color stability than similar meat with an ultimate pH of 5.6 (*22*). Lactate must improve meat color and MRA by some mechanism other than altering pH, however, because NLC steaks were significantly more discolored than L2.5 steaks on day 14, although their pH values were similar ($p > 0.05$). Lawrence et al. (*1*) found injection-enhanced beef steaks with calcium ascorbate and calcium lactate to have similar pH values, but steaks with calcium lactate were significantly more color stable. Papadopoulos et al. (*2*) also noted that sodium lactate promoted color stability in cooked, vacuum-packaged beef roasts, with no effect on pH. Potassium lactate also improved injection-enhanced beef color stability without affecting pH (*21*). Lawrence et al. (*1*) and Mancini et al. (*21*) hypothesized that lactate's ability to stabilize muscle color and maintain ferrous myoglobin is linked to regeneration of NADH via LDH.

NADH Concentration. There were no significant differences for NADH contents among treatments on day 2. At the end of display, however, steaks with L2.5 had greater ($p < 0.05$) NADH than treatments without lactate (**Figure 2**). Steaks enhanced with 2.5% lactate had higher NADH on day 14 than on day 2. Thus, as time progressed, L2.5 steaks regenerated more NADH, which would provide electrons for reducing MMb. Although the difference was not significant, L1.5 and LACE treatments had greater ($p > 0.05$) NADH concentration than did NEC and NLC treatments.

MRA. An interaction between treatment and time occurred ($p < 0.05$) for MRA (**Table 5**). Steaks enhanced with L2.5 and NLC steaks had the least ($p < 0.05$) initial (day 2) MRA. As storage and display time increased, however, NLC steaks decreased ($p < 0.05$) in MRA, whereas L1.5 and L2.5 steaks retained more MRA. During storage and display, the L2.5 steaks were the only steaks not to decrease in MRA ($p > 0.05$). In display, L1.5 steaks did not decrease in MRA ($p > 0.05$). Stable MRA over the 14 days in steaks with L2.5 and the overall elevated MRA with any lactate inclusion are supporting pieces of evidence for the role of increased NADH through lactate-induced LDH activity to reduce discoloration. Steaks with L2.5 had more than 2.5 times as much MRA on day 14 as NLC steaks did.

LDH Activity 1 (Lactate to Pyruvate). A prime objective of this study was to evaluate if enhancement of beef longissimus lumborum with lactate increases post-mortem LDH activity. There was a significant ($p = 0.06$) treatment by time interaction for LDH 1 (lactate to pyruvate) activity. Steaks from the NEC and L1.5 initially had greater ($p < 0.05$) LDH activity than did NLC and LACE steaks on day 2 (**Table 5**). The LDH activity of L2.5 was not different ($p > 0.05$) from that of other treatments. LDH activity of all treatments increased ($p < 0.05$) from days 2 to 9 and was similar ($p > 0.05$) among all treatments. No further increases were noted for any treatment from days 9 to 14 in display ($p < 0.05$), but L2.5 steaks had greater LDH activity than did L1.5, NLC, and NEC steaks on day 14. In consequence, increased LDH activity might explain the increases in NADH, which would be available for MMb reduction (**Figure 1**).

Table 5. LSMeans ($n = 8$) for Metmyoglobin-Reducing Activity (MRA) and Lactic Dehydrogenase (LDH) Activity of Injection-Enhanced Beef Longissimus Lumborum Steaks at Days 2 (Initial), 9 (Start of Display), and 14 (End of Display)

		treatments[a]					
trait	day	NEC	NLC	L1.5	L2.5	LACE	SE[b]
MRA[c]	2	100.0 fx	83.5 gx	99.9 fx	80.9 gx	97.4 fx	9.00–9.18
	9	87.9 fx	42.4 gy	73.0 fy	82.0 fx	85.0 fx	
	14	59.7 gy	30.0 hy	68.2 fgy	77.1 fx	59.3 gy	
LDH-1[d]	2	127.2 fgx	108.1 hx	132.8 fgx	120.4 ghx	114.5 hx	8.97–9.17
	9	149.6 fy	152.8 fy	156.5 fy	162.0 fy	152.8 fy	
	14	145.4 gy	146.5 gy	151.5 gy	171.7 fy	160.5 fgy	
LDH-2[e]	2	113.4 fgx	101.8 fx	129.9 gx	103.1 fx	101.2 fx	13.98–14.04
	9	149.6 fy	152.5 fy	155.9 fy	162.7 fy	152.0 fy	
	14	161.2 gy	161.7 gy	163.8 gy	212.6 fz	182.3 gz	

[a] NEC, non-enhanced control; NLC, no-lactate control; L1.5, 1.5% potassium lactate, L2.5, 2.5% potassium lactate; LACE, 1.5% potassium lactate, 0.1% sodium acetate. All enhanced treatments contained 0.3% NaCl, 0.3% phosphate, and 0.058% rosemary extract. Means within a row with different letters (f–h) are different ($p < 0.05$). Means in a column within a trait with different letters (x–z) are different ($p < 0.05$). [b] Standard error. [c] [(Initial % surface metmyoglobin − final % surface metmyoglobin) ÷ initial % surface metmyoglobin] × 100. [d] Conversion of lactate to pyruvate (units of LDH activity as μmol of NADH/min/g of sample). [e] Conversion of pyruvate to lactate (units of LDH activity as μmol of NADH/min/g of sample).

Different LDH activity, which implies a different rate of NADH production, depends on the muscle fiber type. Longissimus lumborum, a whiter and more color-stable muscle, had greater LDH activity compared with psoas major, which is redder and less color stable (*23*). Therefore, muscle LDH activity may be related to the color stability of post-mortem muscle.

There has been speculation regarding the location in post-mortem muscle of the NADH pool that could be involved with MMb reduction. Watts et al. (*8*) discussed a possible relationship of LDH activity with post-rigor meat-color stability and indicated that it is well-established that the oxidation of NADH to NAD^+ could lead to the reduction of MMb in the presence of intermediate electron carriers such as other reductases. In addition, many enzymes of glycolysis, including LDH, the Krebs cycle, and the electron-transport chain, remain active in post-mortem meat (*24*). Furthermore, Saleh and Watts (*19*) tested several glycolytic and Krebs cycle intermediates for their ability to increase MMb reduction in ground beef, and they concluded that the reducing activity of most meat samples can be increased by appropriate substrate addition.

The glycolytic pathway may have a crucial role in enzymatic MMb reduction by supplying cytoplasmic NADH for the NADH−cytochrome b_5 reductase system in muscle (*25*). The addition of 2-deoxy-D-glucose, an inhibitor of the glycolytic pathway, to the cell suspension completely inhibited MMb reduction, whereas a citric acid cycle inhibitor (malonic acid) did not inhibit this reaction. Therefore, they concluded that active glycolytic flux may be necessary for activity of the MMb reduction system in muscle cells. L-Lactic acid concentration increased with increasing levels of lactate enhancement (up to 3%) and decreased during storage, whereas D-lactic acid content increased during storage, likely due to the numbers of lactic acid bacteria (*2*), because D-lactate is less optimal than L-lactate for utilization by LDH (*17*, *18*). These data lend support to our hypothesis that lactate added during injection enhancement could be related to the replenishment of NADH via LDH activity.

LDH Activity 2 (Pyruvate to Lactate). LDH activity 2 (pyruvate to lactate) followed the same trends as LDH 1, with a significant treatment by time interaction. LDH activity of all treatments increased ($p < 0.05$) during storage and display (**Table 5**). By day 14, steaks with L2.5 had significantly greater LDH 2 activity than did other treatments because they had undergone the greatest increase in LDH activity. Overall, LDH 2 (pyruvate to lactate) activity was higher than LDH 1 (lactate to pyruvate) activity because the reaction favors NAD oxidation rather than reduction. The activity of LDH increases with extended storage time up to 10 days at 4 °C (*26*). During post-mortem aging of muscles, enzymatic degradation of the muscle structure occurs; thus, soluble proteins, including LDH, may be released from their normal location within living muscle (*26*).

The results of both research experiments in this study suggest that lactic dehydrogenase plays a role in lactate-mediated stabilization of beef color in high-oxygen MAP. Lactate seems to promote color stability via increased lactic dehydrogenase activity, through the conversion of lactate to pyruvate and the concomitant regeneration of NADH. The NADH subsequently is available to reduce metmyoglobin to either oxy- or deoxy-myoglobin. Steaks enhanced with 2.5% lactate had the most stable color, greatest metmyoglobin-reducing activity, and greatest LDH activity during storage and display, providing evidence for the proposed LDH−lactate mechanism of beef injection enhanced with solutions containing lactate.

ABBREVIATIONS USED

MMb, metmyoglobin; DMb, deoxymyoglobin; OMb, oxymyoglobin; MRA, metmyoglobin-reducing activity; EDTA, ethylenediaminetetraacetic acid; NAD^+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; LDH, lactic dehydrogenase; FMN, flavin mononucleotide; USDA, U.S. Department of Agriculture; MAP, modified-atmosphere packaging; NEC, nonenhanced control; NLC, no lactate control; L1.5, 1.5% lactate; L2.5, 2.5% lactate; LACE, lactate + acetate; LSMeans, least significant means; SE, standard error.

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LITERATURE CITED

(1) Lawrence, T. E.; Dikeman, M. E.; Hunt, M. C.; Kastner, C. L.; Johnson, D. E. Effects of calcium salts on beef *longissimus* quality. *Meat Sci.* **2003**, *64*, 299−308.

(2) Papadopoulos, L. S.; Miller, R. K.; Acuff, G. R.; Vanderzant, C.; Cross, H. R. Effect of sodium lactate on microbial and chemical composition of cooked beef during storage. *J. Food Sci.* **1991**, *56*, 341−347.

(3) Papadopoulos, L. S.; Miller, R. K.; Ringer, L. J.; Cross, H. R. Sodium lactate effect on sensory characteristics, cooked meat color and chemical composition. *J. Food Sci.* **1991**, *56*, 621−626.

(4) Mancini, R. A.; Hunt, M. C.; Hachmeister, K. A.; Seyfert, M.; Kropf, D. H.; Johnson, D. E.; Cusick, S.; Morrow, C. The utility of lactate and rosemary in beef enhancement solutions: effects on *longissimus* color changes during display. *J. Muscle Foods* **2005**, *16*, 27−36.

(5) Knock, R. C.; Seyfert, M.; Hunt, M. C.; Dikeman, M. E.; Unruh, J. A.; Higgins, J. J.; Monderen, R. A. Effects of potassium lactate, sodium chloride, sodium tripolyphosphate, and sodium acetate on colour, colour stability, and oxidative properties of injection-enhanced beef rib steaks. *Meat Sci.* **2006**, *74*, 312−318.

(6) Ledward, D. A. Post-slaughter influences on the formation of metmyoglobin in beef muscles. *Meat Sci.* **1985**, *15*, 149−171.

(7) Mancini, R. A.; Hunt, M. C. Current research in meat color. *Meat Sci.* **2005**, *71*, 100−121.

(8) Watts, B. M.; Kendrick, J.; Zipser, M. W.; Hutchins, B. K.; Saleh, B. Enzymatic reducing pathways in meat. *J. Food Sci.* **1966**, *31*, 855−861.

(9) Cheah, K. S. Formation of nitrosylmyoglobin in bacon involving lactate dehydrogenase. *J. Food Technol.* **1976**, *11,* 181−186.

(10) Farnsworth, D. *The Farnsworth−Munsell 100-Hue Test for the Examination of Color Discrimination*; Macbeth: Newsburgh, NY, 1957.

(11) Klingenberg, M. Nicotinamide-adenine dinucleotides (NAD, NADP, NADH, NADPH). Spectrophotometric and fluorimetric methods. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Ed.; Academic Press: New York, 1974; Vol. 4, pp 2045−2048.

(12) McCormick, D. B.; Lemuel, D. W. Nicotinic acid: analogs and coenzymes. In *Methods Enzymology. Vitamins and Coenzymes, XVIII*; Colowick, S. P., Kaplan, N. O., Eds.; Academic Press: New York, 1971; pp 26−27.

(13) AMSA. Guidelines for meat color evaluation. *Reciprocal Meat Conf. Am. Meat Sci. Assoc., Proc.* **1991**, *44*, 1−17.

(14) Wahlefeld, A. W. Lactate dehydrogenase. UV-method with L-lactate and NAD. In *Methods of Enzymatic Analysis*, 3rd ed.; Bergmeyer, H. U., Ed.; Plenum: New York, 1983; Vol. 3, pp 126−132.

(15) Vassault, A. Lactate dehydrogenase. UV-method with L-Lactate and NAD. In *Methods of Enzymatic Analysis*, 3rd ed.; Bergmeyer, H. U., Ed.; Plenum: New York, 1983; Vol. 3, pp 118−125.

(16) SAS Institute, Inc. *SAS User's Guide*; Cary, NC, 1996.

(17) Branden, C. I.; Eklund, H. Structure and mechanism of liver alcohol dehydrogenase, lactate dehydrogenase and glyceraldehydes-3-phosphate. In *Dehydrogenases Requiring Nicotinamide Coenzymes. Dehydrogenases I*; Jeffrey, J., Ed.; Birkhauser Verlag: Basel, Switzerland, 1980; pp 41−75.

(18) Hall, G. Lactate as a fuel for mitochondrial respiration. *Acta Anaesthesiol. Scand.* **2000**, *168*, 643−656.

(19) Saleh, B.; Watts, B. M. Substrates and intermediates in the enzymatic reduction of metmyoglobin in ground beef. *J. Food Sci.* **1968**, *33*, 353−358.

(20) Madhavi, D. L.; Carpenter, C. E. Aging and processing affect color, metmyoglobin reductase and oxygen consumption of beef muscles. *J. Food Sci.* **1993**, *58*, 939−942, 947.

(21) Mancini, R. A.; Kim, Y. H.; Hunt, M. C.; Lawrence, T. E. How does lactate enhancement improve beef color stability? In *Proceedings of the 50th International Congress of Meat Science and Technology*; University of Helsinki Department of Food Technology: Helsinki, Finland, 2004; p 41.

(22) Ledward, D. A.; Dickinson, R. F.; Powell, V. H.; Shorthose, W. R. The colour and colour stability of beef longissimus dorsi and semimembranosus muscles after effective electrical stimulation. *Meat Sci.* **1986**, *16*, 245−265.

(23) Stalder, J. W.; Smith, G. L.; Keeton, J. T.; Smith, S. B. Lactate dehydrogenase activity in bovine muscle as a means of determining heating endpoint. *J. Food Sci.* **1991**, *56*, 895−898.

(24) Andrews, M. M.; Guthneck, B. T.; Mcbride, B. H.; Schweigert, B. S. Stability of certain respiratory and glycolytic enzyme systems in animal tissues. *J. Biol. Chem.* **1952**, *194*, 715−719.

(25) Arihara, K.; Itoh, M.; Kondo, Y. Contribution of the glycolytic pathway to enzymatic metmyoglobin reduction in myocytes. *Biochem. Mol. Biol. Int.* **1996**, *38*, 325−331.

(26) Collins, S. S.; Keeton, J. T.; Smith, S. B. Lactate dehydrogenase activity in bovine muscle as a potential heating endpoint indicator. *J. Agric. Food Chem.* **1991**, *39*, 1291−1293.

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