Effect of calcium chloride, zinc chloride, and water infusion on metmyoglobin reducing activity and fresh lamb color

A. E. D. Bekhit*[1], M. A. Ilian*, J. D. Morton*, L. Vanhanan*, J. R. Sedcole†, and R. Bickerstaffe*

*Molecular Biotechnology Group, Agriculture and Life Sciences Division; and †Applied Computing, Mathematics, and Statistics Group, Applied Management and Computing Division, Lincoln University, Canterbury, New Zealand

ABSTRACT: Calcium chloride ($CaCl_2$), zinc chloride ($ZnCl_2$), or water infusions were used to investigate the biochemical factors that affect fresh lamb color, and to examine the role of metmyoglobin-reducing activity in regulating this important quality attribute. Immediately after exsanguination, lamb carcasses (n = 6 per treatment) were infused (10% of BW) with 0.3 *M* $CaCl_2$, 0.05 *M* $ZnCl_2$, or water via a catheter inserted into the left carotid artery. The right LM was excised at 24-h postmortem and divided into two halves. The caudal portion was cut into 2.5-cm-thick chops and displayed for 6 d under 1,076 lx of white fluorescent lighting at 2°C, whereas the cranial half was vacuum-packaged and stored at 2°C for 3 wk before retail display. Objective color measurements and samples for biochemical analysis were taken at 0, 1, 3, and 6 d of display. In infused carcasses, pH decline was more rapid ($P < 0.05$) than in untreated controls, and it was greatest for $CaCl_2$-infused carcasses. Calcium chloride-infused carcasses had lower ($P < 0.01$) NAD and higher ($P < 0.001$) NADPH concentrations than water- and $ZnCl_2$-infused or untreated control carcasses. The negative effects of calcium infusion on fresh lamb color, higher ($P < 0.01$) metmyoglobin accumulation rate, and lower ($P < 0.01$) L*, a*, and b* color measurements could be explained by the lower amounts of unbound water ($P < 0.01$), shorter sarcomere length ($P < 0.01$), lower NAD concentrations ($P < 0.01$), and higher lipid peroxidation ($P < 0.01$). Zinc and water-infusions produced less ($P < 0.01$) lipid oxidation and improved the color and color stability of fresh lamb ($P < 0.001$). Rate of lipid oxidation in LM chops was greater ($P < 0.01$) after 3 wk of vacuum-packaged storage than 24-h postmortem. Metmyoglobin-reducing activities (sarcoplasmic and myofibrillar) were decreased in response to infusion treatments ($P < 0.001$), and $ZnCl_2$ infusion resulted in the lowest metmyoglobin-reducing activities ($P < 0.001$). A significant association between the myofibrillar metmyoglobin-reducing activity and lipid peroxidation was observed, but metmyoglobin-reducing activities were not associated with any improvement in lamb color. Strategies to increase the antioxidant levels in lamb are very important to improve lamb quality, especially during vacuum-packaging storage.

Key Words: Calcium Chloride, Color, Infusion, Lamb, Metmyoglobin-Reducing Activity

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Introduction

Consumers often equate meat color to the freshness of meat and consequently rely on color as a visual measure of quality (Faustman and Cassens, 1990b). Maintaining the cherry-red color of bloomed fresh meat will increase its desirability. Accumulation of metmyoglobin (**MetMb**) at the meat surface during storage leads to the discoloration of fresh meat (Ledward, 1971). The oxidation of myoglobin can be reversed under specific conditions depending on MetMb-reducing activity, and the availability of cofactors; however, the role of MetMb-reducing activity in the maintenance of fresh meat color is a matter of debate. Some investigators (Ledward, 1985; Zhu and Brewer, 1998) have reported that MetMb-reducing activity is the controlling factor retarding the accumulation of MetMb, whereas others (Echevarne et al., 1990; Madhavi and Carpenter, 1993) have found no evidence to support this theory.

Application of $CaCl_2$ to meat has been reported to improve the tenderness of meat (Koohmaraie et al., 1988); however, $CaCl_2$ may have a negative effect on color and color stability (Wheeler et al., 1996). Infusion of water into animal muscles also has been reported to

[1]Correspondence: P.O. Box 84 (phone: 64 (03) 325 3838, ext. 8427; fax: 64 (03) 325 3851; e-mail: bekhita2@lincoln.ac.nz).

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improve tenderness (Karmas, 1970), whereas the infusion of $ZnCl_2$ toughens meat (Koohmaraie, 1990). Apart from the negative effect of $ZnCl_2$ on meat tenderness, Zn is a well-known membrane stabilizer and contributes to the maintenance of membrane structure and function (Bettger and O'Dell, 1981). There is, therefore, the potential to use Zn in the investigation of the factors that influence color and color stability. Thus, the objective of this research was to investigate the effects of prerigor infusion of these compounds on color and color stability, as well as the biochemical factors which could affect the color of fresh lamb, and to determine the role of MetMb-reducing activity in the maintenance of fresh lamb color.

Materials and Methods

Animals and Infusion Treatments

This experiment was part of a larger study to investigate the effect of $CaCl_2$ and $ZnCl_2$ on proteolysis and meat tenderness (Ilian et al., 2004). Twenty-four lambs (9 mo old and an average live weight of 36.3 ± 3.3 kg) were assigned randomly to four postmortem treatment groups (six lambs per group). The lambs were purchased at auction, and their background was unknown. Lambs were slaughtered humanely using standard captive-bolt stunning procedures at the Lincoln University facilities. Two lambs from each group were killed on each of three consecutive days. Lamb carcasses were infused with water, 0.05 *M* $ZnCl_2$, 0.3 *M* $CaCl_2$, or not infused (controls). Vascular infusion was performed as described by Koohmaraie (1990). The left jugular vein of the lambs assigned to the vascular infusion treatments were severed for exsanguination. An incision was then made in the left carotid artery, and a 0.4-cm-diameter catheter was inserted for the delivery of infusion solution. Carcasses were infused with 10% of their BW with the appropriate solution (20°C) using a flow inducer (MHRE, Watson-Marlow Ltd., Cornwall, U.K.) at a flow rate of 14.4 L/h, held in a 15°C cooler for 4 h after dressing, and then moved to a 2°C cooler for 7 d. The left LM was sampled immediately after dressing (0 h) and at 5 and 10 h postmortem, as well as at 24 h postmortem, for measurement of the nucleotides. These samples were snap-frozen immediately in liquid N_2 and stored in a –80°C until analyzed.

Sample Preparation

Carcass pH and temperature were measured immediately after dressing and every 30 min during the first 10 h postmortem, as well as at 24 h postmortem, in the LM between the 12th and 13th ribs using a combination puncture pH electrode (InLab 427, Mettler-Toledo Process Analytical Inc., Wilmington, MA) attached to a pH meter (Hanna HI 9025, Hanna Instruments, Woonsocket, RI).

The right LM was excised at 24 h postmortem and divided into two portions. The caudal section was used to examine the biochemical factors, color, and color stability at 24 h postmortem, whereas the cranial section was vacuum-packaged to examine the treatment effects after 3 wk of vacuum-packaged storage at 2°C. Samples were cut into 2.5-cm-thick chops and placed in polystyrene trays covered with O_2-permeable polyvinyl chloride film (O_2 transmission rate = >2,000 mL/(m^2·atm) for 24 h at 25°C; AEP FilmPac, Ltd., Auckland, N.Z.), and stored for 6 d at 2°C in a white fluorescent illuminated (1,076 lx) open-front display cabinet (Osram Lumilux, Osram Australia Pty Ltd., New South Wales, Australia). Samples taken for biochemical analyses after 0, 1, 3, and 6 d of display were vacuum-packed, rapidly frozen in liquid N_2, and stored at –80°C until analyzed. Measurements were performed in duplicate for each sample, and the mean value was used for statistical analyses.

Metmyoglobin-Reducing Activities

Metmyoglobin reductase extracts were obtained as described by Echevarne et al. (1990), with the modifications of Bekhit et al. (2003). Sarcoplasmic MetMb-reducing activity (**SMRA**) and myofibrillar MetMb-reducing activity (**MMRA**) were determined as described by Bekhit et al. (2003).

Validation of MetMb-Reducing Activity Assay

Use of the chelating agent EDTA (1 m*M*), and a reducing agent, dithiothreitol (**DTT**; 1 m*M*), was essential to obtain maximum MetMb reducing activities (Arihara et al., 1989). These compounds, however, may interfere with the effects of the infused ions in the present study, especially MetMb-reducing activities. Therefore, we investigated the effects of these compounds on SMRA and MMRA of lamb samples from $CaCl_2$- and $ZnCl_2$-infused carcasses. Inclusion of EDTA during the extraction of the enzyme and in the enzyme assay increased ($P < 0.01$) SMRA (6.2%) and MMRA (8%) of $CaCl_2$-infused lamb samples. Only MMRA was increased ($P < 0.001$; 52%) in $ZnCl_2$-infused lamb samples. Addition of DTT in the extraction buffers significantly increased SMRA (7%) in $CaCl_2$-infused carcasses and MMRA (10%) in the lamb samples from $ZnCl_2$-infused carcasses. Because these compounds were found to alter and interfere with the MetMb-reducing activities as associated with the infusion treatments, EDTA and DTT were removed from the extraction buffers, dialysis buffer, and enzyme assay.

Total Pigment, Myoglobin Concentration, Heme Iron, and Metmyoglobin Percent

Total pigments were determined as described by Fleming et al. (1960) and Rickansrud and Henrickson (1967). Total molar concentration of pigments was calculated using the molar extinction coefficient of 11.3×10^3 for cyanmetmyoglobin (Drabkin, 1950), and the to-

tal pigments were calculated as milligrams of total pigments/kilogram, from the following formula:

$$\text{Total pigments, mg/kg} = \{(A/11{,}300) \times [17{,}000 \times (0.05 + d) \times 1{,}000/\text{sample wt}]\}/1{,}000$$

where A = absorbance at 540 nm; 11,300 = the molar extinction coefficient of cyanmetmyoglobin at 540 nm; 17,000 = MW of pigments; 0.05 = volume (L) of the extract; and d = volume increase (25 mL × 2) due to addition of cyanides.

Myoglobin concentration was determined according to the procedure of Sammel et al. (2002). Calculations were made using a molar extinction coefficient of 7.6×10^{-3} (Bowen, 1949) and a MW of 16,110 Da for myoglobin (Drabkin, 1978). Myoglobin concentrations were expressed as milligrams per kilogram. Heme iron calculations were based on myoglobin containing 0.35% iron (Drabkin, 1978), and heme iron concentration was expressed as micrograms per gram. Metmyoglobin percent was determined as described by Krzywicki (1982) at 0, 1, 3, and 6 d of display for 24-h postmortem samples, and after 1 and 6 d of display for 3-wk vacuum-packaged samples.

NAD, NADP, NADH, and NADPH Analyses

Nucleotides concentrations were determined by reverse-phase chromatography as described by Noack et al. (1992). Nucleotides were extracted from lamb samples by the phenol-chloroform-isoamyl alcohol method of Gellerich et al. (1987), with the modifications of Noack et al. (1992). Concentrations of NAD, NADH, NADP, and NADPH were determined on control samples at 0, 5, and 10 h postmortem, and during display time at 0, 1, 3, and 6 d for 24-h postmortem samples of all infused samples.

Thiobarbituric Acid Reactive Substances Analysis

Thiobarbituric acid reactive substances (**TBARS**) after 0, 1, 3, and 6 d of display for 24-h postmortem samples, as well as after 1 and 6 of display for 3-wk vacuum-packaged samples, were determined using the method of Witte et al. (1970), with the modification of Siu and Draper (1978). Thiobarbituric acid reactive substances were calculated as milligrams of malondialdehyde/kilogram of sample, and the mean of the six measurements per sample was used for the statistical analyses.

Color Measurements and Sarcomere Length

Objective color determinations were performed as previously described (Bekhit et al., 2001) on 2.5-cm-thick LM chops after allowing a 2-h bloom period, and then after 1, 3, and 6 d of display at 2°C in the illuminated display cabinet. Lamb color (L*, a*, and b*) measurements were collected using a Minolta chromameter (CR-210; Minolta Camera Co., Ltd., Osaka, Japan) with a 2° observer and illuminant D_{65}. Three replicate L*, a*, and b* measurements were taken for each sample, and averaged for statistical analyses. Moreover, myofibrils were isolated from the LM according to the procedure of Culler et al. (1978), and sarcomere length was determined as described by Geesink et al. (2001).

Statistical Analyses

Data were analyzed as a split-split-plot design, with infusion treatment as the whole plot, postmortem time (24 h vs. 3 wk of vacuum-packaged storage) as the subplot, and display time (0, 1, 3, or 6 d) as the repeated measures sub-subplot. Each infusion treatment assigned to lambs was treated as a completely randomized block design, with individual lamb was the experimental unit. Data were analyzed using the REML routine in GenStat (GenStat Release 6.1, Lawes Agricultural Trust, VSN Int. Ltd., Rothamsted, U.K.), and the significance of treatment terms and their interactions were determined by Wald tests. In the REML analysis, treatment, postmortem time, and display time were set as fixed factors, whereas animals and slaughter day were set as random factors using the VCOMPONENTS directive. Model terms were sequentially added to the fixed model to test for fixed effects. The statistical model was $Y_{ijk} = \mu + (T)_i + (PM)_j + (DT)_k + (T \times PM)_{ij} + (T \times DT)_{ik} + (PM \times DT)_{jk} + (T \times PM \times DT)_{ijk} + \varepsilon_{ijk}$, where T = infusion treatment, PM = postmortem time, and DT = display time. Data were subjected to regression analysis to adjust correlation coefficient for confounding effects of lamb, treatment, display time, and their interactions (Welham and Thompson, 1997). Means and SEM were those estimated by the REML routine. Data for meat pigments were analyzed using one-way ANOVA using PROC GLM with infusion treatment as the lone main effect in the model. An α level of 0.05 was used to determine statistical significance.

Results and Discussion

Average Live Weight of Lambs and Concentration of Infused Ions

No significant differences were found in the live weight of the animals assigned to various treatments or control (36.5 ± 4.05; 36.2 ± 3.72; 36.33 ± 3.19; 36.23 ± 3.06 kg for water-, $ZnCl_2$-, $CaCl_2$-, and noninfused treatments, respectively). The efficacies of the infusion treatments were evaluated by determining the content of Zn and Ca ions in the LM from all the carcasses. Treatments increased ($P < 0.001$) the concentration of Zn and Ca ions in their respective infused lamb carcasses (Ilian et al., 2004).

LM pH Decline

All infused carcasses had a more ($P < 0.05$) rapid pH decline compared with the noninfused control (Figure

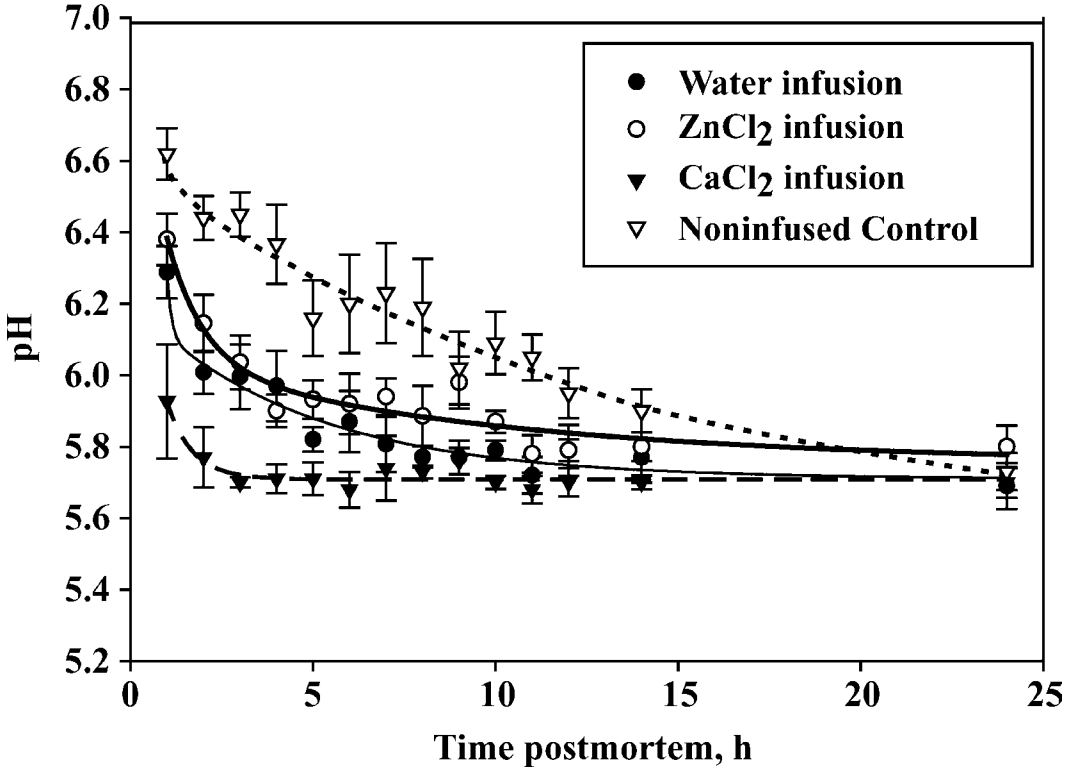

Figure 1. Effect of prerigor vascular infusion with water, calcium chloride ($CaCl_2$), or zinc chloride ($ZnCl_2$) on postmortem LM pH decline. Within a specific time postmortem, SE bars that do not overlap indicate that means differ, $P < 0.05$.

1). Whereas $CaCl_2$-infused carcasses reached ultimate pH after 2.5 h postmortem, water- and $ZnCl_2$-infused carcasses reached their ultimate pH values after 7 and 11 h postmortem, respectively. All infused carcasses had lower ($P < 0.05$) pH values than noninfused carcasses during the first 12 h postmortem (Figure 1). Calcium chloride-infused carcasses had lower ($P < 0.05$) pH values than $ZnCl_2$- and water-infused carcasses during the first 5 h postmortem.

Ultimate (24 h) LM pH was not different among treatments, with mean values of 5.72 ± 0.14, 5.81 ± 0.12, 5.72 ± 0.07, and 5.77 ± 0.14 for water-infused, $ZnCl_2$-infused, $CaCl_2$-infused, and noninfused carcasses, respectively. However, the rapid pH decline in the LM of infused carcasses occurred when the carcass temperatures were relatively high compared with noninfused carcasses (15.21, 10.0, 6.2, and 4.4°C when carcasses achieved their ultimate pH for $CaCl_2$-, water-, and $ZnCl_2$-infused or noninfused carcasses, respectively; results not shown). High rates of pH decline may produce meat with abnormal textural and water-binding properties, similar to PSE pork (Wang et al., 1995), and may alter the perceived color independently of MetMb formation (Ledward, 1985). Additionally, the combination of rapid pH decline and elevated muscle temperature results in conditions favorable for protein denaturation (Hunt et al., 2003), and denaturation causes myofibrillar lattice shrinkage, which results in a lighter appearance (Young and West, 2001).

TBARS

Infusion treatments, display time, aging time, and the interactions among these main effects affected ($P < 0.001$) TBARS values. There were no differences between infusion treatments on 0 and 1 d of display for the 24-h postmortem LM samples; however, chops from $CaCl_2$-infused carcasses had higher ($P < 0.01$) TBARS values after 3 d of display than water-infused, $ZnCl_2$-infused, or noninfused carcasses (Figure 2A). After 6 d of display, chops from $ZnCl_2$-infused carcasses exhibited the least ($P < 0.01$) TBARS values, whereas chops from $CaCl_2$-infused carcasses had the greatest ($P < 0.01$) TBARS values; however, LM TBARS values were not different between $CaCl_2$-infused and noninfused carcasses (Figure 2B).

Because initial (d 0) TBARS values were comparable between 24-h postmortem and 3-wk vacuum-packaged LM chops, it was evident that lipid oxidation did not occur during storage, but was accelerated upon exposure to oxygen (Figure 2B). It is likely that the changes in the cellular and tissue structure during the vacuum-packaged aging enable the pro-oxidants to interact directly with the cellular lipids, which leads to increased lipid oxidation. Alternatively, the endogenous antioxidant capacity of lamb could be depleted during aging.

After 1 d of display, TBARS values of vacuum-packaged samples from carcasses infused with $ZnCl_2$ were less ($P < 0.01$) than those from water- and $CaCl_2$-infused

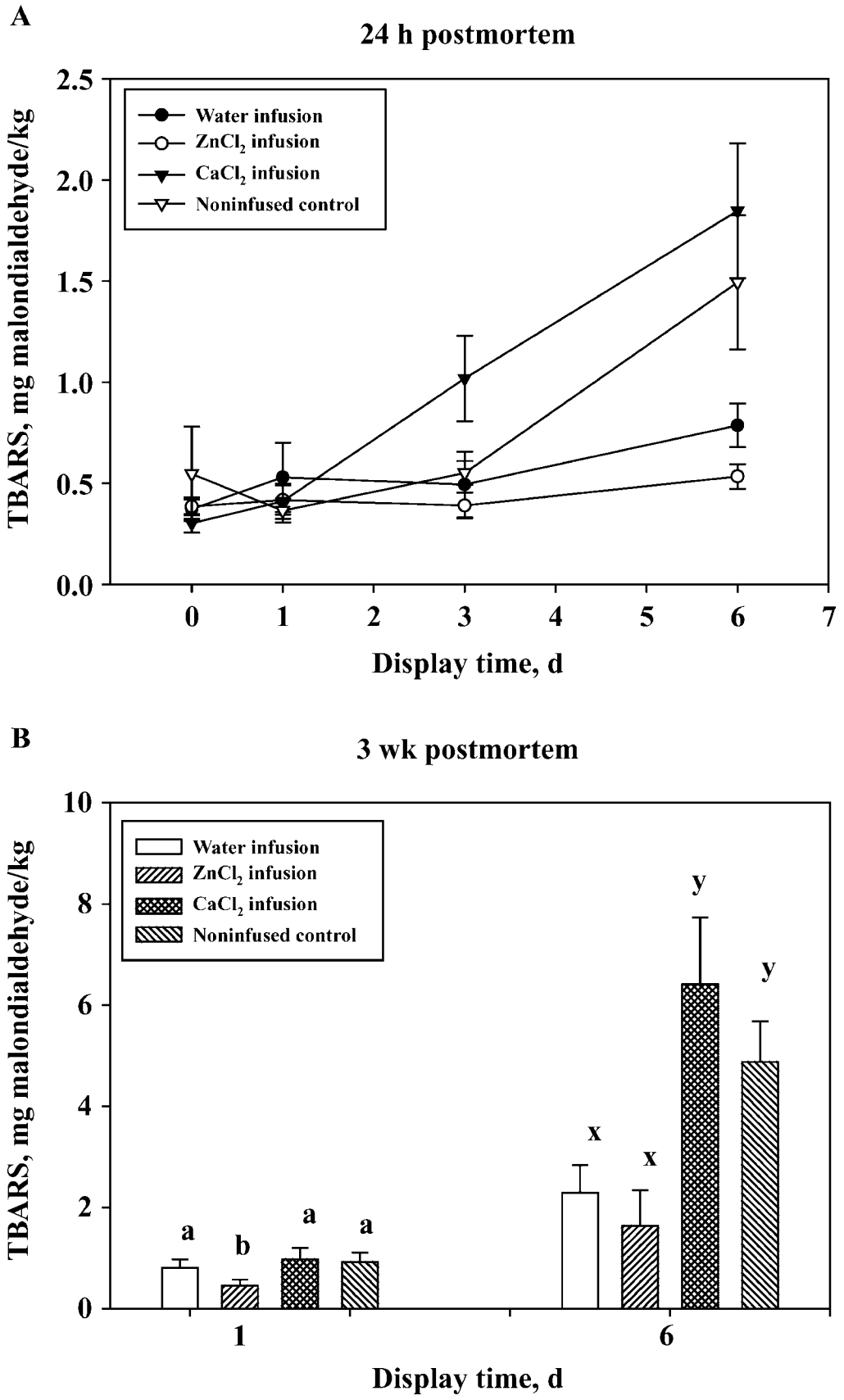

Figure 2. Interactive effects of display day and pre-rigor vascular infusion with water, calcium chloride ($CaCl_2$), or zinc chloride ($ZnCl_2$) on thiobarbituric acid reactive substances (TBARS, mg malondialdehyde/kg of fresh tissue) values of the LM aged: A) 24 h or B) 3 wk. In Panel A, within a specific display day, SE bars that do not overlap indicate that means differ, $P < 0.05$, whereas in Panel B, bars that do not have a common letter differ, $P < 0.01$.

carcasses, as well as noninfused carcasses (Figure 2B). By the end of display (d 6), chops from $ZnCl_2$- and water-infused carcasses had lower ($P < 0.001$) TBARS values than either those from $CaCl_2$-infused and noninfused carcasses. There was no difference in TBARS values between chops from water- and $ZnCl_2$-infused carcasses after 6 d of display (Figure 2B).

The role of reactive oxygen species in promoting the oxidative processes is well known (Grandemer, 1998; Morrissey et al., 1998). The effects of $CaCl_2$ infusion in our study agree with those of St. Angelo et al. (1991) and Harris et al. (2001). Increased lipid oxidation in $CaCl_2$-treated lamb was suggested to be due to the stimulation of lipoxygenase activity by Ca (St. Angelo et al., 1991). Calcium also has been demonstrated to stimulate the mitochondrial respiration process (Carafoli and Gazzotti, 1970). Free radicals are the products of the respiration processes and initiate the oxidation processes (Di Meo and Venditti, 2001). On the other hand, water and $ZnCl_2$ acted as antioxidants, which was evident from the lower TBARS values throughout display for chops from both the 24-h and 3-wk aged LM. Water quenches both the high- and low-energy states of singlet oxygen, and it constitutes a very effective primary defense against this oxidant (Forman and Fisher, 1981); however, because water constitutes 70% of meat, that line of reasoning may not be plausible. It is more likely that a diluting effect on the prooxidants (meat pigments) accounts for the effect of water on lipid oxidation. Moreover, there are several reports on the antioxidant properties of Zn and its effect on antioxidant enzymes in biological systems (Bettger and O'Dell, 1981).

Sarcomere Length

It has been reported that prerigor $CaCl_2$ addition, whether by injection (Geesink et al., 1994) or infusion (Hunt et al., 2003; Dikeman et al., 2003), induces extreme sarcomere contraction. In this study, shorter ($P < 0.001$) sarcomeres were observed for up to 7 d postmortem in chops from $CaCl_2$-infused carcasses compared with chops from water-infused, $ZnCl_2$-infused, and noninfused carcasses (Figure 3).

The closer spacing of fibrils also produces less light reflectivity, and, as a consequence, the meat is more translucent (Swan, 1993; Young and West, 2001). Under conditions of extreme myofibril contraction, the light penetrates the meat more deeply, and myoglobin absorbs the light strongly, which causes the darker color appearance of the meat. As a result, LM chops from $CaCl_2$-infused carcasses appeared darker than any other treatment.

NAD, NADP, NADH, and NADPH Concentrations

Nucleotides were significantly affected by postmortem time, infusion treatments, and the interaction between the two factors. Concentrations of NAD in LM chops decreased ($P < 0.05$) with increasing time postmortem (Figure 4B). Chops from $CaCl_2$-infused carcasses had lower ($P < 0.01$) NAD concentrations than chops from water-infused, $ZnCl_2$-infused, and noninfused carcasses (Figure 4A). Carafoli and Gazzotti (1970) demonstrated that Ca stimulated mitochondrial respiration. Atkinson and Follett (1973) also found that oxygen uptake of lamb, pork, and beef muscle was correlated with NAD concentrations during 6 d of storage at 0 to 4°C. This was supported by an earlier observation by Watts et al. (1966), who demonstrated that oxygen consumption in meat increased on the addition of NAD or NADH. Hence, an increase in mitochondrial respiration may explain the lower ($P < 0.01$) NAD concentration in the LM of $CaCl_2$-infused carcasses.

Concentrations of NADP in LM chops from water-infused carcasses and noninfused carcasses decreased with increasing time postmortem, whereas in LM chops from $ZnCl_2$- and $CaCl_2$-infused carcasses, NADP concentrations increased with increasing time postmortem. Concentrations of NADP were undetectable in the LM from $CaCl_2$-infused carcasses at 10 h postmortem.

In agreement with the results of Renerre (1984) and Faustman and Cassens (1990a), NADH concentrations decreased rapidly with time postmortem, and plateaued after 24 h postmortem. With the exception of the lower ($P < 0.05$) NADH concentration for the LM from water-infused carcasses at 10 h compared with that of $ZnCl_2$-infused carcasses, no differences were detected among infusion treatments. It has been shown that the addition of NADH to minced meat increased the oxygen consumption rate (Atkinson and Follett, 1973). The concentration of NADH would have consequential effects on the color of meat, as well as the activity of the enzymatic systems that require NADH as cofactors.

Reduced NADPH concentrations in chops from $CaCl_2$-infused carcasses were higher ($P < 0.001$) than chops from water- and $ZnCl_2$-infused carcasses at 5, 10, and 48 h postmortem (Figure 4B). Nucleotides are important cofactors for the enzymatic reduction of MetMb. Metmyoglobin-reducing systems, which use NAD and NADP as cofactors, were reported by Rossi-Fanelli et al. (1957). Other MetMb reductases that require NADH and an appropriate mediator also have been reported by Hagler et al. (1979), Levy et al. (1985), and Matsui et al. (1975). Moreover, it has been shown that NAD and NADH are directly associated with color stability of meat (Madhavi and Carpenter, 1993; Renerre and Labas, 1987).

Pong et al. (2000) observed an increase in the NADH level during the first 3 d of storage of tuna muscle, followed by a dramatic decrease; conversely, NADPH concentrations decreased rapidly during storage. They postulated that these changes in NADH and NADPH occurred as a result of the decreased availability of NADH and NADPH kinase during the glycolytic pathway of the postmortem reaction, in which the reversal of electron transport in the cytosol allows the reduction of NAD to NADH. However, their hypothesis was based on the profiles of NADH and NADPH concentrations

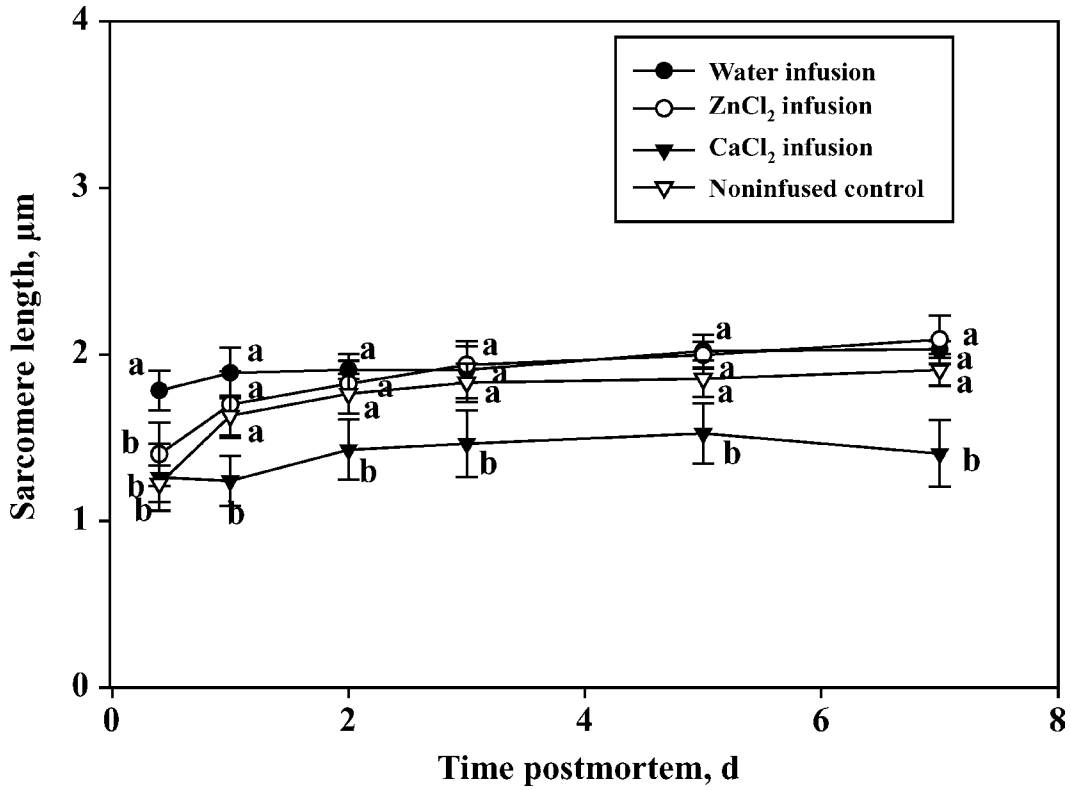

Figure 3. Interactive effects of time postmortem and prerigor vascular infusion with water, calcium chloride ($CaCl_2$), or zinc chloride ($ZnCl_2$) on sarcomere length. Within a specific time postmortem, datum points lacking a common letter differ, $P < 0.05$.

during storage and was not supported by any statistical evidence. Pong et al. (2000) suggested that a constant level of electron donors (either by NADH or NADPH) would maintain the redox pool of the cells. Some early researchers postulated regeneration of NADH by reversal of electron transport (Bodwell et al., 1965; Klingenberg, 1968; Giddings, 1974). If that were the case, a significant negative correlation should exist between NAD and NADH and between NADP and NADPH to indicate the increase or decrease of one component at the expense of the other. In the present study, NAD and NADH concentrations during the first 24 h, and NADP and NADPH concentrations during postmortem time period, demonstrate that the increase in NAD and NADP concentrations coincides with the decrease in NADH and NADPH concentrations, respectively. The correlation coefficients between these compounds for all treatments, whether during the first 24 h postmortem or during the 7 d postmortem, were significant for NAD and NADH (r = 0.425; $P < 0.001$), NAD and NADPH (r = 0.261; $P = 0.011$), NADPH and NADP (r = –0.208; $P = 0.043$), and NADPH and NADH (r = 0.374; $P < 0.001$).

Meat Pigments and Heme Iron

Even though infusing carcasses decreased LM myoglobin concentrations, only chops from water-infused carcasses had lower ($P < 0.05$) myoglobin concentrations than those of noninfused carcasses (Table 1). The ability of the infused solutions to extract myoglobin from the muscles during the infusion process was dependent on ionic strength. The low ionic solution (water) resulted in high amounts of myoglobin being solubilized from the meat samples, whereas the high ionic solution (0.3 *M* $CaCl_2$) resulted in low amounts of myoglobin being extracted from the meat. The infusion of 0.05 *M* $ZnCl_2$ resulted in an intermediate amount of extracted myoglobin. Chops from $CaCl_2$-infused carcasses had numerically higher heme iron concentrations than chops from water- and $ZnCl_2$-infused carcasses, but chops from carcasses infused with water or $ZnCl_2$ had lower ($P < 0.05$) heme iron concentrations than chops from noninfused carcasses. Some investigators have suggested that infusion could induce a lighter color in lamb meat due to the dilution of muscle pigments (Farouk and Price, 1994). Nonetheless, Schoenbeck (1998) found no differences in total muscle pigments in muscles from infused and noninfused beef carcasses. The first suggestion, a dilution effect, could explain the lighter color associated with water or Zn treatments in the present study.

The amount of nonmyoglobin pigments (mainly hemoglobin) was not affected by the infusion treatments (Table 1). Some investigators have suggested that the psoas major from $CaCl_2$-infused beef carcasses contained more hemoglobin than that from non-infused

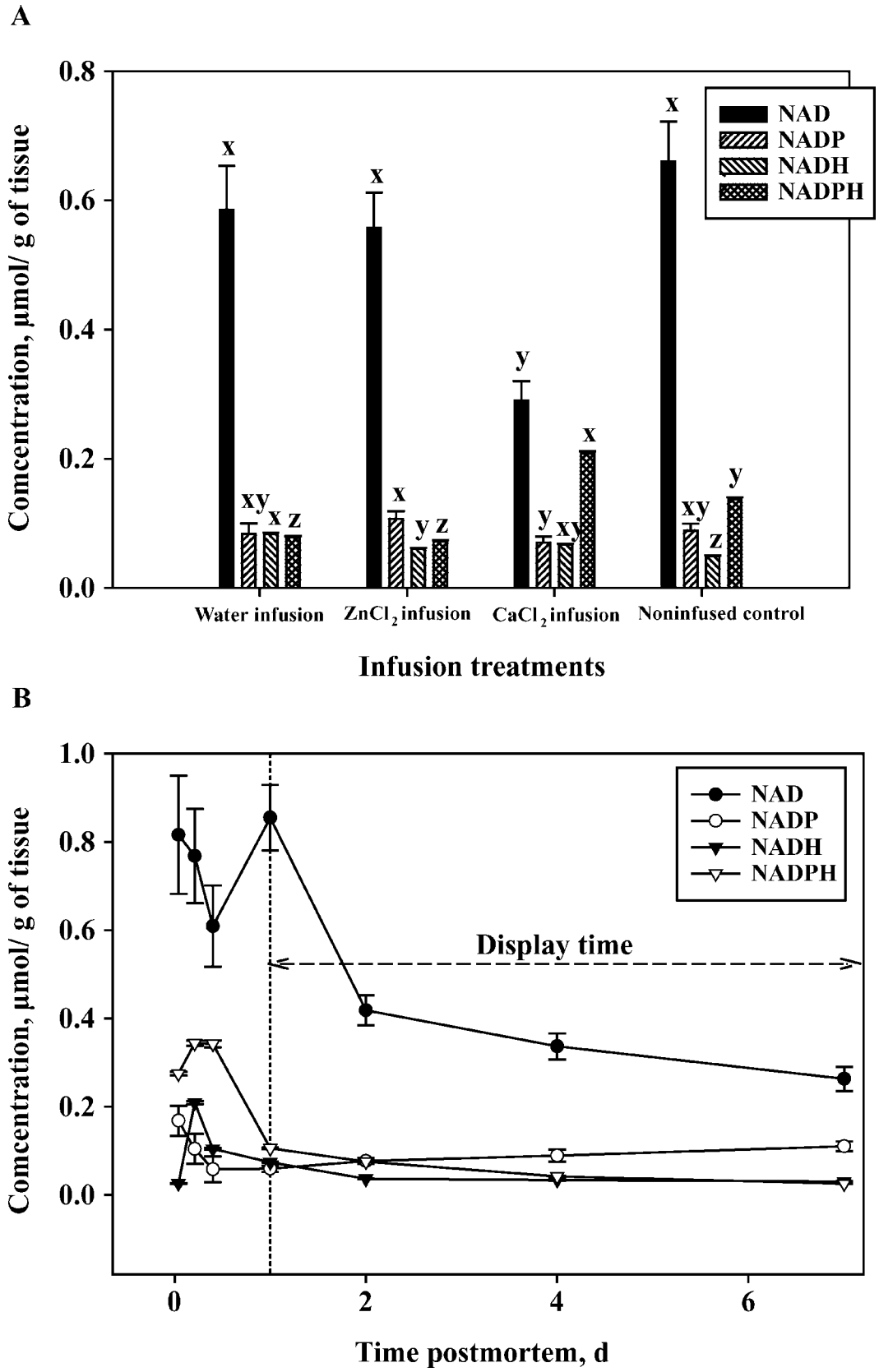

Figure 4. Effect of A) prerigor vascular infusion with water, calcium chloride ($CaCl_2$), or zinc chloride ($ZnCl_2$) and B) time postmortem on LM NAD, NADP, NADH, and NADPH concentrations (µmol/g of fresh tissue). In Panel A, bars that do not have a common letter differ, $P < 0.01$, whereas in Panel B, within a specific time postmortem, points that do not have a common letter differ, $P < 0.05$.

Table 1. Effect of prerigor vascular infusion with water, calcium chloride ($CaCl_2$), or zinc chloride ($ZnCl_2$) on meat pigment and heme iron concentrations (mg/g of fresh tissue) of LM aged 24-h postmortem

Treatment	Myoglobin content, mg/g	Total pigments, mg/g	Non-myoglobin pigments, mg/g	Heme iron, μg/g
Water infusion	2.10 ± 0.19^{x}	2.38 ± 0.21^{x}	0.28 ± 0.02	7.35 ± 0.16^{x}
$ZnCl_2$ infusion	2.24 ± 0.26^{xy}	2.52 ± 0.30^{xy}	0.28 ± 0.06	7.81 ± 0.22^{x}
$CaCl_2$ infusion	2.64 ± 0.19^{xy}	2.97 ± 0.21^{xy}	0.34 ± 0.06	9.22 ± 0.16^{xy}
Noninfused control	3.54 ± 0.55^{y}	3.88 ± 0.59^{y}	0.34 ± 0.06	12.38 ± 0.45^{y}

^{x,y}Within a column, means that do not have a common superscript letter differ, $P < 0.05$.

carcasses (Schoenbeck, 1998). It is not clear how an infused carcass can contain more hemoglobin than a noninfused carcass, unless the bleeding process was slowed as an effect of Ca. This is unlikely, as the severe muscle contractions observed during the infusion of $CaCl_2$ in our study and in others (Hunt et al., 2003; Dikeman et al., 2003) would be expected to improve the exsanguination process.

Metmyoglobin Accumulation

The main effects of infusion treatment, display time, and aging, as well as the first-order interactive effects, altered ($P < 0.01$) MetMb accumulation in the LM. For LM aged 24 h, chops from $ZnCl_2$-infused carcasses had lower ($P < 0.01$) percentages of MetMb than chops from $CaCl_2$-infused and noninfused carcasses throughout the 6 d of display (Figure 5A). Lamb chops from water-infused carcasses had a lower ($P < 0.01$) MetMb percentage than chops from $CaCl_2$-infused and noninfused carcasses at 0 and 3 d of display. After 3 wk of vacuum-packaged aging, $ZnCl_2$-infusion produced lamb with a lower ($P < 0.01$) MetMb percentage than $CaCl_2$-infused lamb (Figure 5B); however, after 6 d of display, there was no difference among infusion treatments. The accumulation of MetMb on the surface of meat is the main cause of meat discoloration (Dean and Ball, 1960; Schwimmer, 1981; Renerre, 1999). It is generally regarded that the net amount of MetMb formed is the result of its formation by the autoxidation of oxymyoglobin and myoglobin and reduction of MetMb by MetMb-reducing activity (Zimmerman and Snyder, 1969; Giddings, 1974). The reciprocal autoxidation between the lipid and the pigments in meat is well known (Renerre, 1999). Therefore, it was not surprising that $CaCl_2$-infused and noninfused treatments, which had higher amounts of pigments and TBARS values, also exhibited a higher percentage of MetMb accumulation.

Objective Color

Chops from $CaCl_2$-infused carcasses aged only 24 h had lower ($P < 0.001$) L* values than chops from water-infused carcasses at 0, 3, and 6 d of display (Figure 6A). Lamb from water- and $ZnCl_2$-infused carcasses was lighter ($P < 0.001$) than that of $CaCl_2$-infused carcasses after 6 d of display. Chops from noninfused and $CaCl_2$-infused carcasses reached their maximum L* values after 24-h of display, and L* values decreased as the duration of display progressed. Chops from carcasses infused with water attained maximum L* values after 3 d of display and then started to decrease. Conversely, L* values for chops from $ZnCl_2$-infused carcasses continued to increase throughout the display period.

Differences between treatments were more pronounced after 3 wk of vacuum-packaged aging (Figure 6D). Chops from $CaCl_2$-infused carcasses had lower ($P < 0.001$) L* values than the other treatments, including noninfused controls, from the beginning to the end of retail display. Chops from carcass infused with water or $CaCl_2$, as well as noninfused controls, reached their maximum L* values after 3 d of display, whereas L* values of chops from $ZnCl_2$-infused carcasses continued to increase during the display time.

It has been suggested that the lighter color associated with infused lamb carcasses compared with noninfused carcasses was due to light scattering or the dilution of muscle pigments by the infused solutions (Farouk and Price, 1994). In beef carcasses, Hunt et al. (2003) suggested that the water added during infusion and/or the rapid pH decline were the likely reasons for the lighter color associated with infused carcasses. Neither reason could explain the darker LM color associated with the $CaCl_2$-infusion treatment in this study because the $CaCl_2$ treatment had the most rapid LM pH decline, and carcasses were infused at a rate of 10% of BW, regardless of solution treatment. However, other factors may contribute, including the amount of unbound water in meat and the increase in the oxygen consumption rate of the mitochondria. High oxygen consumption rate in meat has been associated with dark meat color (Atkinson and Follett, 1973; O'Keeffe and Hood, 1982). The severe muscle contraction in $CaCl_2$-infused lamb carcasses, as revealed by shorter sarcomeres, may have contributed to observed darker-color LM by creating a compacted structure with less reflective light.

Redness (a* values) of LM chops was affected ($P < 0.01$) by infusion treatments, display time, and the interactive effects of aging, treatments, and display time. When chops were aged only 24 h postmortem, the water- and $ZnCl_2$-infusion treatments produced LM chops with higher ($P < 0.001$) a* values than the $CaCl_2$-infusion and noninfused treatments during retail display

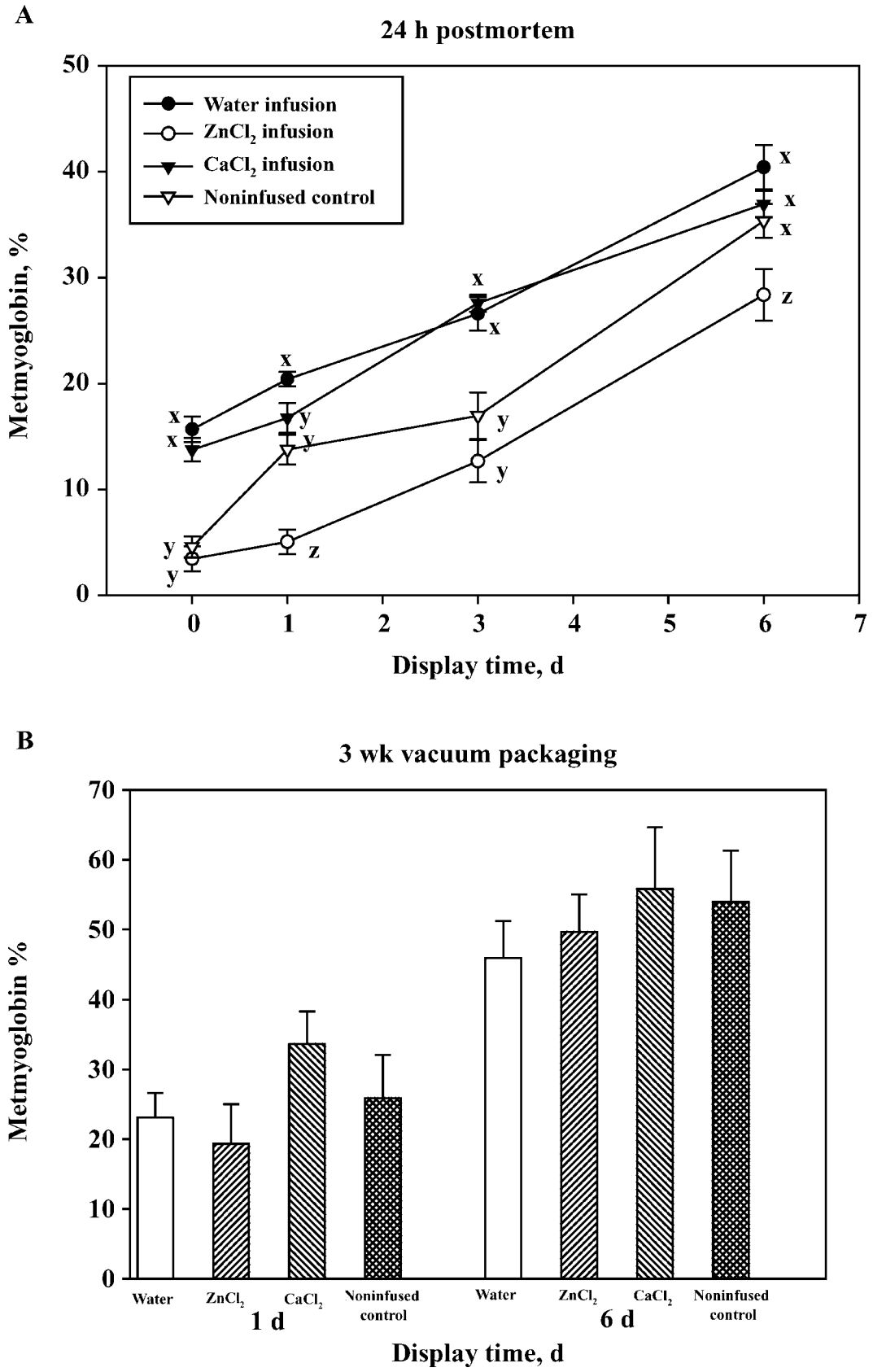

Figure 5. Interactive effects of display day and prerigor vascular infusion with water, calcium chloride ($CaCl_2$), or zinc chloride ($ZnCl_2$) on metmyoglobin percent accumulation in LM fresh tissue aged: A) 24-h; or B) 3 wk. In Panel A, within a specific display day, datum points that do not have a common letter differ, $P < 0.05$.

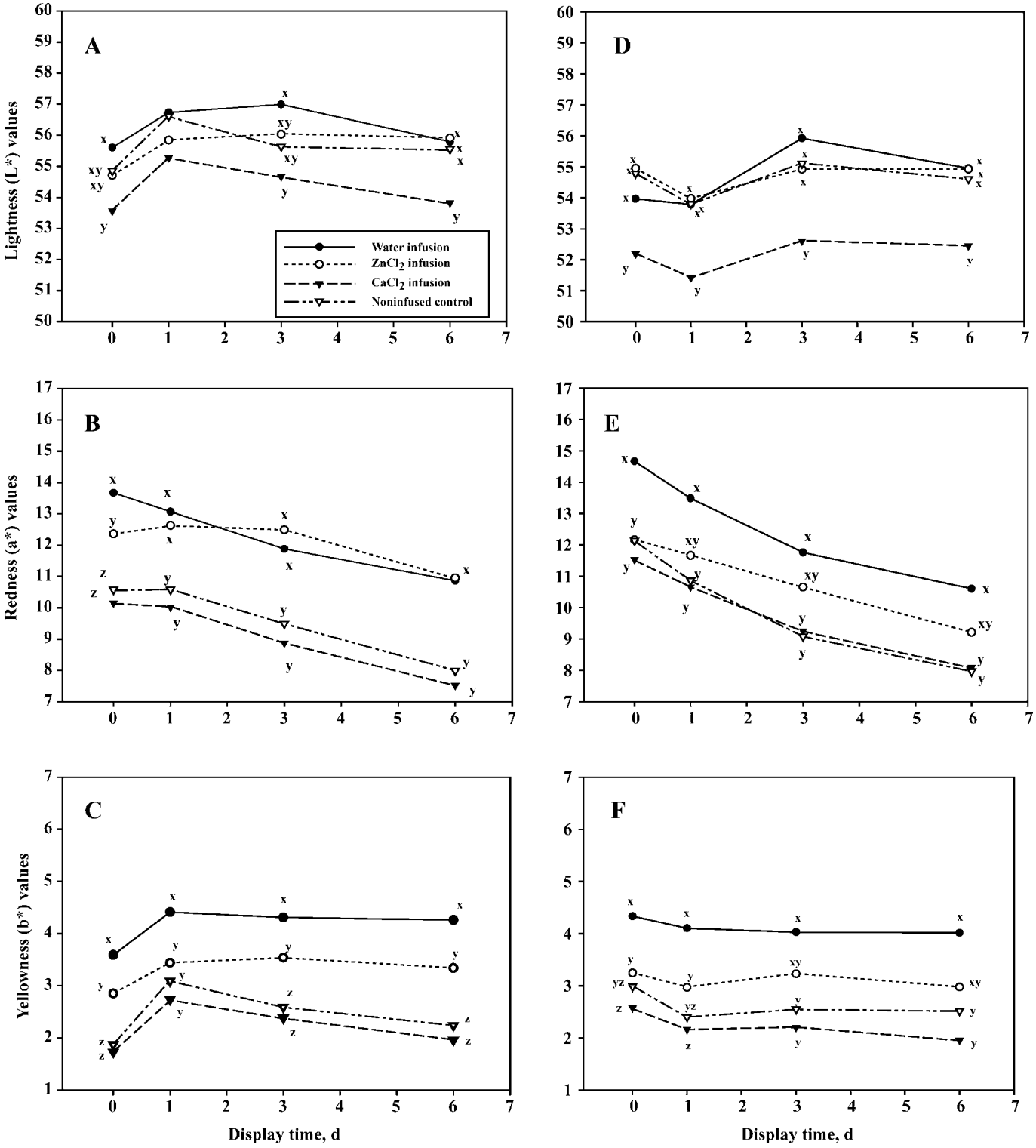

Figure 6. Interactive effects of display day and prerigor vascular infusion with water, calcium chloride ($CaCl_2$), or zinc chloride ($ZnCl_2$) on lightness (L*), redness (a*), and yellowness (b*) values for LM aged 24 h (A, B, and C, respectively) or 3 wk (D, E, and F, respectively). The L* values measure darkness/lightness spectrum (higher L* values indicate a lighter color); a* values measure redness (higher a* values indicate a redder color); and b* values measure yellowness (higher b* values indicate a more yellow color). Within a specific display day, points that do not have a common letter differ, $P < 0.05$.

(Figure 6B). Although LM chops from water-infused carcasses were initially redder ($P < 0.05$) than those from $ZnCl_2$-infused carcasses, infusion treatment did not affect a* values after 1, 3, or 6 d of retail display. Infusion of carcasses with $ZnCl_2$ caused a lower ($P < 0.01$) rate of change in redness of chops aged 24-h (1.41 units drop during 6 d of display compared with the 2.81, 2.63, and 2.59 units drop for chops from water-infused, $CaCl_2$-infused, and noninfused carcasses, respectively). After 3 wk of vacuum-aging, chops from $ZnCl_2$-infused carcasses had lower ($P < 0.01$) ranges of change in redness (2.95 units drop during 6 d of display compared with 4.07, –4.18, and 3.42 units drop for chops from water-infused, $CaCl_2$-infused, and noninfused carcasses, respectively), indicating that the differences among water-infused, $CaCl_2$-infused, and noninfused carcasses were mainly due to the differences in initial a* values. In agreement with an earlier observation (Bekhit et al., 2001), the 3-wk vacuum-aged samples had higher initial a* values and greater rates of redness change than the 24-h postmortem samples.

Yellowness (b*) and the rate of change in b* values were affected ($P < 0.001$) by infusion treatments and display time ($P < 0.001$) in the 24-h and 3-wk vacuum-packaged aged samples (Figure 6C and F, respectively). Infusing carcasses with water resulted in more ($P < 0.01$) yellow LM chops than those from $CaCl_2$-infused, $ZnCl_2$-infused, and noninfused carcasses across the 6 d of display, whereas chops from $CaCl_2$-infused and noninfused carcasses were consistently the least ($P < 0.01$) yellow in color.

Color measurements and color stability of LM were affected by prerigor infusion treatments and display time. The negative effects of $CaCl_2$ infusion on color in the current study agree with those in other reports (Geesink et al., 1994; St. Angelo et al., 1991; Hunt et al., 2003); however, Harris et al. (2001) reported that post-rigor $CaCl_2$ injection resulted in lighter, redder beef than that from untreated carcasses over 3 d of retail display at 4°C. The differences in the induction technique (infusion vs. injection), species (lambs vs. beef), and $CaCl_2$ induction time (prerigor vs. post-rigor) used in the present study and that of Harris et al. (2001) cannot explain the differences in the effect of $CaCl_2$ on meat color observed in the two studies. Another study (Geesink et al., 1994), which used the same conditions as Harris et al. (2001), did not find any positive effect from injecting $CaCl_2$ on beef meat color.

Autoxidation of myoglobin and oxymyoglobin can be accelerated by lipid peroxidation (Renerre, 1999). The negative effect of $CaCl_2$ on meat color may be due to the acceleration of MetMb formation (Geesink et al., 1994) by increasing the autoxidation of lipid/pigments, probably through the stimulation of lipoxygenase by Ca (St. Angelo et al., 1991). The negative effects of $CaCl_2$ on the physical state of meat (e.g., muscle contraction) also contribute to the production of the dark color associated with the $CaCl_2$ treatment. Moreover, the possible increase in oxygen consumption rate due to mitochondrial respiration, as evident from the lower NAD concentration with this treatment, may explain the lower a* values associated with $CaCl_2$-infused lamb.

Zinc ions, in the current study, may maintain and/or enhance lamb color through one or more systems. For instance, Zn was found to bind to myoglobin and increase the oxygen affinity (oxygenation) of myoglobin (Rifkind et al., 1977). Additionally, Zn ions inhibit mitochondrial respiration (Selwyn et al., 1993; Saris and Niva, 1994); therefore, diminishing the mitochondrial oxygen consumption rate and maintaining meat color (O'Keefe and Hood, 1982). Furthermore, Zn has been reported to prevent the formation of reactive oxygen species through a mechanism that may involve protection of sulfhydryl groups against oxidation (Bettger and O'Dell, 1981), and/or displacement of redox transition metals from site-specific loci. Essentially this means that Zn exerts its antioxidant action by occupying Fe- and Cu-binding sites in lipids and proteins (Stohs and Bagchi, 1995; Bray and Bettger, 1990; Powell, 2000). In addition, it has been reported that Zn has a synergistic action with α-tocopherol (a lipid-soluble antioxidant) and epicatechin (a water-soluble antioxidant) that prevents lipid oxidation (Zago and Oteiza, 2001). Free radicals produced in oxidizing lipids can oxidize and degrade the heme pigments, which results in an undesirable color (Haurowitz et al., 1941; Koizumi et al., 1973; Harris et al., 2001).

MetMb-Reducing Activities

Sarcoplasmic MetMb-reducing activity was affected by the infusion treatments ($P < 0.001$) and aging ($P < 0.05$). For both 24-h (Figure 7) and 3-wk (Figure 8) aged LM chops from $ZnCl_2$-infused carcasses had consistently lower ($P < 0.001$) SMRA compared with noninfused carcasses. Sarcoplasmic MetMb-reducing activity for chops from water- and $CaCl_2$-infused carcasses did not differ from either $ZnCl_2$-infused or noninfused carcasses. After 6 d of retail display, chops from $ZnCl_2$-infused and noninfused carcasses aged 3 wk had lower ($P < 0.05$) SMRA than chops aged only 24 h.

Myofibrillar MetMb-reducing activity was ($P < 0.001$) affected by infusion treatments, display time, and aging. No differences were observed in MMRA among treatments on d 0 and 1 of retail display for 24-h-aged LM (Figure 7). After 3 d of display, however, the $CaCl_2$ and $ZnCl_2$ infusion treatments produced lower ($P < 0.01$) MMRA than noninfused controls, but only chops from $ZnCl_2$-infused carcasses had lower ($P < 0.01$) MMRA than noninfused carcasses at the end of retail display. After 3 wk of vacuum-aging, all infusion treatments produced greater ($P < 0.001$) MMRA values on d 1 and 6 of display compared with those of 24-h aged LM on the same days of display. All infused carcasses had lower ($P < 0.05$) MMRA than noninfused controls after 3 wk of aging on d 1 of display (Figure 8). The $ZnCl_2$ infusion treatment produced lower ($P < 0.05$) MMRA than the water- and $CaCl_2$-infusion treatments,

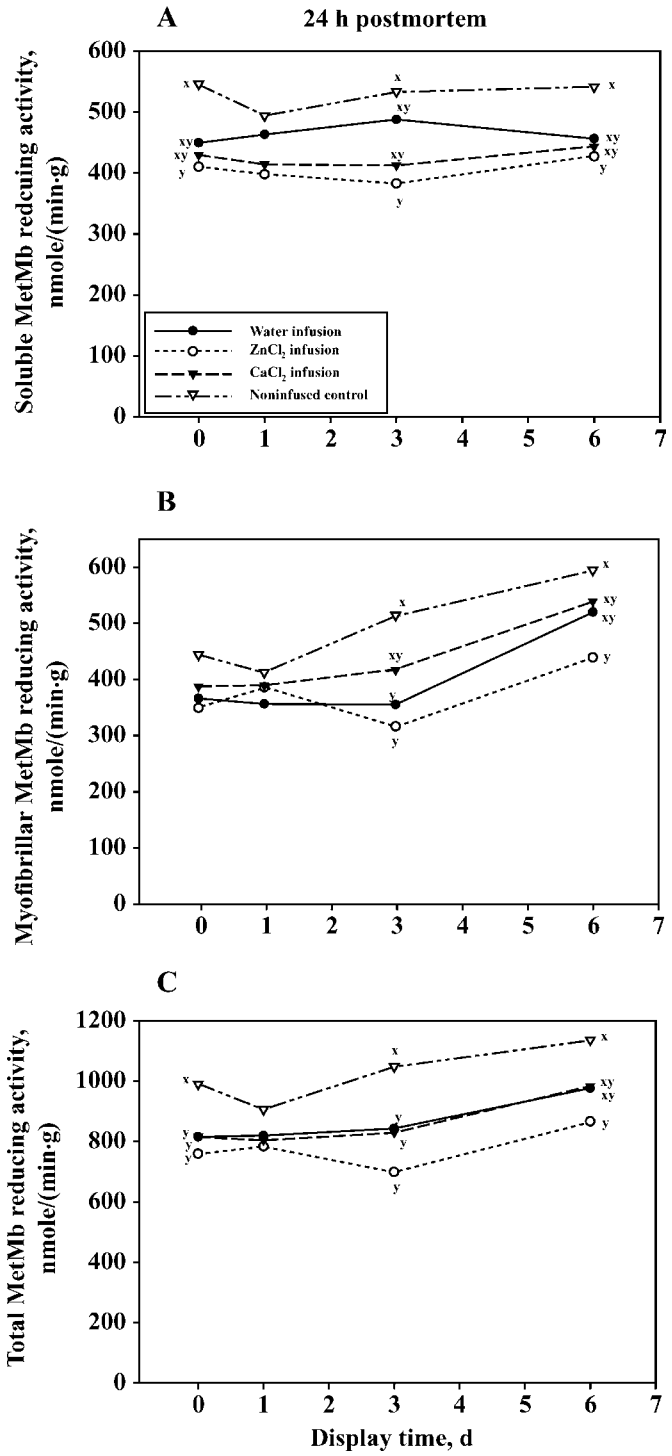

Figure 7. Interactive effects of display day and prerigor vascular infusion with water, calcium chloride ($CaCl_2$), or zinc chloride ($ZnCl_2$) on metmyoglobin (MetMb) reducing activities, nmole/(min·g fresh tissue), of LM aged 24 h. Within a specific display day, points that do not have a common letter differ, $P < 0.05$.

with MMRA being lower ($P < 0.05$) in chops from both $CaCl_2$- and $ZnCl_2$-infused carcasses compared with noninfused controls. Myofibrillar MetMb-reducing activity was increased ($P < 0.001$) due to vacuum-aging after 6 d of display.

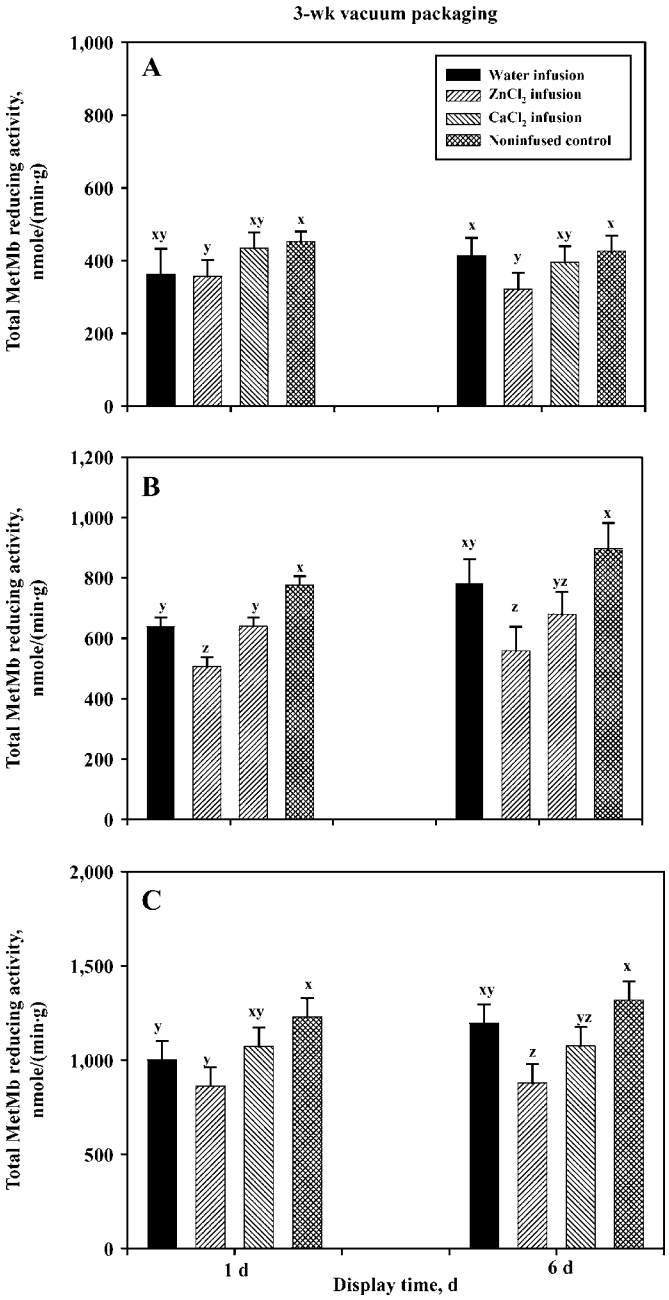

Figure 8. Interactive effects of display day and prerigor vascular infusion with water, calcium chloride ($CaCl_2$), or zinc chloride ($ZnCl_2$) on metmyoglobin (MetMb) reducing activities, nmole/(min·g fresh tissue), of LM vacuum-aged for 3 wk. Within a specific display day, bars that do not have a common letter differ, $P < 0.05$.

Lamb from infused carcasses had numerically lower total MetMb-reducing activity (**TMRA**) than noninfused controls; however, only 24-h-aged LM from $ZnCl_2$-infused carcasses had lower ($P < 0.001$) TMRA than that from noninfused carcasses. Total MetMb-reducing activity for 3-wk vacuum-aged LM was similar to MMRA, with TMRA increasing ($P < 0.01$) with increasing aging time.

The inhibitory effect of Zn and Ca ions on MetMb-reducing activity in our study agrees with results re-

ported by Al-Shaibani et al. (1977) and Hagler et al. (1979). A higher level of inhibition by Ca was reported by Hagler et al. (1979), which was probably due to the effect of the ion on purified enzyme.

The association of MetMb-reducing activities with color and other biochemical factors affecting lamb color was investigated by examining the correlations between display time, MetMb percent, TBARS, L*, SMRA, MMRA, and TMRA in 24-h postmortem and 3-wk vacuum-aged ovine LM. Data were subjected to regression analysis to calculate accumulated ANOVA and estimates of parameters to adjust for the interactions of sheep, infusion treatments, and display time. Although SMRA decreased with extended aging ($r = -0.300$; $P < 0.001$), increases in MMRA ($r = 0.666$; $P < 0.001$) and TMRA ($r = 0.436$; $P < 0.001$) were associated with increased postmortem aging. Myofibrillar MetMb-reducing activity and TMRA were found to increase with the increase in display time ($r = 0.298$ and 0.316, respectively; $P < 0.001$). This finding contradicts our hypothesis that the increase in SMRA observed with storage time (Bekhit et al., 2002, 2003) was a response to relocalization of MMRA due to structural disintegration. Nonetheless, the correlation was observed with all infusion treatments; thus, the effect could be the result of the treatment itself. Both $ZnCl_2$ and $CaCl_2$ have opposite effects on muscle tissue (Koohmaraie, 1990; Koohmaraie et al., 1990). Calcium ions accelerate the disintegration of the muscle tissue and Zn acts as a membrane stabilizer (Bray and Bettger, 1990), and also competitively bind to Ca sites to inhibit the effects of Ca in many enzyme systems, especially the calpains (Koohmaraie, 1990; Koohmaraie et al., 1990). These limiting effects are not dependent on time, and both cations interfere with the natural course of proteolysis and subsequent release of cell contents.

An increase of MetMb percentage and TBARS values was associated ($P < 0.001$) with the increase in MMRA ($r = 0.526$ and 0.407, respectively) and TMRA ($r = 0.481$ and 0.367, respectively). Partially purified cytochrome b_5 reductase and cytochrome b_5 from beef liver have been found to increase lipid peroxidation in frozen beef patties in the presence of NADH (Mikkelsen and Skibsted, 1992). Earlier immunochemical studies on the pathway of electron flow in NADH-dependent microsomal lipid peroxidation by Hirokata et al. (1978) has shown that the presence of Fe^{3+} ions can support NADH lipid peroxidation of liver microsomes. These authors pointed out that the electrons from NADH were supplied to the lipid peroxidation reaction via NADH-cytochrome b_5 and cytochrome b_5 because antibodies for these proteins inhibit NADH-dependent lipid peroxidation. It is more likely that in the current study the increase in lipid oxidation, which resulted from higher cytochrome b_5-MetMb reductase system activity, was the cause of the increase on MetMb percentage because TBARS and MetMb percentage were strongly correlated.

Lightness (L*) values were decreased as MMRA ($r = -0.294$; $P = 0.005$) and TMRA ($r = -0.370$; $P < 0.001$) increased. The results indicate that the increase of MetMb-reducing activities may promote meat discoloration indirectly via increased lipid oxidation.

Pearson correlations were used to investigate the relationship between the nicotinamide derivatives and the other biochemical factors in 24-h postmortem lamb samples during 6 d of storage at 2°C. Only NAD concentrations were found to have a significant correlation with the studied factors. Nicotinamide adenine dinucleotide concentrations were negatively correlated to TBARS ($r = -0.296$; $P = 0.004$) and MMRA ($r = -0.217$; $P = 0.035$).

Implications

Prerigor water and zinc chloride infusions improved the color and color stability of ovine LM, whereas calcium chloride infusion decreased the color and the color stability of the muscle. The effects of water, zinc chloride, and calcium chloride on color stability seem to be due to their effects on the oxidative processes, by decreasing pigment concentration and by altering the physical state of the meat. Given the expected negative effect of zinc chloride on meat tenderness and the positive effect of prerigor water infusion on meat tenderness, prerigor water infusion may be a method to improve both the color and tenderness of lamb. Results of this study indicate an increased susceptibility of meat lipids to oxidation after prolonged periods of vacuum-packaged storage; thus, strategies to suppress oxidation (modified atmosphere packaging and/or the use of antioxidants) should be considered for vacuum-aged lamb destined for retail display.

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