Effects of a multielement trace mineral injection and vitamin E supplementation on performance, carcass characteristics, and color stability of strip steaks from feedlot heifers

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ABSTRACT: The objective was to evaluate the interaction of a trace mineral (**TM**) injection (Multimin 90) and a supranutritional concentration of dietary vitamin E (**VITE**) on performance, carcass characteristics, and color stability of strip steaks from feedlot heifers. Prior to trial initiation, Angus × Simmental cross heifers (N = 48) were managed on a common diet supplemented to meet the NRC recommendations. Heifers were stratified by BW and allotted to a 2 × 2 factorial arrangement: 1) no supplemental vitamin E and saline injection (**CONT + SAL**), 2) 1,000 IU vitamin E·heifer^{-1}·d^{-1} and saline injection (**VITE + SAL**), 3) no supplemental vitamin E and TM injection (**CONT + MM**), or 4) vitamin E and TM injection (**VITE + MM**). Trace mineral injection contained 15, 10, 5, and 60 mg/mL of Cu, Mn, Se, and Zn, respectively, and TM injection or saline injection (1 mL/68 kg BW) were given on day 0 of the 89-d finishing period. All heifers were fed a common diet containing a basal concentration of 19.8 IU/kg DM vitamin E. Heifers were slaughtered and loins sections were collected. Strip steaks were cut and placed in overwrap trays for evaluation of color stability for 16 d. Data were analyzed using the MIXED procedure of SAS. Color stability data were analyzed as repeated measures. Neither TM injection nor VITE had an effect on final BW, DMI, or G:F (P ≥ 0.12). There was a tendency (P = 0.09) for TM injection to increase ADG. A tendency (P = 0.08) was observed for TM injection to increase DMI of heifers receiving supranutritional VITE. Trace mineral injection and VITE had no effect on HCW, yield grade, 12th-rib backfat thickness, or ribeye area (P ≥ 0.34). Marbling scores tended to increase (P = 0.08) in VITE heifers compared with control-fed heifers. Vitamin E supplementation decreased final lipid oxidation (1.00 vs. 1.97 µg malondialdehyde/g fat, P = 0.03) and total visual discoloration (15.82% vs. 33.96%, P = 0.04) of steaks compared with steaks from nonsupplemented heifers. Heifers fed supranutritional VITE produced steaks that maintained retail color longer shown by lower hue angle values (38.17° vs. 38.66°, P < 0.01) than nonsupplemented heifers. A TM injection × vitamin E × day interaction (P < 0.01) revealed by day 16 steaks from the CONT + MM heifers exhibited greater discoloration than VITE + SAL and VITE + MM steaks with CONT + SAL intermediate. Overall, VITE improved color stability and TM injection appeared to increase discoloration of strip steaks from feedlot heifers after day 14 of display.

Key words: beef, color stability, heifers, trace mineral, vitamin E

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INTRODUCTION

Fresh meat color is one of the driving forces behind consumer purchasing intent. As a result, premature discoloration of steaks and roasts results in annual revenue losses of $1 billion

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(Smith et al., 2000). Therefore, preslaughter strategies with potential to slow visual discoloration would decrease losses associated with discounted, reworked, or discarded product (Zerby et al., 1999). Although the effects of vitamin E supplementation on color stability of beef products have been well established (Faustman et al., 1998), the potential for vitamin E and trace mineral (**TM**) supplementation to additively extend color stability is unknown.

Oxidative challenges associated with transit and handling (Genther-Schroeder and Hansen, 2015) and dietary inclusion of feedstuffs high in polyunsaturated fatty acids (Gill et al., 2008) may decrease color stability of steaks from finishing cattle. Recently, a TM injectable has been increasingly used to overcome issues such as dietary antagonists, TM deficiencies in feedstuffs and forages, and variation in TM intake. Trace mineral injection has been demonstrated to increase the activity of TM-containing antioxidant enzymes such as Mn-superoxide dismutase (Genther and Hansen, 2014b) and Se-containing glutathione peroxidase (Pogge et al., 2012). Selenium deficiencies routinely identified in Midwestern U.S. soils and forages (USDA, 2006), increase the likelihood these antioxidant systems may be compromised in deficient cattle. The hypothesis was that supranutritional vitamin E and TM injection would have little effect on growth performance; however, they may additively improve production of antioxidant enzymes, thereby decreasing oxidative stress and improving color stability of strip steaks. The objective was to evaluate the interaction of TM injection and supranutritional concentration of dietary vitamin E on growth performance, carcass characteristics, and color stability of strip steaks from feedlot heifers.

MATERIALS AND METHODS

All protocols were approved by the University of Illinois Institutional Animal Care and Use Committee (Protocol #15008).

Experimental Design and Cattle Management

A total of 48 Angus × Simmental heifers were used in a randomized complete block design with a 2 × 2 factorial arrangement with treatment factors including daily dietary inclusion of the control, basal vitamin E (19.8 IU/kg DM) or targeting 1,000 IU vitamin E·heifer^{-1}·d^{-1} [109.8 IU/kg DM (dl-α-tocopheryl acetate)], and subcutaneous injection of a saline or TM injection (Multimin 90; Multimin USA, Fort Collins, CO) at a dosage of 1 mL/68 kg BW. In total, 4 treatment combinations were used (n = 12): 1) no supplemental vitamin E and saline injection (**CONT + SAL**), 2) vitamin E and saline injection (**VITE + SAL**), 3) no supplemental vitamin E and TM injection (**CONT + MM**), or 4) vitamin E and TM injection (**VITE + MM**). Trace mineral injection delivered 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA).

Prior to trial initiation, heifers were managed as a group on a common diet fed to meet the NRC (2000) requirements containing 50% high-moisture corn, 20% corn silage, 20% modified wet distillers grains with solubles, and 10% supplement. Diets prior to trial initiation contained a basal vitamin E concentration of 19.8 IU/kg DM. Heifers were administered 140-mg trenbolone acetate and 14-mg estradiol (Component TE-H; Elanco Animal Health, Greenfield, IN) on day 0 of the trial. After being weighed on days −1 and 0, heifers were stratified by BW and allotted across 8 pens (6 heifers per pen). Injection treatment was applied to the individual heifer on day 0 of the trial. Each pen housed heifers receiving both injection treatments (SAL and MM). Dietary vitamin E treatment was applied to pen; however, individual animal intakes were collected using a GrowSafe feeding system (GrowSafe Systems Ltd., Airdrie, AB, Canada). Heifers were weighed at 28-d intervals and fed for ad libitum intake at 1,000 h daily for a total of 89 d on feed. Heifers were housed in 4.88 × 4.88 m^2 pens in a confinement barn with slatted, concrete floors covered by interlocking rubber matting.

Feed Management

Diets were the same for both dietary treatments with the exception of vitamin E inclusion in feed supplements. Diets were formulated to meet or exceed the NRC (2000) recommendations and contained 20% corn silage, 35% modified wet distillers grains with solubles (**MWDGS**), 35% dry-rolled corn, and 10% supplement (DM basis; Table 1). Dry supplement (10%) served as the carrier for vitamin E to ensure uniform mixing in diets. The basal diet was formulated to provide 44 mg Mn/kg DM [as manganese sulfate and Availa-4 (Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN)], 67 mg Zn/kg DM (as zinc sulfate and Availa-4), 9 mg Cu/kg DM (as copper sulfate), and 0.4 mg Se/kg DM (as sodium selenite; Table 1). The MWDGS used in this study contained 8.5% fat.

Table 1. Ingredient and nutrient composition of diets fed for 89 d before slaughter

	Dietary treatment	
	Control	Vitamin E[1]
Ingredient	% of diet DM	
Dry-rolled corn	35	35
Modified wet distillers grains[2]	35	35
Corn silage	20	20
Control supplement[3]	10	—
Vitamin E supplement[4]	—	10
Analyzed nutrient content	% of diet DM	
CP, %	14.61	14.72
NDF, %	26.39	26.32
ADF, %	10.03	10.19
Fat, %	4.82	4.88
Calculated nutrient content[5]		
Vitamin E, IU/kg DM	19.8	109.8
S, % of diet DM	0.31	0.31
Cu, mg/kg DM	9.32	9.31
Mn, mg/kg DM	43.54	43.51
Se, mg/kg DM	0.43	0.42
Zn, mg/kg DM	66.53	66.44

[1]Heifers were supplemented with no additional vitamin E in the diet (Cont) or 1,000 IU of vitamin E·heifer^{-1}·d^{-1} (Vit E) for 89 d prior to slaughter.

[2]Modified wet distillers grains contained 8.5% fat.

[3]Supplement contained 75.4% ground corn, 22.7% limestone, 0.9% trace mineral salt (20% $CaCO_3$, 15.43% Availa-4 Zinpro, 14.16% KCl, 8.75% MgO, 8.00% $MnSO_4$, 6.74% $FeSO_4$, 6.55% rice hulls mineral oil, 5.95% S prilled, 4.41% vitamin E, 1.50% Se, 1.03% $MgSO_4$ and KSO_4, 0.88% $CuSO_4$, 0.22% vitamin A, 0.13% vitamin D_3 500, 0.04% $Ca(IO_3)_2$, yielding 277 mg/kg Co, 5,000 mg/kg Cu, 250 mg/kg I, 20,200 mg/kg Fe, 30,000 mg/kg Mn, 150 mg/kg Se, 30,000 mg/kg Zn, 2,205 KIU/kg vitamin A, 662 KIU/kg vitamin D_3, 22,000 IU/kg vitamin E), 0.8% liquid fat, 0.15% Rumensin 90 (200 g monensin/kg DM; Elanco Animal Health, Greenfield, IN), and 0.10% Tylan 40 (88 g tylosin/kg DM; Elanco Animal Health).

[4]Supplement contained 70.9% ground corn, 22.7% limestone, 4.5% vitamin E supplement (20,000 IU/kg dl-α-tocopheryl acetate; ADM Alliance Nutrition, Quincy, IL), 0.9% trace mineral salt, Rumensin 90 (200 g monensin/kg DM; Elanco Animal Health, Greenfield, IN), 0.10% Tylan 40 (88 g tylosin/kg DM; Elanco Animal Health).

[5]Nutrients were calculated using NRC values (NRC, 2000).

Percentage of MWDGS inclusion was intentionally higher to challenge color stability and increase the likelihood of observing treatment effects should they exist. Feed samples were collected every 2 wk throughout the duration of the trial. Samples were stored in a −20 C freezer until further analysis. Equal proportions from each collection were composited for laboratory analysis. All samples were dried and ground through a Wiley mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA). Ingredients were analyzed for DM (24 h at 103 C), NDF and ADF (using Ankom Technology method 5 and 6, respectively; Ankom200 Fiber Analyzer, Ankom Technology), CP (Leco TruMac, LECO Corporation, St. Joseph, MI), fat (ether extract, Ankom method 2; Ankom Technology), and ash (600 °C for 2 h; Thermolyte muffle oven Model F30420C; Thermo Scientific, Waltham, MA).

Slaughter and Sample Preparation

On day 90, heifers were transported approximately 300 km to a commercial slaughter facility and were humanely slaughtered under USDA inspection. Two heifers were removed from the trial, one prior to slaughter due to laminitis and another for pericarditis observed during slaughter, leaving 46 heifers for use in the experiment. At approximately 24-h postmortem, carcasses were evaluated for 12th-rib backfat thickness, ribeye area (**REA**), percent KPH, ribeye lean maturity score, and ribeye marbling score via camera grading. A 5-cm section of longissimus lumborum was excised between the 12th- and 13th-rib section by facility staff, stored on wet ice, and transported to the University of Illinois Meat Science Laboratory. Two 1.9-cm-thick steaks were cut using a gravity slicer. The first was used for initial lipid oxidation and proximate analysis and the second for retail display and final lipid oxidation. Steaks for retail display were placed on soaker pads in foam trays (27.3 × 14.9 cm, Dynea-pak, Laval, Quebec, Canada) and overwrapped with a polyvinyl chloride film (oxygen-permeable polyvinyl chloride fresh meat film; 1,629 mL O_2·m^{-2}·d^{-1} at 23 °C) to be serially evaluated for color stability beginning on day 2 postmortem. Steaks were rotated daily to minimize the effect of location and light exposure. Lighting was provided by 122-cm-long 32-W fluorescent bulbs (Ecolux with Starcoat, 3000K, General Electric, Boston, MA). All steaks were removed from retail display and final lipid oxidation was evaluated after the group reached an average of approximately 25% discoloration, at day 16 retail display.

Instrumental Color Analysis

For each day of serial color stability evaluation, surface color was evaluated on steaks, through overwrap film, using a HunterLab Miniscan XE Plus spectrophotometer Model 45/0 (HunterLab Associates, Reston, VA) with a 2.54-cm-diameter aperture, A illuminant, and 10° observer angle. All measurements, including reflectance at 580 and 630 nm and CIE L^* (lightness), a^* (redness), and b^* (yellowness), were measured on 2 random locations of the steak and averaged for analyses. The ratio of reflectance at 630/580 nm was used to instrumentally

measure fresh meat color change with ratios closer to 1.0 indicating greater discoloration due to metmyoglobin formation (Strange et al., 1974). Hue angle was also used to evaluate shifts in color over time toward discoloration with greater values indicating less red and greater metmyoglobin formation (Bernofsky et al., 1959). Chroma, or saturation index, was used to assess color intensity with greater values indicating greater saturation of the principle hue on the steak (AMSA, 2012). Hue angle and saturation index (chroma) were calculated using the following equations: $\text{Hue angle} = [\arctan(b^*/a^*)]$ and $\text{Chroma} = (a^{*2} + b^{*2})^{0.5}$.

For each day of display, the percentage steak surface discoloration was also recorded by 2 evaluators with extensive experience evaluating meat color changes throughout shelf life. Evaluations of discoloration were averaged for analysis.

Lipid Oxidation

Initial and final lipid oxidation of steaks was evaluated using the thiobarbituric acid reactive substances (**TBARS**) assay. Steaks were trimmed of external fat, and the entire steak was homogenized in a Waring blender (WaringPro, Torrington, CT) using the pulse function (grind 1 s, rest 3 s) until homogenous to prevent overheating. Duplicate 5-g samples were collected from the ground homogenate of each steak and subjected to extraction by aqueous acidic solution protocol using a modified procedure described by Leick et al. (2010). A standard curve (R^2 = 0.9989) was made to represent 0, 1.25, 2.5, 5, and 7.5 mg malondialdehyde (**MDA**)/mL using 25 µM 1,1,3,3-tetraethoxypropane. After incubation, 150 µL of sample, blank and standard were pipetted into 96-well flat-bottomed plates (12.8 × 8.5 cm; ThermoFisher, Rochester, NY), and absorbance was measured at 530 nm in a plate reader (Synergy HT Multi-Model Microplate Reader, Bio-Tek, Winooski, VT) to determine sample absorbance. Samples were compared to a standard curve (0 to 7.5 mg MDA/mL), and TBARS were calculated and expressed as mg MDA/kg of meat. Lipid oxidation was also corrected for lipid content and expressed as a percentage of weight using data from proximate analysis. Adjusted TBARS, expressed as µg MDA/g of fat, were calculated using the following equation:

$$\frac{\text{mg MDA}}{\text{kg meat}} \times \frac{\text{kg meat}}{\text{g fat}} \times \frac{1{,}000\ \mu\text{g MDA}}{\text{mg MDA}} = \frac{\mu\text{g MDA}}{\text{g fat}}$$

Proximate Composition

The remaining steak homogenate after initial TBARS analysis (day 0 retail display) was used for moisture and extractable lipid determination. Duplicate 10-g samples were oven dried at 110 °C for at least 24 h and weighed to determine the percentage of moisture content. Dried samples were then washed in warm chloroform–methanol solvent, as described by Novakofski et al. (1989), and weighed to determine the percentage of total extractable lipid.

Statistical Analyses

The experiment was conducted as a 2 × 2 factorial arrangement of treatments in a randomized complete block design with vitamin E treatment applied to the pen and injection applied to the individual heifer. Data were analyzed using the MIXED procedure of SAS (v 9.4; SAS Institute Inc., Cary, NC). The model for live performance, carcass characteristics, and lipid oxidation included fixed effects of dietary treatment and TM injection, their interaction, as well as the random effect of pen. Heifer served as the experimental unit as individual intake data were collected. Initial and final instrumental color and discoloration parameters were analyzed independently as a 2 × 2 factorial. Color stability over the course of the 16-d retail display period was analyzed as repeated measures using an unstructured covariate structure determined using Akaike's information criterion to minimize variance. The model included treatment, day, and their interaction, as well as individual heifer, as a random effect. The slice option was used to evaluate the effect of day on instrumental color and color stability data, and least square means were calculated. Treatment effects and interactions were considered significantly different at $P \leq 0.05$, and trends were discussed at $P < 0.10$.

RESULTS

Growth Performance

There were no interactions ($P \geq 0.62$) between Vitamin E supplementation and TM injection for BW at any point during the 89-d feeding period (Table 2). Neither vitamin E supplementation ($P \geq 0.79$) nor TM injection ($P \geq 0.51$) had an effect on BW.

From day 0 to day 28 after trial initiation, TM injection increased DMI of VITE + MM heifers,

Table 2. Effects of trace mineral injection and dietary vitamin E supplementation on growth performance of feedlot heifers[1]

	Treatment[2,3]					*P*-value		
Item	Cont + Sal	Cont + MM	Vit E + Sal	Vit E + MM	SEM	Diet	Injection	Diet × Injection
Total head, *n*	12	12	11	11				
BW, kg								
Initial	425	422	427	421	9.1	0.98	0.66	0.89
Day 28	469	464	464	469	9.7	0.99	0.95	0.62
Day 56	500	499	502	503	9.5	0.79	0.99	0.93
Final	538	543	536	544	10.3	0.98	0.51	0.91
Total BW gain, kg	112	121	110	123	6.4	0.94	0.09	0.72
Days 0 to 28								
ADG, kg/d	1.53	1.44	1.31	1.64	0.14	0.96	0.36	0.12
DMI, kg/d	8.7^{ab}	8.3^{ab}	7.5^{b}	8.9^{a}	0.5	0.67	0.27	0.03
G:F	0.17	0.17	0.16	0.18	0.01	0.98	0.48	0.44
Days 29 to 56								
ADG, kg/d	1.09	1.26	1.33	1.22	0.12	0.48	0.73	0.12
DMI, kg/d	8.8	8.7	8.6	9.5	0.4	0.55	0.23	0.13
G:F	0.12^{b}	0.14^{ab}	0.16^{a}	0.13^{ab}	0.01	0.31	0.74	0.02
Days 57 to 89								
ADG, kg/d	1.17	1.37	1.07	1.29	0.12	0.47	0.07	0.93
DMI, kg/d	8.6	8.8	8.2	9.4	0.3	0.81	0.04	0.17
G:F	0.14	0.15	0.13	0.14	0.01	0.47	0.29	0.49
Days 0 to 89								
ADG, kg/d	1.26	1.36	1.23	1.38	0.07	0.94	0.09	0.73
DMI, kg/d	8.7	8.6	8.1	9.3	0.4	0.96	0.12	0.08
G:F	0.15	0.16	0.15	0.15	0.01	0.80	0.29	0.23

[1]Trace mineral injection included 15 mg/mL of Cu, 10 mg/mL of Mn, 5 mg/mL of Se, and 60 mg/mL of Zn.

[2]Heifers were supplemented with no additional vitamin E in the diet (Cont) or 1,000 IU of vitamin E·heifer^{-1}·d^{-1} (Vit E) for 89 d prior to slaughter. Heifers received saline (Sal) or trace mineral injection (TM) at a dosage of 1 mL/68 kg BW at day 1 of the 89 d feeding period.

[3]Treatments within day lacking common superscripts differ ($P < 0.05$).

whereas it decreased DMI in CONT + MM heifers ($P \geq 0.03$). Unsurprisingly, given this DMI interaction, during day 29 to day 56, TM injection resulted in slightly decreased ($P = 0.02$) G:F in VITE + MM heifers and increased G:F of CONT + MM heifers. From day 57 to day 89, heifers receiving TM injection had 8.6% greater DMI (9.11 vs. 8.39 kg/d, $P = 0.04$) and a tendency for greater ADG ($P = 0.07$) than heifers receiving saline injection. Overall (day 0 to day 89), there was a tendency for TM injection to increase (1.37 vs. 1.25 kg/d, $P = 0.09$) ADG compared with saline injection. Furthermore, there was tendency for TM injection to increase DMI of VITE + MM heifers, whereas TM injection resulted in decreased DMI in CONT + MM heifers ($P = 0.08$), mirroring the interaction from day 0 to day 28 (Table 2). Vitamin E supplementation had no main effect on growth performance ($P \geq 0.31$).

Carcass Characteristics

There were no interactions ($P \geq 0.49$) between vitamin E supplementation and TM injection on carcass characteristics. Similarly, TM injection had no main effect ($P \geq 0.19$) on any carcass characteristics. Interestingly, there was a tendency for heifers fed supranutritional vitamin E to have greater marbling scores (560 vs. 490, $P = 0.09$) than control-fed heifers. However, there was no difference (8.62% vs. 7.44%, $P = 0.18$) in the percentage of extractible lipid between vitamin E and control-fed heifers (Table 3).

Instrumental Color and Color Stability

Initially, supplemental vitamin E and TM injection had no main effects on day 1 strip steak lightness (L^*), yellowness (b^*), chroma, or visual discoloration ($P \geq 0.10$). There was a tendency for strip steaks from vitamin E supplemented heifers to exhibit slightly greater 630/580 ratios ($P = 0.09$); however, differences were modest (Table 4). Steaks from heifers receiving TM injection exhibited a tendency for greater hue angles ($P = 0.08$), indicative of greater metmyoglobin formation, than steaks from heifers receiving the control saline injection.

Table 3. Effects of trace mineral injection and vitamin E supplementation on carcass characteristics of feedlot heifers[1]

	Treatment[2]					*P*-value		
Item	Cont + Sal	Cont + MM	Vit E + Sal	Vit E + MM	SEM	Diet	Injection	Diet × Injection
Ending live wt, kg	538	543	536	544	10	0.98	0.51	0.91
HCW, kg	340	340	338	341	6	0.99	0.83	0.79
Carcass yield %	63.2	62.5	63.0	62.7	0.4	0.95	0.19	0.65
REA, cm^2	82.8	84.0	82.0	84.6	2.1	0.97	0.35	0.72
12th-rib fat, cm	1.2	1.3	1.3	1.3	0.1	0.65	0.96	0.49
KPH, %	2.2	2.1	2.2	2.3	0.1	0.09	0.31	0.81
Calculated yield grade	3.1	3.1	3.2	3.0	0.1	0.85	0.56	0.62
Marbling score[3]	480	500	565	550	35	0.08	0.89	0.54
Extractible lipid, %	7.1	7.7	8.5	8.7	0.8	0.18	0.58	0.75

[1]Trace mineral injection included 15 mg/mL of Cu, 10 mg/mL of Mn, 5 mg/mL of Se, and 60 mg/mL of Zn.

[2]Heifers were supplemented with no additional vitamin E in the diet (Cont) or 1,000 IU of vitamin E·heifer^{-1}·d^{-1} (Vit E) for 89 d prior to slaughter. Heifers received saline (Sal) or trace mineral injection (TM) at a dosage of 1 mL/68 kg BW at day 1 of the 89-d feeding period.

[3]Marbling scores: 400 = Small^{00} (low choice) and 500 = Modest^{00} (average choice).

Table 4. Effects of trace mineral injection and vitamin E supplementation on color stability of strip steaks from feedlot heifers at days 1 and 16 of retail display[1]

	Treatment[2]					*P*-value		
Item	Cont + Sal	Cont + MM	Vit E + Sal	Vit E + MM	SEM	Diet	Injection	Diet × Injection
Initial, day 1 retail display								
Lightness, *L**	43.3	42.1	43.4	44.3	1.0	0.23	0.88	0.29
Redness, *a**	30.0	29.6	28.4	30.3	0.8	0.63	0.28	0.09
Yellowness, *b**	22.0	22.2	20.7	23.2	0.9	0.89	0.11	0.15
630/580 nm	5.8	6.1	5.2	5.7	0.3	0.09	0.15	0.71
Chroma	37.2	36.9	35.2	38.2	1.1	0.75	0.17	0.10
Hue angle	36.2	36.8	35.8	37.4	0.6	0.92	0.08	0.40
Visual discoloration, %	0.0	0.0	0.0	0.0	—	—	—	—
Final, day 16 retail display								
Lightness, *L**	43.1	41.9	43.6	45.3	1.3	0.11	0.85	0.24
Redness, *a**	22.3	20.5	23.3	23.8	1.0	0.04	0.52	0.25
Yellowness, *b**	19.8	18.3	19.1	19.8	0.7	0.64	0.61	0.13
630/580 nm	2.8	2.6	3.0	3.2	0.2	0.10	0.98	0.47
Chroma	29.8	27.5	30.1	30.9	1.2	0.13	0.54	0.19
Hue angle	41.7	41.9	39.4	39.7	0.5	<0.0001	0.62	0.85
Visual discoloration, %	28.0	40.0	16.0	15.0	8.0	0.02	0.47	0.41

[1]Trace mineral injection included 15 mg/mL of Cu, 10 mg/mL of Mn, 5 mg/mL of Se, and 60 mg/mL of Zn.

[2]Heifers were supplemented with no additional vitamin E in the diet (Cont) or 1,000 IU of vitamin E·heifer^{-1}·d^{-1} (Vit E) for 89 d prior to slaughter. Heifers received saline (Sal) or trace mineral injection (TM) at a dosage of 1 mL/68 kg BW at day 1 of the 89-d feeding period.

Furthermore, there was tendency for TM injection to increase strip steak redness of VITE + MM heifers at day 1, whereas TM injection decreased redness of steaks from CONT + MM heifers ($P = 0.09$).

On day 16, when steaks were removed from retail display, the average percentage of visual discoloration was 25%. Despite the initial interaction for redness between supplemental vitamin E and TM injection, no interaction ($P = 0.25$, Table 4) was demonstrated at day 16. Nonetheless, there was a main effect of supranutritional vitamin E on redness at day 16, with strip steaks from heifers receiving supplemental vitamin E exhibiting greater surface *a** values (*a** = 23.52 vs. 21.42 units, $P = 0.04$) than control-fed heifers. Steaks from vitamin E supplemented heifers also exhibited lesser hue angles (39.56° vs. 41.82°, $P < 0.0001$) and visual discoloration (15.82% vs. 33.96%, $P = 0.02$) than those from control-fed heifers. Additionally, steaks from vitamin E supplemented heifer less lipid oxidation ($P = 0.03$) than control-fed heifers (Fig. 1). Trace mineral injection had no effect ($P \geq 0.47$) on day 16 instrumental color, 630/580 nm ratio, hue angle, chroma, or visual discoloration.

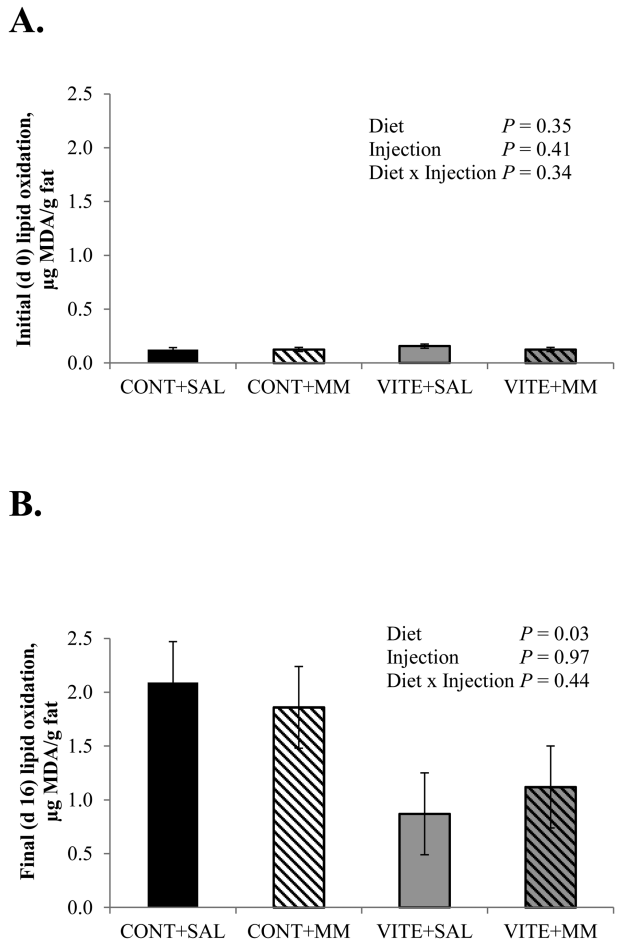

Figure 1. Effects of trace mineral injection (containing Cu, Mn, Se, and Zn) and supranutritional dietary vitamin E supplementation on (A) initial lipid oxidation at day 2 postmortem and (B) final lipid oxidation at day 16 postmortem, of beef strip steaks as determined by the thiobarbituric acid reactive substances (TBARS) assay. Oxidation was quantified in µg malondialdehyde (MDA)/g fat to correct for differences in extractable lipid content.

Furthermore, there were no interactions ($P \geq 0.13$) between vitamin E supplementation and TM injection at day 16 (Table 4).

Similar findings were shown when color stability was evaluated across the full 16-d duration of retail display using repeated measures. As expected, there was an interaction between day of retail display and vitamin E supplementation with steaks from heifers receiving supranutritional vitamin E having decreased visual discoloration at later days of retail display ($P < 0.0001$; Fig. 2) than steaks from control-fed heifers. In support of this finding, steaks from vitamin E heifers also exhibited smaller hue angles at later days of retail display ($P < 0.01$; Fig. 3). Because no interactions between vitamin E supplementation and TM injection were shown ($P \geq 0.12$), LS means for the interactions between day and vitamin E supplementation were statistically separated by day. Steaks from heifers receiving supranutritional vitamin E had smaller hue angles at days 15 and 16 ($P < 0.05$; Fig. 3) and decreased visual discoloration compared with the control-fed heifers at days 14 to 16 of retail display ($P < 0.05$; Fig. 2).

Although no main effect of TM injection was ($P \geq 0.70$) demonstrated, an interaction between day of retail display and TM injection was shown ($P = 0.02$; Fig. 2) for visual discoloration. However, TM injection had increased visual discoloration at later days of retail display than steaks from heifers receiving saline injection. When evaluated by day, steaks from heifers receiving TM injection had a tendency for greater visual discoloration at day 14 ($P = 0.09$) and greater discoloration at day 15 (20.20% vs. 10.74%, $P = 0.04$) of retail display than steaks from heifers receiving saline injection.

The 3-way interaction of supplemental vitamin E, TM injection, and day of retail display resulted in a significant effect on the stability of steak surface redness ($P = 0.04$) and extent of visual discoloration ($P < 0.01$). The 3-way effect on steak surface redness was primarily driven by changes at late retail display (days 11 to 16) with steaks from the CONT + SAL and VITE + MM treatments demonstrating a moderate decline in a^* value from day 11 to day 16 ($\Delta a^* = 7.06$ and 5.42 units, respectively), CONT + MM steaks experiencing a greater decline ($\Delta a^* = 9.13$ units), and VITE + SAL treatment maintaining redness ($\Delta a^* = 3.94$ units) from day 11 to day 16 (Fig. 4).

The 3-way effect of supplemental vitamin E, TM injection, and day of retail display on visual discoloration was driven by differences between treatments in both point at which initial discoloration occurred and the total extent of discoloration at the end of retail display. No differences in visual discoloration were shown until day 14 of retail display; however, steaks from the CONT + MM treatment showed numerically greater discoloration by day 13 of retail display indicating an earlier point of initial discoloration than the other 3 treatments (Fig. 2). At days 14 and 15 of retail display the CONT + MM treatment showed greater discoloration ($P < 0.01$) than the other 3 treatments (Fig. 2). By day 16, there was a tendency ($P = 0.08$) for discoloration of steaks from the CONT + SAL treatment to be intermediate to the CONT + TM and VITE + SAL treatments.

DISCUSSION

The relatively limited number of studies evaluating the effects of vitamin E supplementation on

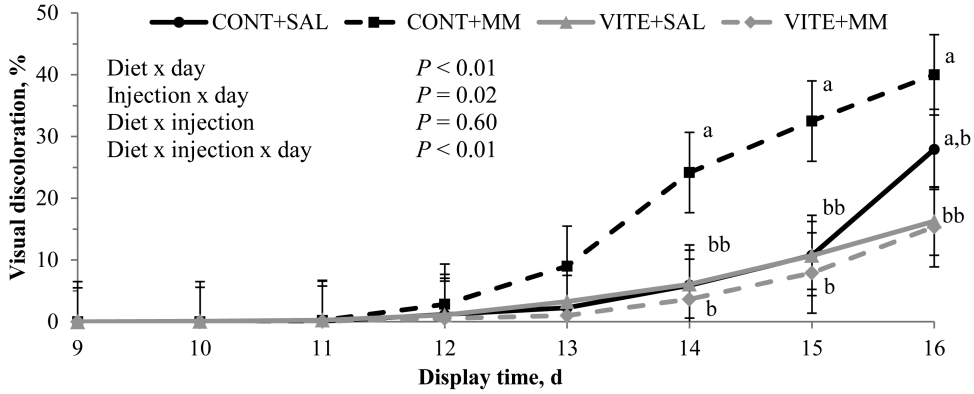

Figure 2. Effects of supranutritional dietary vitamin E supplementation on calculated hue angle of beef strip steaks across 16 d of retail display. Treatments within day lacking common superscripts differ ($P < 0.05$).

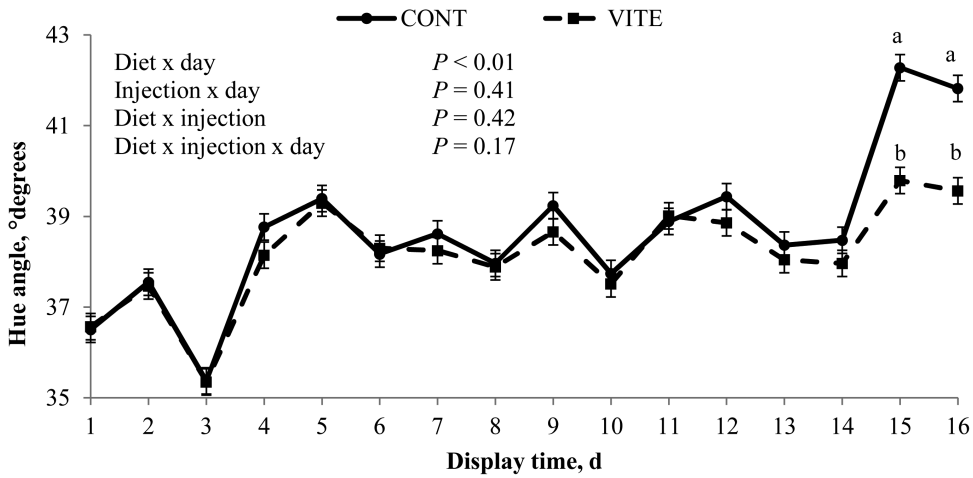

Figure 3. Effects of trace mineral injection (containing Cu, Mn, Se, and Zn) and supranutritional dietary vitamin E supplementation on visual surface discoloration of beef strip steaks across 16 d of retail display. Treatments within day lacking common superscripts differ ($P < 0.05$).

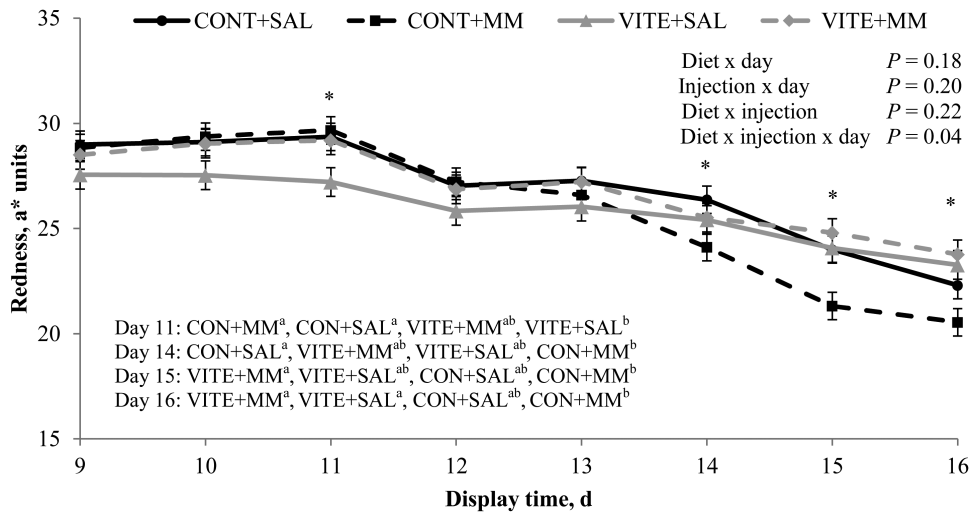

Figure 4. Effects of trace mineral injection (containing Cu, Mn, Se, and Zn) and supranutritional dietary vitamin E supplementation on surface redness (a^* value) of beef strip steaks across 16 d of retail display. Asterisks indicate significance within day with treatments lacking common superscripts differing ($P < 0.05$).

growth performance in feedlot cattle demonstrate considerable variation in response. A meta-analysis conducted by Cusack et al. (2009) indicated feeding vitamin E at concentrations greater than the 1996 NRC recommendation of 15 to 60 IU/kg DM does not improve feedlot performance, mirroring

much of the research in feedlot cattle showing little effect of vitamin E supplementation on final BW, ADG, DMI, and G:F (Arnold et al., 1992; Garber et al., 1996). However, a previous review (Secrist et al., 1997) reported vitamin E supplementation improved ADG by 2.9%. Although both reviews suggest the performance response associated with vitamin E is probably dependent on supplementation concentration (Secrist et al., 1997; Cusack et al., 2009), supplemented heifers in this study received vitamin E well in excess of the industry's average inclusion of 30 IU/kg DM in finishing diets as reported in the feedlot survey of Samuelson et al. (2016) for 90 d prior to slaughter and demonstrated no greater performance traits than nonsupplemented heifers.

Considerably less research assessing the potential for TM injection to improve performance of feedlot cattle exists. Nonetheless, numerical differences in the current study show slight improvements in ADG of TM-injected heifers compared with saline-injected heifers. Arthington et al. (2014) reported a tendency for TM injection to increase ADG of growing heifers. Genther and Hansen (2014b) also demonstrated a tendency for TM supplementation to improve carcass adjusted ADG of steers in the feedlot setting. In a group of steers managed prior to trial initiation on a TM maintenance diet, numerical differences in ADG of saline- and TM-injected steers amounted to a 3.9% improvement in ADG (Genther and Hansen 2014b). However, when steers were managed on a TM-deficient diet prior to trial initiation, those receiving TM injection demonstrated a 17.7% improvement in ADG compared with saline-injected steers (Genther and Hansen 2014b). Although not statistically different, the improvement shown in the present study amounted to a 9.6% increase in ADG of feedlot heifers receiving TM injection. This finding suggests that although the heifers in the current study were not managed to be TM deficient, improvements in ADG associated with TM injection may have been the result of improved in TM status in a group of heifers with lesser TM stores; however, TM status was not assessed via liver biopsy.

Interestingly, heifers supplemented with vitamin E had numerically increased marbling score and is in contrast with the majority of the literature showing little effect of vitamin E supplementation on marbling deposition (Arnold et al., 1992; Liu et al., 1996; Burken et al., 2012). Although it should be noted, the increase of extractible lipid was not statistically different between supplemented and nonsupplemented heifers in this study. Even so, the magnitude of difference in extractible lipid between vitamin E and control-fed heifers was 1.18 units. This change in lipid content is representative of the difference between Modest (average choice) and Moderate (high choice) marbling scores according to Savell et al. (1986). To add further credence to this finding, 76.3% of the variability (R^2 = 0.76) in camera-assigned marbling score was explained by the percentage of extractible lipid observed in the cut surface of the steaks cut from the ribbed carcass. It should be noted that the Secrist et al.'s (1997) review reported marbling score increased numerically with greater vitamin E supplementation. However, increased lipid deposition of vitamin E supplemented cattle observed by Secrist et al. (1997) is less surprising given the concurrent improvements in performance of those cattle.

Of the limited research evaluating effects of TM injection on carcass characteristics, there is considerable variation of response. A study by Genther and Hansen (2014b) reported greater REA and marbling scores in feedlot steers receiving TM injection 90 d prior to slaughter. As expected, those steers that were maintained on a TM-deficient diet prior to trial initiation had greater REA response to TM injection than steers that were control fed (Genther and Hansen 2014b). The same study reported TM injection also resulted in greater marbling scores and a shift in distribution to higher-quality grades; however, the marbling score change between TM-injected and saline-injected steers amounted to less than 1 quality grade improvement (Genther and Hansen 2014b). In another study by Genther-Schroeder and Hansen (2015), TM injection had no effect on HCW, BF, REA, or marbling score. The current study agrees with the findings presented previously with little effect of TM injection on carcass characteristics. As it is generally difficult to quantify the TM status of feedlot cattle upon receiving from an industry standpoint, differences in response to TM injection are likely due to differences in TM status on arrival.

Any technology that adds value to 1 or more segments of the industry with minimal risk has the potential for large-scale adoption. Specific to the retailer, management strategies with the potential to slow the rate of visual discoloration in the retail case would decrease losses associated with discounted, reworked, or discarded product (Zerby et al., 1999). It is been established that vitamin E supplementation results in greater retail shelf life by decreasing the rate of visual discoloration in both whole muscle cuts and ground products (Arnold

et al., 1992; Zerby et al., 1999; Bloomberg et al., 2012), and the results of this study support those findings. However, common finishing diets have not historically included dietary vitamin E concentrations great enough to elicit greater retail shelf life (Samuelson et al., 2016), probably a result of poor investment returns in the form of greater associated diet costs coupled with limited performance benefits.

Trace minerals included in the TM injection used in this study all play roles in the production and activity of antioxidants important in counteracting oxidative stress. Markers of oxidative stress and inflammation were shown to be greater during periods of intense stress such animal transit (Chirase et al., 2004), making receiving cattle more prone to disease challenge and poor performance. Selenium is a component of the antioxidant enzyme glutathione peroxidase and has an important role in the conversion of superoxide radicals to hydrogen peroxide, protecting cellular membranes from free radical damage (Kincaid, 1995). Given the selenium deficiencies of many Midwestern U.S. soils and forages (USDA, 2006), there is a greater likelihood these antioxidant systems may be compromised in deficient cattle. Another such group of antioxidant enzymes are the superoxide dismutases, which are dependent on manganese, copper, and zinc status (Weisiger and Fridovich, 1973; Xin et al. 1991). Steers that received TM supplementation via injection exhibited greater Mn-superoxide dismutase (Genther and Hansen, 2014a) and glutathione peroxidase activity (Pogge et al., 2012) in red blood cell lysate than saline-injected steers, adding credence to the idea that TM injection might provide additional oxidative stability to the adipose tissue of strip steaks.

Despite the hypothesis that vitamin E and TM injection would additively improve color stability and delay the point of initial discoloration, steaks from control-fed heifers that received TM injection discolored both at an earlier point in retail display and to a greater extent than their saline-injected counterparts. In a study of product acceptability and discoloration in the retail case, Gill and Jones (1994) reported products were rated as undesirable after reaching >20% surface discoloration. Applying this measure of acceptability to the current study, steaks from the CON + MM treatment would have been rated as undesirable 3 days faster than steaks from the other 3 treatments. A potential explanation for the inability of an injectable TM to improve color stability is the low concentrations of selenium and zinc maintained in skeletal muscle compared with liver or kidney (Jensen-Waern et al., 1998; Lawler et al., 2004).

Furthermore, the detrimental effect of TM injection to retail color stability may be attributable to copper supplementation. Engle and Spears (2000) reported that dietary copper supplementation in finishing steers altered lipid metabolism. This same study showed greater concentrations of polyunsaturated fatty acids and a tendency for decreased saturated fatty acids concentrations in the longissimus muscle of steers receiving copper supplementation (Engle and Spears, 2000). Trace mineral injection has been demonstrated to increase liver copper and selenium concentrations as well as plasma selenium concentrations in both control and TM-deficient steers (Genther and Hansen, 2014a). A possible explanation for changes in discoloration of steaks from heifers receiving TM injection is that bolus delivery may also be increasing tissue deposition thereby influencing the initial point of discoloration.

Given dietary inclusion of distillers grains with solubles can also increase the concentration of less oxidatively stable polyunsaturated fatty acids (Gill et al., 2008), one hypothesis is that compounded effects of TM injection and 35% MWDGS inclusion resulted in decreased oxidative and color stability in this study. However, it is important to note MWDGS used in the present study contained only 8.5% fat, the lower end of the range in distiller's grains crude fat content (USGC, 2012). More interesting yet is the observation that steaks from VITE + MM heifers did not experience the same decline in color stability shown in steaks from CONT + MM heifers. This explanation is supported by the observation that steaks from the VITE + MM treatment actually discolored to the same extent as steaks from the VITE + SAL treatment. Therefore, it appears as though vitamin E supplementation had a stabilizing effect on the cellular membranes of heifers that received MM injection. This explanation is supported by the observation of numerical differences in lipid oxidation in which steaks from the VITE + MM treatment were intermediate to the CON + MM and VIT + SAL treatments.

CONCLUSIONS

Vitamin E supplementation and TM injection had little effect on growth performance of feedlot heifers. Although there was a tendency for minor improvements in growth of feedlot heifers receiving TM injection, this response may be greater in finishing cattle with subacute TM deficiencies. As expected, VITE supplementation improved color stability and

resulted in greater retail display life than steaks from control-fed heifers. When used in conjunction with a diet higher in MWDGS inclusion, TM injection appeared to increase discoloration of strip steaks from feedlot heifers after 14 d of retail display.

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