Meat and Muscle Biology™

Beef Color Stability and Composition in Cattle Fed High Levels of Vitamin E Following Prolonged Aging

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Abstract: This study aimed to evaluate supplementing high levels of vitamin E (α-tocopherol; 2,200 international units [IU]/d) to cattle and its impact on retaining ideal beef color after prolonged aging times. Fifteen Low-Choice strip loins from cattle fed high levels of vitamin E for the final 100 d on feed and 15 Low-Choice strip loins selected from a commercial lot of cattle were obtained. Loins were split into 3 equal portions, and sections were randomly assigned to 21, 42, or 63 d of wet aging under vacuum packaging, giving 6 treatments (Control-21, Control-42, Control-63, Vitamin E-21, Vitamin E-42, Vitamin E-63). Vitamin E–supplemented loins showed greater α-tocopherol content and less discoloration following retail display across 21, 42, and 63 d aging than control loins ($P < 0.05$). Concurrently, vitamin E loins exhibited greater a^* (redness) and lower malonaldehyde (lipid oxidation) content and hue angle values during retail display across 21, 42, and 63 d aging than control loins ($P < 0.05$). Greater protein ($P < 0.0001$) and tendencies for lower moisture ($P = 0.07$) content were found in vitamin E loins compared to control. Subcutaneous fat samples from vitamin E–supplemented loins had a greater percentage of saturated fatty acids ($P = 0.008$), while control loins had greater percentages of branched-chain, cis-monounsaturated, t16:1, atypical dienes, conjugated linoleic acid, and omega-3 and omega-6 fatty acids ($P < 0.02$). This implies subtle dietary differences between fed cattle treatments. No differences in composite fatty acids were found in lean tissue ($P > 0.05$). Although minor changes in fatty acid and proximate composition exist across treatments, results suggest that vacuum-packaged beef from cattle fed high levels of vitamin E can sustain color stability and retail display life after prolonged aging, extending the time of ideal product quality in commodity beef products.

Key words: aging, beef, color, quality, α-tocopherol
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Introduction

Acceptable color of fresh beef in the retail case is a major factor used by consumers, as the bright cherry-red color of fresh beef is associated with freshness and wholesomeness (Hood and Riordan, 1973; Faustman et al., 1998). In commodity export products, which undergo multiple weeks of vacuum-packaged aging, fresh beef is susceptible to accelerated discoloration and oxidation of lipids. This is due, in part, to the degradation of mitochondrial activity and subsequent depletion of reducing compounds, which results in accelerated oxidation of proteins and lipids (Mitacek et al., 2019). Discoloration is a result of the oxidation of myoglobin, the sarcoplasmic protein primarily responsible for fresh meat color, into metmyoglobin (Seideman et al., 1984). This can result in reduced shelf life for fresh beef and is an inherent challenge in commodity products requiring long periods of time for transportation prior to reaching the retail sales counter (e.g., export markets). To counteract this, antioxidant applications have been investigated to reduce the rate of oxidation of lipids and proteins, prolonging product quality prior to

purchase. Previous research has reported that daily supplementation of vitamin E (α-tocopherol) to feedlot cattle is an effective method to delay oxidative reactions in fresh beef. A lipid-soluble, polyphenolic free radical scavenger, vitamin E works to inhibit free radical peroxidation of unsaturated fatty acids found in plasma membranes of meat tissue (Suman et al., 2014). Vitamin E functionality is seen within the phospholipid membrane of meat, quenching radical ions to delay autoxidation within phospholipids and sustaining myoglobin's red pigment in fresh beef (Faustman et al., 1998). Knowing this, daily supplementation of vitamin E at ∼1,000 IU has been a popular application in cattle finishing, and its effects have been investigated across multiple dietary strategies (Faustman et al., 1998; Roeber et al., 2001; Sales and Koukolová, 2011); however, the reduction of antioxidant capacity from vitamin E after prolonged, refrigerated storage is not well defined (Liu et al., 1996; Yang et al., 2002).

There is limited information on the impact of supplementing vitamin E above 1,000 IU/d on the quality of beef after prolonged aging, similar to what occurs with exported products. Therefore, our objective was to investigate cattle supplemented with high levels of vitamin E across beef aged for moderate to long periods of time to increase the understanding of meat quality as it relates to the retention of ideal meat color in commodity export products.

Materials and Methods

Sample collection

Cattle ($n = 90$) across 9 pens were grain finished with 2,200 IU of vitamin E (α-tocopherol) daily for the final 100 d on feed. All methods of live animal handling for vitamin E–fed cattle were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee (IACUC #1785). All animals harvested for this study were slaughtered humanely, under U.S. Department of Agriculture (USDA) guidelines, at a commercial beef harvest facility in Omaha, NE. One USDA Low-Choice strip loin (Institutional Meat Purchase Specifications #180; USDA, 2020) was selected from one carcass per pen, totaling 9 loins, along with 9 Low-Choice strip loins selected from a single, randomly selected lot of commercially fed cattle to act as controls. Loins were vacuum packaged and transported with dry ice to the University of Nebraska meat laboratory. Upon arrival, loins were immediately removed from vacuum packages and cut into 3 equal sections (approximately 7.2 cm in length), repackaged under vacuum (3 mil STD barrier, Ultra Source, Kansas City, MO, USA), and randomly assigned to one of 3 lengths of wet aging: 21, 42, or 63 d of aging at 3°C. This totaled 6 different treatments: Control-21, Control-42, Control-63, Vitamin E-21, Vitamin E-42, and Vitamin E-63. At the completion of each aging period, loin sections were opened and fabricated in the following sequence (anterior to posterior): One 2.54-cm-thick steak was cut for instrumental color measurement and subjective discoloration scoring during 7 d of retail display. Fat caps from these steaks were removed, and vacuum packaged and stored at −80°C for fatty acid analysis. Two 1.27-cm-thick steaks were made, denuded of subcutaneous fat, and cut in half. One of the 4 halved portions was selected for laboratory proximate composition, and the remaining 3 portions were randomly assigned to lipid oxidation after 0, 4, or 7 d of retail display. One 1.27-cm-thick steak was cut for α-tocopherol quantification at 0 d of retail display.

Instrumental color during simulated retail display

Objective color measurements were taken once daily every 24 h for 7 d after fabrication. Steaks were placed on Styrofoam trays (21.6 × 15.9 × 2.1 cm, Styro-Tech, Denver, CO, USA), overwrapped with oxygen-permeable polyvinyl chloride film (Prime Source PSM 18#75003815, Bunzl Processors Division, North Kansas City, MO, USA; oxygen transmission rate = 2.25 mL/cm^2/24 h at 23°C and 0% relative humidity; water vapor transfer rate = 496 g/m^2/24 h at 37.8°C and 90% relative humidity), and placed under retail display conditions (3°C under white fluorescent lighting at 1,650–2,000 lux). Objective color was measured using L^*, a^*, b^* scales using a Minolta CR-400 colorimeter (Illuminant D65, 8-mm diameter aperture, 2° standard observer angle; Minolta, Osaka, Japan) in accordance with Ribeiro et al. (2021). Delta E (overall color change over time) was calculated for steaks across all aging periods and days of retail display in accordance with King et al. (2023). Delta E values were calculated using the following equation: $\Delta E = [(\Delta L^{*2}) + (\Delta a^{*2}) + (\Delta b^{*2})]^{0.5}$. Delta E was used to determine the overall color change from the initial (day 0) to the final (day 7) day of retail display. Hue angle (the reduction of red pigment to brown in meat) was calculated using the following equation: $Hue\ Angle = Degree([arctangent(\frac{b}{a})])$.

Subjective color

Visual discoloration was assessed daily every 24 h (±1 h) during the 7 d of retail display. Panelist training for the color panel was described by Herrera et al. (2021). All spaces used during retail display were within the acceptable light intensity range described by King et al. (2023).

Lipid oxidation

Lipid oxidation was determined using thiobarbituric acid reactive substances (TBARS) values measured for each aging period at 0, 4, and 7 d of retail display in accordance with the procedure from Ahn et al. (1998), as modified by Henriott et al. (2020). Results were expressed in mg of malonaldehyde per kg of tissue.

Alpha-tocopherol content

Muscle samples were saponified as described by O'Fallon et al. (2007) with extraction modifications as described by Du and Ahn (2002). Briefly, samples were trimmed of all external fat and connective tissues and weighed by 1 g into a 20-mL flat-bottom borosilicate vial with a Teflon®-lined screw cap, to which α-tocopherol-d6 was added as an internal standard, 10 N KOH, methanol, and 20% ascorbic acid were subsequently added. The mixture was homogenized for 15 s, saponified at 55°C, and then held in a water bath at 55°C for 1.5 h. After cooling the vial in cold water, 5 mL of water and 5 mL of hexane were added, and the mixture was vortexed vigorously for 30 s. Tocopherol liberated during saponification was extracted in hexane, transferred into a 2-mL amber gas chromatography (GC) vial with a Teflon®-lined screw cap, and stored at −20°C until determination by gas chromatography-mass spectrometry (GC-MS). Tocopherol was determined by an Agilent 7890A GC system equipped with a customized DB-5 capillary column (10 m × 0.25 mm × 0.25 µm), an autosampler, and a split/splitless injector, and an Agilent 5975C inert XL MSD with triple-axis mass detector. Tocopherol was separated in a 3-min temperature-gradient program with helium as the carrier gas flowing at a constant rate of 1.5 mL/min. The transfer line, ion source, and quadrupole were heated at 280, 230, and 150°C, respectively. Ionization was performed in an electron impact mode at 70 eV. Mass-to-charge (m/z) 430 (tocopherol) and 436 (tocopherol-d6) were monitored in single ion monitoring (SIM) mode with dwell time being optimized for at least 20 data points across peak width. Tocopherol was quantified by an internal standard calibration method and expressed as mg/kg of muscle (ppm).

Proximate composition

The proximate composition of moisture, fat, ash, and protein was evaluated as described by Hart et al. (2019) and AOAC (1990).

Fatty acid composition (subcutaneous fat and muscle)

Analysis of subcutaneous fatty acid composition was conducted in accordance with Klopatek et al. (2022) and Dugan et al. (2007).

Fatty acid composition and total lipids of muscle tissue were extracted following the procedure described by Folch et al. (1957) with minor revisions. After extraction, lipids were converted to fatty acid methyl esters according to Morrison and Smith (1964) and Metcalfe et al. (1966), with modifications as described by Hart et al. (2019). Percentages of fatty acids were determined by the peak areas in the chromatograph. Finally, values were adjusted according to the percent fat and converted to mg/100 g tissue by the following equation: fatty acid mg/100 g tissue = (fatty acid peak area * fat of sample) * 1,000.

Statistical analysis

Fatty acid and proximate compositions were analyzed as a completely randomized design. The L^*, a^*, and b^* values and subjective discoloration data were analyzed as a split-plot design with repeated measures, with dietary treatment assigned as the whole plot, aging period as the split plot, and day of retail display considered as a repeated measure. Delta E values, hue angle, and α-tocopherol content were analyzed as a split-plot design with dietary treatment as the whole plot and aging period as the split plot. Lipid oxidation (TBARS) data were analyzed as a split–split plot design with dietary treatment as the whole plot, aging period as the split plot, and day of retail display as the split–split plot. Loin section was considered the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS with the LS MEANS statement. Statistical significance was determined at $P < 0.05$ with trends discussed when the P value was between 0.05 and 0.10.

Results and Discussion

Objective color

Overall, all objective color values had a day effect ($P < 0.0001$), as L^*, a^*, and b^* decreased as display continued (Table 1). In addition, L^* and a^* values decreased ($P < 0.0001$) as total days aged increased

Table 1. Analysis of objective color (L^*, a^*, b^*) and subjective discoloration during retail display.

		Days of Retail Display									
Variable	Treatment	0	1	2	3	4	5	6	7	SEM	*P* value
L^*[1]	Control	45.75	44.90	44.68	44.29	43.51	43.63	43.40	44.10	.89	.5708
	Vitamin E	46.70	45.84	45.45	45.54	45.00	44.40	44.15	43.83		
a^*[1]	Control	20.51^{a}	20.21^{a}	18.77^{bc}	17.77^{d}	15.90^{e}	13.16^{f}	11.12^{g}	9.59^{h}	.31	<.0001
	Vitamin E	20.65^{a}	20.39^{a}	19.30^{b}	18.18^{cd}	17.39^{d}	15.64^{e}	13.15^{f}	11.98^{g}		
b^*[1]	Control	9.30	9.48	8.88	8.68	8.13	7.97	7.88	7.81	.38	.10
	Vitamin E	10.14	10.24	9.65	9.34	9.12	8.78	8.32	7.82		

			Days of Retail Display									
	Treatment	Age	0	1	2	3	4	5	6	7	SEM	*P* value
Percent Discoloration (%)	Control	21	0.51^{E}	0.60^{E}	0.60^{E}	1.47^{E}	5.6^{E}	23.13^{CD}	29.71^{CD}	39.31^{C}		
	Vitamin E	21	0^{F}	0^{F}	0^{F}	0.04^{F}	0.38^{E}	0.56^{E}	3.71^{E}	13.22^{D}		
	Control	42	0^{F}	0^{F}	0.36^{F}	0.96^{E}	14.31^{D}	55.44^{B}	81.58^{A}	87.60^{A}	7.54	<.0001
	Vitamin E	42	0^{F}	0^{F}	0^{F}	0.13^{F}	1.18^{E}	8.69^{DE}	29.11^{CD}	52.89^{B}		
	Control	63	0^{F}	0.04^{F}	0.20^{F}	0.93^{E}	4.38^{E}	35.80^{C}	77.53^{AB}	95.29^{A}		
	Vitamin E	63	0.04^{F}	0.04^{F}	0.04^{F}	0.04^{F}	0.67^{E}	13^{D}	34.27^{C}	59.73^{B}		

[a–g]Means within the same variable with different superscripts denote treatment*day interactions ($p < 0.05$).

[A–F]Means within the same variable with different superscripts denote treatment*age*day differences ($p < 0.05$).

[1]L^*: Black to white color space; 100 = light (white), 0 = dark (black); a^*: Red to green color space; + value (red), − value (green); b^*: Yellow to blue color space; + value = yellow, - value = blue.

[2]SEM: Standard error of the mean.

(63 d < 42 d < 21 d). For redness (a^*) values, a dietary treatment-by-day of retail display interaction ($P < 0.0001$) was found, as steaks from vitamin E–fed cattle exhibited greater, more acceptable red color following 3 d of retail display compared to control samples. These data are supported by hue angle values (Figure 1), where a treatment-by-age-by-day interaction ($P = 0.0013$) revealed greater hue angles in control-63 and control-42 after 5 d of retail display than all other treatments. At 6 and 7 d of retail display, Vitmain-63 samples were statistically similar to Control-42, with Control-63 having a greater hue angle than any other treatment. Knowing this, vitamin E samples were shown to have sustained red color over prolonged aging

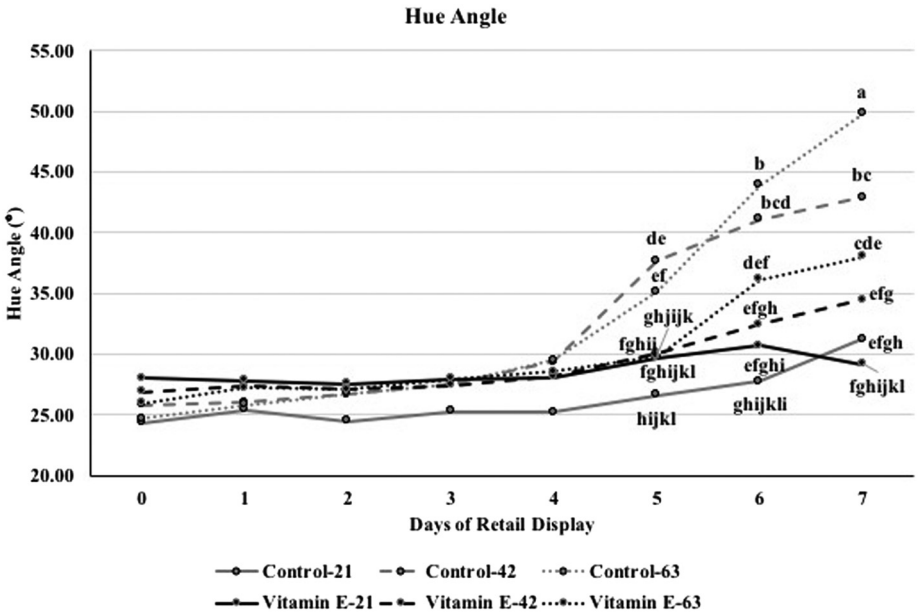

Figure 1. Hue angle (°) of control and vitamin E steaks aged 21, 42, or 63 d after 7 d of retail display [control SEM: 2.3115; vitamin E SEM: 1.2666; $P = 0.0013$]. [a–l]Means with different superscripts denote day-by-dietary treatment differences ($P < 0.05$).

compared to control treatments with the same aging time. The b^* values tended ($P = 0.10$) to be greater in vitamin E samples across all days of retail display. Overall, the change in b^* values from 0 to 7 d was greater in vitamin E ($\Delta = 2.32$) compared to control samples ($\Delta = 1.49$). These data were supported by a significant difference ($P = 0.0008$) in delta E values with control loins having a larger delta E value and greater overall color change, compared to vitamin E samples (18.78 and 11.43, respectively).

Using a colorimeter, it was reported by Holman et al. (2017) that a relationship between a^* and consumer acceptance of beef color exists. Beef color was considered acceptable (with 95% acceptance) when a^* values were equal to or above 14.5. In the present study, the days required for each treatment to reach an a^* value of 14.5 was calculated by interpolating between the 2 days with values above and below 14.5. The 14.5 color threshold value was no longer met by steaks from the control and vitamin E treatments after 4.96 and 5.15 d of retail display, respectively ($P < 0.0001$). This suggests beef from vitamin E–fed animals should sustain acceptable redness scores about a day longer than control loins, regardless of 21, 42, or 63 d of aging.

Subjective color (discoloration)

A 3-way interaction between dietary treatment-by-days of aging-by-days of retail display interaction ($P < 0.0001$) was found for subjective discoloration (Table 1). In general, percent discoloration gradually increased as the aging period and retail display increased, regardless of treatment type. Loins from cattle fed high levels of vitamin E had significantly lower percent discoloration in steaks across 21, 42, and 63 d of aging. Given that meat is typically discounted when reaching 20% discoloration, we assessed the average length of time required to surpass 20% discoloration (data not shown). An aging effect ($P < 0.0001$) was found, as beef aged longer had reduced time to surpass 20% discoloration. Beef fed vitamin E did not surpass this threshold with 6 d of retail display following 42 and 63 d of aging. The 20% threshold was not exceeded for vitamin E samples after 21 d of aging. Beef from cattle fed vitamin E and aged for either 42 or 63 d required 6 d of retail display prior to surpassing 20% discoloration. For comparison, control steaks surpassed 20% discoloration after 5 d of retail display, regardless of aging period; however, this was not supported by a treatment-by-aging interaction ($P = 0.45$).

It has been reported by Hood and Riordan (1973) that about 20% surface discoloration in retail display can result in a 50% decline in consumer purchasing decisions for beef. Prior to reaching this level, consumers can begin to discriminate against discolored meat, opting for non-discolored products if both packages are viewed in retail display. This requires retailers to drastically reduce the cost of fresh beef in order to promote consumer purchasing of discolored products. According to Ramanathan et al. (2022), approximately 2.55% of all ground beef and beef steaks in the United States are discarded in a 52-wk period, resulting in beef industry losses of $3.73 billion annually due to discoloration. The presence of vitamin E greatly reduces the level of oxidation in myoglobin, prolonging ideal beef color during retail display; however, the quicker discoloration rate observed as aging increased, regardless of treatment, could be attributed to depletion of mitochondrial activity and reducing compounds and exhaustion of vitamin E content due to aging time (Mitacek et al., 2019). Discoloration is caused by an accumulation of metmyoglobin on the meat surface due to the oxidation of myoglobin. Muscle ability to convert metmyoglobin (ferric iron state, Fe^{3+}) to the reduced ferrous state (Fe^{2+}) through metmyoglobin-reducing activity (MRA) is limited. This capacity is continually reduced as time postmortem progresses, in part due to the decreasing functionality of mitochondria, which produces many of the reducing compounds necessary for color stability (Mancini and Hunt, 2005). Reduction of metmyoglobin formation is crucial to meat color stability and greatly depends on muscle oxygen-scavenging enzymes, reducing enzyme systems, and the nicotinamide adenine dinucleotide hydride (NADH) pool. Once NADH and exogenous antioxidants like vitamin E are depleted, MRA is limited and accumulation of metmyoglobin on the meat surface occurs.

Lipid oxidation

A treatment-by-day interaction ($P < 0.001$) was found (Figure 2) for lipid oxidation. In general, lipid oxidation increased from day 0 to day 7 in both treatments ($P < 0.05$). Steaks from control samples had statically greater malonaldehyde content at 4 and 7 d of retail display compared to all days tested for vitamin E samples, with control samples at 4 and 7 d possessing 4 and 5 times the level of malonaldehyde versus vitamin E samples at the same days, respectively. Across days of retail display, steaks from cattle supplemented with vitamin E showed statistically similar malonaldehyde content to control samples at day 0.

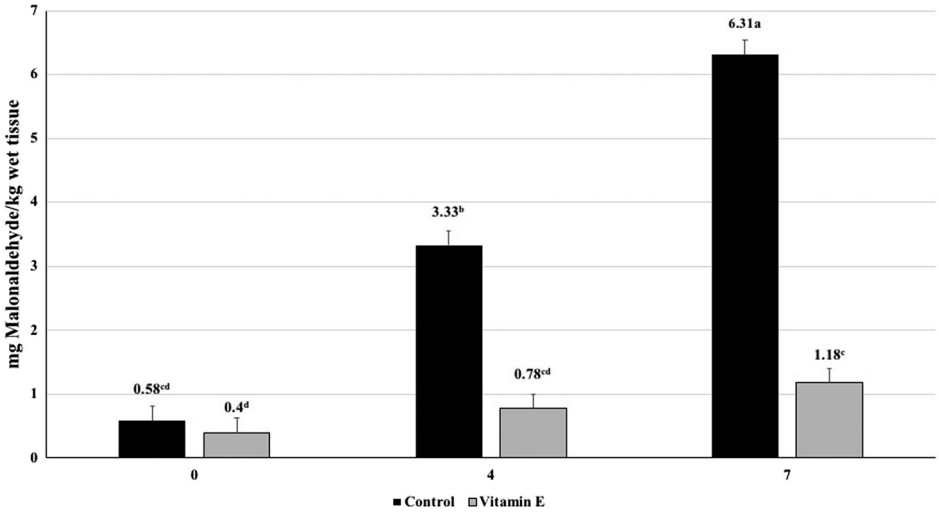

Figure 2. Thiobarbituric acid reactive substances (TBARS) of loins from cattle fed with or without vitamin E, mg malonaldehyde per kg of wet tissue [SEM: 0.2263; $P < 0.001$]. [a–d]Means with different superscripts denote dietary treatment differences ($P < 0.05$).

This demonstrates vitamin E samples at increased retail display up to 7 d showed the same level of oxidation of control samples at 0 d of retail display when lipid oxidation was at its lowest throughout retail display.

Oxidation of muscle lipids is promoted by multiple factors, including but not limited to total fat content, composition of fatty acids, heavy metal ions such as iron or calcium in meat that can increase the rate of oxidation, atmospheric oxygen in contact with the meat surface, and light via photooxidation. Depending on these conditions, the rate of oxidation can accelerate rapidly, resulting in increased free radical formation, accelerated meat discoloration, and development of product off-flavors that negatively impact cooked beef flavor. Living muscle is capable of producing endogenous enzymes, including superoxide dismutase, catalase, and glutathione, which eliminate free radicals formed during oxidation through the formation of water and energy metabolism (Morrissey et al., 1998; Dröge, 2002). In postmortem meat, however, the balance of pro-oxidants and antioxidants that exist in living muscle is disrupted as the functionality of antioxidant-producing organelles declines, thus favoring oxidation damage lipids and proteins (Min and Ahn, 2005). Oxidation of lipids and myoglobin, in particular, often appear to be linked, and the oxidation of one leads to the formation of chemical species that can aggravate oxidation of the other, and vice versa (Baron and Andersen, 2002; Faustman et al., 2010). As a whole, these mechanisms can quickly result in poor meat color and develop potent off-flavors. This is in agreement with our study, where greater TBARS values and discoloration scores were observed in the control sample compared to their vitamin E counterparts. Under similar packaging and storage conditions, the presence of α-tocopherol plays an essential role. Feeding vitamin E to cattle increases the deposition of α-tocopherol content within phospholipid membranes of muscle cells, allowing radical quenching to occur in the presence of pro-oxidants. This phenomenon of antioxidant activity is reflected in our study, as a notable reduction in lipid oxidation occurred. Alpha-tocopherol can regain antioxidant functionality after it reduces oxidative compounds via regaining of its hydrogen electron. If reconfiguration reactions within tocopherols occur as a result of oxidation, it loses its function as an antioxidant.

Campo et al. (2006) considered TBARS values exceeding 2.28 mg of malonaldehyde per kg as unacceptable for beef as rancid flavors begin to develop at this threshold, overpowering beef flavor with rancid notes. Hughes et al. (2015) considered levels between 2.60 and 3.11 mg of malonaldehyde per kg as acceptable to consumers. It is important to note there were differences in matrix aging times and cookery methods for consumer assessment across these studies, which may explain the differences in their findings.

In this study, the limiting threshold of 2 mg of malonaldehyde per kg was not met by steaks from the vitamin E treatment in any aging period following retail display, suggesting that cattle fed high levels of vitamin E can be merchandized in the retail case up to 7 d without detrimental effects to flavor from lipid oxidation. In contrast, control loins exceeded the malonaldehyde limit set by Campo et al. (2006) for 21 d after 4 d of retail display. Also, the threshold was surpassed in both 42 and 63 d aging in control samples after 4 d of retail display. This suggests that lipid stability is

strongly maintained in beef from cattle fed vitamin E, greatly reducing the potential for off-flavor development and maintenance of beef flavor.

Alpha-tocopherol content

A treatment effect ($P < 0.0001$) was found for α-tocopherol content (Figure 3), as cattle fed vitamin E were drastically higher compared to control loins (15.22 mg/kg and 2.7 mg/kg, respectively). This was expected, as 2,200 IU/head/d over the last 100 d on feed is a high level of vitamin E supplementation for finishing cattle, compared to ~50 IU/head/d traditionally found in finishing rations. However, the level of α-tocopherol deposited in muscle tissue can be dependent on more than supplementation level. Ripoll et al. (2013) illustrated increasing time supplementing α-tocopherol increased deposition levels in muscle tissue and decreased development of lipid oxidation products in lamb during retail display. Additionally, Yang et al. (2002) found supplementation of vitamin E to increase tocopherol content in grain-fed beef more so than pasture-fed. This may be due to the presence of tocopherol compounds already present in grasses and legumes contributing to tocopherol content in grass-fed beef, whereas grain-fed beef deposited tocopherols primarily through feed supplementation.

An aging effect ($P = <0.01$) was found, where tocopherol content in 21 and 63 d were statistically lower than 42 d aging (8.56 mg/kg, 11.07 mg/kg, and 7.25 mg/kg, respectively). No treatment-by-age interaction was present ($P = 0.15$), though vitamin E samples had numerically greater tocopherol content across all aging periods. Changes in tocopherol content due to aging time are still unexplained. Yang et al. (2002) found no significant changes in tocopherol content across 0 and 47 d aging, although some comparisons showed numerical increases in tocopherol content after aging. This conflicts with Lagerstedt et al. (2011), as their findings showed cumulative days of aging lowered tocopherol content in longissimus muscle of young bulls. Lagerstedt et al. (2011) aged *longissimus dorsi* muscle from 3 to 18 d under hybrid vacuum-modified atmospheric packaging conditions. When only in vacuum packaging, samples aged 18 d lose less than 2% of total α-tocopherol content compared to minimal-aged products. Compared to our study, as much as 14.9% of tocopherol content was lost after aging. It is valuable to note, however, that our study assessed samples from cattle fed high levels of vitamin E and aged notably longer than Lagerstedt et al. (2011). These studies examined tocopherol content in fresh beef across differing days of aging and packaging strategies (vacuum vs vacuum/modified atmospheric packaging hybrid), and results cannot be directly compared.

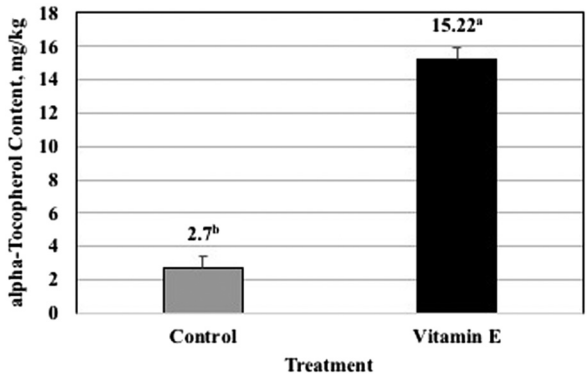

Figure 3. Alpha-tocopherol content of control and vitamin E steaks [SEM: 0.6921; $P < 0.001$] [a,b]Means with different superscripts denote dietary treatment differences ($P < 0.05$).

Proximate composition

In the present study, dietary treatments were significantly different ($P < 0.05$) in protein content (Table 2), as samples from cattle fed vitamin E had greater protein composition compared to controls (23.43% and 20.76%, respectively). This difference may, in part, be related to differences in cattle populations sampled, as all control cattle were chosen randomly, provided they achieved identical quality grades. Conversely, vitamin E–fed cattle were selected from a controlled population of cattle with known dietary composition. Control loins tended ($P = 0.07$) to have greater moisture content (70.23%) compared to vitamin E samples (68.40%). These differences may be related, in part, to differences in cattle populations sampled, as all control cattle were chosen randomly, provided they achieved identical quality grades. It should be noted that protein content was calculated as the difference remaining in product composition. No significant differences ($P > 0.05$) were found in fat or ash, with meat sample averages of of 7.42% and 1.175%, respectively, across treatments.

Fatty acid composition (muscle)

No differences ($P > 0.05$) were found across treatments in saturated, unsaturated, monounsaturated, polyunsaturated, or ratio of saturated-to-unsaturated fatty acids. A few differences ($P < 0.05$) in the content of specific fatty acids in muscle tissue were found (Table 2). Control loins had greater contents ($P < 0.05$) of palmitic acid (C16:0), and cis-11-Eicosenoic (C20:1) acid. Loins from cattle supplemented with vitamin E had a greater

Table 2. Analysis of proximate composition and fatty acid composition[1] of subcutaneous adipose tissue and lean tissue from cattle fed commercial finishing diet with or without 2,200 IU of vitamin E.

Variable	Treatment			
Proximate composition	Control	Vitamin E	SEM	*P* value
Protein	20.76[b]	23.43[a]	0.27	<0.0001
Moisture	70.23	68.40	0.65	0.07
Fat	7.84	7.00	0.65	0.38
Ash	1.18	1.17	0.03	0.92
Composition of selected fatty acids in subcutaneous fat,% of total fatty acids				
SFA	40.91[b]	43.52[a]	0.65	0.008
cis-MUFA	48.41[a]	45.42[b]	0.83	0.02
Trans-t16:1	0.36[a]	0.32[b]	0.01	0.0019
Trans-t18:1	3.30	4.10	0.31	0.08
CLA	0.62[a]	0.50[b]	0.02	0.0011
n-6 PUFA	2.83[a]	2.33[b]	0.13	0.0096
n-3 PUFA	0.23[a]	0.19[b]	0.01	0.0066
Composition of selected fatty acids in lean tissues (% of total fatty acids)				
C 14:0	3.26	3.13	0.13	0.50
C cis9-14:1	1.10	1.06	0.10	0.76
C 15:0	0.37[b]	0.53[a]	0.03	0.0004
C 16:0	27.54[a]	26.27[b]	0.32	0.01
C trans9-16:1	0.30	0.20	0.03	0.06
C cis9-16:1	4.03	3.99	0.16	0.87
C 17:0	1.00[b]	1.48[a]	0.06	<0.0001
C cis9-17:1	0.83[b]	1.25[a]	0.05	<0.0001
C 18:0	13.98	13.44	0.49	0.42
C trans10/11-18:1	1.57[b]	2.32[a]	0.22	0.02
C cis9-18:1	26.48	39.93	5.54	0.09
C cis11-18:1	14.68	1.58	5.75	0.11
C 18:2n6	3.32	3.11	0.24	0.52
C 18:3n6	0.02	0.04	0.02	0.44
C 18:3n3	0.17	0.16	0.02	0.65
C cis9-20:1	0.51[a]	0.41[b]	0.02	0.004
C 20:3n6	0.14	0.18	0.02	0.12
C 20:4n6	0.45[b]	0.70[a]	0.07	0.02
C cis-24:1	0.01[b]	0.08[a]	0.01	0.0007
C 22:5n3	0.12	0.11	0.02	0.80
C 22:6n6	0.00	14.75	10.08	0.30
Fatty acid sums and ratios, % of total fatty acids				
ΣSFA	46.15	44.66	0.73	0.16
ΣUFA	53.74	55.34	0.76	0.14
Σcis-MUFA	49.51	50.92	0.74	0.18
ΣPUFA	4.23	4.42	0.31	0.67
Ratio (SFA:UFA)	0.86	0.81	0.03	0.15

[a,b]Means within the same row with different superscripts denote dietary treatment differences ($P < 0.05$).

[1]Percentage value range from 0 to 100.

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; CLA = conjugated linoleic acid; n-6 = omega 6 fatty acids; n-3 = omega 3 fatty acids; UFA: unsaturated fatty acids; PUFA: polyunsaturated fatty acids. ΣSFA = sum of saturated fatty acids (14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 22:0, 24:0); ΣUFA = sum of cis-MUFA, PUFA and CLA; Σcis-MUFA = sum of cis-monounsaturated fatty acids (c9-14:1, c7- 16:1, c9-16:1, c11-16:1, c9-17:1, c9-18:1, c11-18:1, c12-18:1, c13-18:1, c14-18:1, c15-18:1, c16-18:1, c9-20:1, c11-20:1); ΣPUFA = sum of 18:3n-3, 20:5n-3, 22:5n-3, 18:2n-6, 20:3n-6, and 20:4n-6.

content ($P < 0.05$) of pentadecanoic (C15:0), heptadecanoic (C17:0), cis-10-heptadecanoic (C17:1), and arachidonic acid (C20:4n6). Although these differences exist, the percent of difference within a fatty acid is minimal, ranging from 1.27% to as minute as 0.07% in a fatty acid that is statistically significant. Because of this, an assessment of grouped fatty acid composition was conducted. Although significant differences on an individual fatty

acid basis exist, the sum of fatty acids shows no differences in saturated, unsaturated, monounsaturated, and polyunsaturated fatty acid contents. This shows that there were minimal differences in fatty acid composition between the vitamin E–fed versus the control populations, strongly suggesting the differences in color and lipid stability were likely due to the feeding of vitamin E. That being said, vitamin E supplementation in cattle has been found to alter the proteomic profile of mitochondria in meat. Zhai et al. (2019) identified cattle supplemented with vitamin E as having a lower abundance of mitochondrial enzymes present in the electron transport chain, tricarboxylic acid cycle, pyruvate metabolism, and adenosine triphosphate (ATP) regeneration processes compared to cattle not supplemented vitamin E. Primary mitochondrial function in living muscle tissue is to produce energy in the form of ATP using the consumption of oxygen, an oxidizing agent. Although mitochondrial activity can produce nicotinamide adenine dinucleotide (NADH), a coenzyme contributing to metmyoglobin reduction in meat, ATP generation along the electron transport chain requires both oxygen and multiple protein complexes for the exchange of electrons. These protein complexes are sites to promote the generation of reactive oxygen species (ROS) and can increase myoglobin oxidation and meat discoloration. Concurrently, cattle supplemented with vitamin E showed reduced abundance of mitochondrial enzymes linked to minimizing oxidative stress and mitochondria integrity, including creatine kinase s-type and pyruvate dehydrogenase protein X component. This study suggests the deposition of α-tocopherol within mitochondrial membranes could enhance the presence of reducing enzymes naturally occurring with the mitochondria matrix, while also acting as an embedded oxygen scavenger for ROS produced within the electron transport chain during ATP generation.

Fatty acid composition (subcutaneous fat)

Small but significant differences ($P < 0.05$) in subcutaneous fatty acids were found (Table 2). Control subcutaneous fat samples had greater branched-chain fatty acids (BCFA), cis-monounsaturated fatty acids (cis-MUFA), trans isomers of palmitoleic (16:1) and oleic (18:1) acid, atypical dienes, conjugated linoleic acid (CLA), and omega-6 and omega-3 polyunsaturated fatty acids (n-6/n-3PUFA). Subcutaneous fat from cattle fed vitamin E had greater saturated fatty acid (SFA) content. These differences are likely due to the different basal diets between the control and vitamin E–fed groups.

Conclusion

Feeding high levels of vitamin E to cattle sustained meat color and oxidative stability after prolonged aging, as shown by greater redness (a^*) scores, lower percentage discoloration and total color change (delta E), and reduced lipid oxidation. The results from this study provide an industry strategy for fresh beef products that undergo prolonged aging, as occurs in export products, to retain ideal meat quality and consumer appeal during retail display.

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