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Effect of α-tocopherol tissue levels on beef quality

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To evaluate meat quality of beef with different α-tocopherol tissue levels, 55 feedlot steers were fed a barley-based finisher diet with four vitamin E supplementation levels (0, 350, 700 and 1400 IU DL-α-tocopheryl acetate/animal per day) for 120 days. Although the increase in oxidation levels overtime was much smaller ($P < 0.001$) in the high-medium and high groups, α-tocopherol tissue levels did not affect ($P > 0.05$) pH, proximate analysis, drip and cooking losses, and shear force of steaks. No effect of α-tocopherol tissue levels was found in retail evaluation of steaks after a short ageing time of 6 days, but with 21 days of ageing, a delay in formation of metmyoglobin ($P = 0.008$) was observed in steaks with higher tissue levels of α-tocopherol. Similar results were found for ground beef (25% fat) prepared from 6-day aged meat. Thus, higher α-tocopherol tissue levels protect ground beef and long-aged steaks from discolouration and lipid oxidation.

Keywords: ageing, beef, tenderness, vitamin E

Implications

Dietary vitamin E has been shown to have numerous positive effects on meat quality, especially colour and lipid stability. However, there are concerns regarding tenderness and the lack of marketable payback to producers to cover the cost of vitamin E supplementation. This study was conducted to clarify the main effects of dietary vitamin E on beef quality. Quality enhancements can be used for new product differentiation strategies by the beef industry using a new trademark ('E-Beef'), and provide return on investment for vitamin E supplementation.

Introduction

Vitamin E, due to its effects on nutritional myopathy, retinal degeneration, erythrocyte haemolysis and prostaglandin biosynthesis, is an essential nutrient for the growth and health of beef cattle. Vitamin E is also widely used as an antioxidant in biological systems and has a positive impact on colour and lipid stability of fresh and frozen beef (Liu *et al.*, 1995). The discolouration of beef from bright red to brown, which occurs during retail display, is a combined function of myoglobin and lipid oxidation, and supplementation of vitamin E can result in an increasing retail display life of beef by 1.6 to 5 days (Gray *et al.*, 1996; Morrissey *et al.*, 1998). α-Tocopherol preserves the integrity of muscle cell membranes, inhibiting the passage of sarcoplasmic fluid through it, as well as acting as a radical-quenching antioxidant, consequently preventing drip loss and the oxidation of membrane phospholipids during storage (Asghar *et al.*, 1991; Gray *et al.*, 1996; Faustman *et al.*, 1998). Several studies have reported positive effects of dietary vitamin E supplementation in reducing the oxidation of lipids and myoglobin in modified atmosphere-packed beef (Gatellier *et al.*, 2001; Houben *et al.*, 2002) and steaks (Liu *et al.*, 1995; Robbins *et al.*, 2003). Vitamin E effects on membrane integrity may also prevent the rapid dissipation of ion gradients within muscle reducing rates of tenderization, although previous studies have not found any effect of dietary vitamin E on beef texture (Robbins *et al.*, 2003). More recently, Rowe *et al.* (2004a and 2004b) reported a significant increase in proteolysis in steaks from α-tocopherol-fed animals, indicating that the use of antioxidants in meat could improve tenderness.

Among several factors, ageing is known to affect beef colour stability during retail display. Supplementing 500 mg α-tocopheryl acetate/animal per day for 126 days was sufficient to obtain the colour-stabilizing effect of vitamin E for beef aged 14 days (Liu *et al.*, 1995). When beef is to be aged for 56 days (e.g. export), 2000 mg of α-tocopheryl acetate/animal per day for 126 days is recommended (Liu *et al.*, 1996). In the meat distribution chain, during the process of shipping from packing plants to retail display, there is a time

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gap when the product is being submitted to ageing, a well-known process in which meat tenderness has improved. Therefore, a combination of beef ageing and feeding vitamin E could provide further benefits beyond maintaining colour and lipid stability. Thus, this study aimed to evaluate meat quality parameters of beef with different tissue α-tocopherol levels over extended ageing times.

Material and methods

Animals and sampling

A total of 56 feedlot steers were housed in eight feedlot pens (four diets; two pens/dietary treatment; seven animals/pen, 14 animals/dietary treatment) at the Agriculture and Agri-Food Canada Lacombe Research Centre. All finisher diets contained 73% steam rolled barley and 22% barley silage (Table 1; as fed basis). Feed samples were collected weekly, pooled monthly and analysed for dry matter, crude protein, acid detergent fibre (ADF) and neutral detergent fibre (NDF) as described previously by Aldai *et al.* (2010) ($n = 4$). Fatty acid methyl esters from the finishing diets were prepared as described by Dugan *et al.* (2007). Results are shown in Table 1. The control diet provided 340 IU DL-α-tocopheryl acetate/animal perday. Increasing vitamin E levels (0, 350, 700 and 1400 IU DL-α-tocopheryl acetate/animal per day) were top-dressed and mixed into the feed with a pitchfork to deliver four different treatments over the finishing period. Cattle were fed once daily to appetite in order to ensure that all feed provided each day was consumed. The dry matter intake was 10.3 ± 0.5 kg/day. Animals in each pen were group fed and were capable of feeding at the feed bunk at the same time. One animal from the 700 IU group died during the experiment ($n = 13$), reducing the total number to 55 feedlot steers.

Animals were fed experimental diets for an average of 120 days, and all dietary treatments and experimental procedures were approved by the Lacombe Research Centre Animal Care Committee and animals were cared for in accordance with guidelines established by the Canadian Council on Animal Care. Animals were slaughtered over three slaughter dates within a 1-month period (three animals/dietary treatment per slaughter day for the first and third kill, with six animals/dietary treatment for the second kill) at target ultrasound backfat measurements of 6 to 8 mm. At slaughter, live weights were recorded and animals were stunned and exsanguinated. Cooler shrink loss, full Canadian grade data, colour, final (24 h) pH and temperature were recorded as described by Aldai *et al.* (2010). The left *longissimus thoracis* (LT) and right and left *longissimus lumborum* (LL) were removed from carcasses for this study. From the LT, one steak (25 mm) was removed from the posterior end, the lean separated, and this was comminuted (Robot Coupe Blixir BX3; Robot Coupe USA Inc., Ridgeland, MS, USA) and frozen at −80°C for subsequent α-tocopherol determinations. The remaining LT was trimmed of all subcutaneous fat.

A second steak (25 mm) was removed for cooking loss and shear force determinations (2 days of ageing) and the remaining LT vacuum-packaged (Ultravac Model UV2100; Koch Instruments, Kansas City, MO, USA) and placed into a cooler at 2°C with a wind speed of 0.5 m/s for 6 days for subsequent meat quality and retail display analyses. Following the 6-day ageing period, seven steaks (25 mm) were removed from the cut surface of the LT. These steaks were used for 6, 14 and 21 days shear force, cooking loss determinations and retail study. Steaks for retail study were preweighed onto a polystyrene tray with a dri-loc pad (UZ Soaker Ultra Zap Pads, Paper Pak Industries Washington, GA USA), overwrapped with oxygen permeable film (8000 ml/m^2 per 24 h vitafilm choice wrap; Goodyear Canada Inc., Toronto, ON, Canada) and put into a retail display case at 1°C for evaluation after 0, 2 and 4 days of retail ageing. Following the 4 days objective color measurements, the steaks were removed and final weights were recorded to determine drip loss.

From the left LL, ∼300 g of subcutaneous fat and 900 g of lean were vacuum-packaged for each animal and placed in a cooler for 6 days of ageing, as described previously, for subsequent preparation of ground beef patties. The entire vacuum-packaged right LL was aged for 21 days and after

Table 1 *Composition of the basal total mixed ration*

Diet ingredients (% as fed basis)	
Steam-rolled barley	72.8
Barley silage	22.0
Protein supplement[a]	4.36
Canola oil	0.39
Molasses	0.39
Mold inhibitor	0.08
Vitamin E (IU/head per day)	
dl-α-Tocopheryl acetate	340
Nutrient composition (DM basis)	
DM (%)	73.5 ± 1.5
CP (%)	13.7 ± 0.3
ADF (%)	15.7 ± 1.1
NDF (%)	26.6 ± 0.2
TDN (%)[b]	75.0 ± 0.1
ME (MJ/kg)[c]	11.3 ± 0.1
NEgain (MJ/kg)[c]	4.8 ± 0.1
NEmaintenance (MJ/kg)[c]	7.4 ± 0.1
Fatty acid composition (mg/g, DM basis)	
16:0	3.76 ± 0.17
18:0	0.29 ± 0.15
9c-18:1	3.06 ± 0.26
11c-18:1	0.20 ± 0.18
18:2n-6	10.0 ± 0.49
18:3n-3	1.16 ± 0.03
Total fatty acids	19.0 ± 0.93

DM = dry matter; TDN = total digestible nutrients; ME = metabolizable energy; NE = net energy.
[a]Supplement contained 93% DM, 23% CP, 2.7% crude fat, 4.0% crude fibre, 8.7% calcium, 2.2% phosphorus, 1.3% sodium, 0.7% potassium, 0.3% sulphur, 0.2% magnesium, 2800 mg/kg zinc, 2200 mg/kg manganese, 963 mg/kg iron, 907 mg/kg copper, 48.5 mg/kg iodine, 17 mg/kg cobalt, 14.5 mg/kg selenium, 241 000 IU/ kg vitamin A, 45 000 IU/kg vitamin D.
[b]For finishing diets, TDN (%) = 92.2 – 1.12 × ADF (%).
[c]ME and NE values were calculated according to National Research Council 1996. Nutrient requirements of beef cattle, 7th edition. National Academy Press, Washington, DC, USA.

removing from the cooler, one steak from each animal was cut, packaged and put into the retail display case as described previously for the 6-day aged steak.

Ground beef patty preparation
At 6 days, the lean and subcutaneous fat from the left LL were removed from the cooler. The weighed lean and fat were ground through a Butcher Boy meat grinder Model TCA22 (Lasar Manufacturing Co., Los Angeles, CA, USA), initially with a 6-mm grind plate followed by a second grind using a 4-mm grind plate to achieve a 75/25 lean-to-fat grind. Fat content was verified by petroleum ether extraction on moisture-free grind (method 960.39; Association of Official Analytical Chemists (AOAC), 1995) using a Foss Soxtec System Model 2050 (Foss Analytical AB, Hoganas, Sweden). A 125-g patty (11.4 cm diameter × 1.3 cm thickness) was formed using a single hamburger press (Cabelas, Sydney, NE, USA). Five patties for colour and thiobarbituric acid-reactive substance (TBARS) analyses were put into a fan-assisted, horizontal (chest type) retail display case (Hill Refrigeration of Canada Ltd., Barrie, ON, Canada) under fluorescent room lighting (GE deluxe cool white, Mississauga, Ontario, Canada), supplemented with incandescent lighting directly above the display case (GE clear cool beam 150 W/120 V spaced 91.5 cm apart) to provide an intensity of 1076 lux at the meat surface for 12 h/day (Jeremiah and Gibson, 2001).

α-Tocopherol analysis
Muscle levels of α-tocopherol were determined using normal phase HPLC with tocopherol acetate as an internal standard as described by Katsanidis and Addis (1999) and adapted for fluorescence detection by Hewavitharana *et al.* (2004).

Proximate analysis
Moisture content was determined by placing 100 g of the meat grind into stainless steel beakers in a gravity convection-drying oven at 102°C for a 24-h period (VWR Scientific Model 1370FM; Mississauga, ON, Canada). Crude fat content was determined as described previously. Nitrogen content (method 992.15; AOAC, 1995) was determined from fat-free grind (Nitrogen/Protein Determinator CNS2000; Leco Corp., St. Joseph, MI, USA).

Shear force and cooking loss
Shear force analysis was performed on cooked steaks as described by Aldai *et al.*, (2010), using a TA-XT Plus Texture Analyzer equipped with a Warner–Bratzler shear blade at a crosshead speed of 200 mm/min and a 30-kg load cell using Texture Exponent 32 Software (Texture Technologies Corp., Hamilton, MA, USA). Cooking loss was determined through gravimetric weight difference of raw and final cooked weights of the same steaks before shear force analysis.

Retail case display study
All samples (steaks and ground beef patties) were placed into the display case to control for known temperature gradients within the case ensuring as little temperature variation among treatments as possible. On each specific retail ageing day, objective colour measurements using a Minolta CR-300 with Spectra QC-300 Software (Minolta Canada Inc., Mississauga, ON, Canada) were made at three locations across the surface of the 6- and 21-day aged steaks (Commission Internationale de l'Eclairage, 1978) and converted to hue ($H_{ab} = \arctan[b^*/a^*]$) and chroma ($C_{ab} = [a^{*2} + b^{*2}]^{0.5}$). Spectral reflectance readings were also collected at the same time in order to calculate relative contents of metmyoglobin, myoglobin and oxymyoglobin (Krzywicki, 1979). One ground beef patty was placed on a pre-labelled polystyrene tray with a dri-loc pad, overwrapped and put into retail display case for an additional 4 days. Full spectral reflectance readings along with objective colour measurements were taken on the patty at 0, 2 and 4 days of retail display. A sub-sample (~100 g) was comminuted with a Robot Coupe (Jackson, Mississipi, USA) and the concentration of TBARS (0 day in retail) was determined as described by Nielsen *et al.* (1997). Two additional 125-g patties were placed onto pre-labelled trays with dri-loc pads, overwrapped and put into the retail display case for subsequent analyses of TBARS at 2 and 4 days in retail.

Statistical analyses
Statistical analyses were conducted using the MIXED procedure of Statistical Analysis Systems Institute (SAS Institute, 2009). The model included as a fixed effect of the original levels of dietary vitamin E (0, 350, 700 and 1400 IU vitamin E/kg) for carcass data and vitamin E groups, based on α-tocopherol tissue levels (<3 μg/g or low; 3 to 4 μg/g or low-medium; 4 to 5 μg/g or medium-high and >5 μg/g or high) for beef quality traits. When necessary, days of ageing and their interaction as fixed effects for meat quality traits were included in the model. Individual animal nested within group was included as a random effect. Group means were calculated as least square means and separated using the F-test protected LSD procedure ($P \leq 0.05$).

Results and discussion

Animal growth and carcass characteristics
Dietary vitamin E levels did not affect ($P > 0.05$) growth performance, carcass weight and cooler loss, but hot dressing was affected ($P = 0.027$) by the dietary vitamin E supplementation (Table 2). In relation to grading data, grade fat and fat class were lower in the intermediate dietary vitamin E levels ($P = 0.035$ and 0.013, respectively). In a review about the effects of vitamin E on performance of feedlot cattle, Secrist *et al.* (1997) found that indicators of fat deposition (fat thickness, marbling score and yield grade) tended to increase with vitamin E supplementation, but that was not observed in this study. Generally, vitamin E supplementation (vitamin E levels 224 to 1200 IU/animal per day) has not been found previously to affect carcass characteristics or quality and yield grades of beef cattle (Realini *et al.*, 2004; Montgomery *et al.*, 2005; Lee *et al.*, 2008). Effects of vitamin E supplementation depend on its level, previous

Table 2 *Effect of dietary vitamin E supplementation (IU dl-α-tocopheryl acetate/animal per day) on animal growth performance and carcass characteristics*

	Dietary vitamin E supplementation					
Characteristics	0 ($n = 14$)	350 ($n = 14$)	700 ($n = 13$)	1400 ($n = 14$)	s.e.	*P*-value
Growth performance						
Start weight (kg)	431	431	429	430	14.51	0.999
Average daily gain (kg/day)	1.83	1.63	1.74	1.87	0.109	0.174
Carcass data						
Live weight (kg)	633	600	604	613	15.67	0.366
Cooler loss (%)	1.37	1.32	1.30	1.37	0.040	0.246
Commercial weight (kg)	370	358	358	361	9.170	0.722
Hot dressing (%)	58.4[b]	59.6[a]	59.4[a]	58.8[ab]	0.500	0.027
Canadian grade data						
Grade fat	12.7[a]	10.5[b]	10.6[b]	11.6[ab]	1.623	0.035
Muscle score	2.60	2.60	2.63	2.45	0.313	0.968
Fat class	6.10[a]	4.95[b]	4.97[b]	5.52[ab]	0.823	0.013
Cutability estimate	56.3	58.0	57.8	56.9	1.463	0.140
Ribe-eye area (cm^2)	84.6	86.1	85.9	84.2	2.727	0.883
Marbling[c]	523	506	496	526	19.40	0.528

[a,b]Means within the same row with different superscripts are significantly different at $P < 0.05$.
[c]US Department of Agriculture's marbling standards: 300 = traces; 400 = slight; 500 = small; 600 = modest; 700 = moderate; 800 = slightly abundant; 900 = moderately abundant; 1000 = abundant; and 1100 = very abundant.

nutritional history, vitamin E status of the cattle at the start, stress level of animals and vitamin E content of the basal diet, as well as genetics, which is related to the ability of individual animals to tolerate handling and feedlot conditions (Secrist *et al*., 1997). Liu *et al*. (1995) recommend a supplementation strategy of 500 IU of supplemental vitamin E/animal per day for 126 days. Feeding at least 1300 IU/day for 44 days and 1200 IU/day for 38 days elevated the α-tocopherol concentration from 1.4 to 3.5 μg/g and 2.2 to 3.5 μg/g, respectively (Arnold *et al*., 1992 and 1993).

Vitamin E grouping approach

In studies concerned with the effects of beef cattle vitamin E supplementation on meat quality, determining the concentration of α-tocopherol in tissue is of great importance (Faustman *et al*., 1998). In this study, animals were grouped according to their actual tissue α-tocopherol levels, namely low ($n = 10$; <3 μg/g meat), low-medium ($n = 20$; 3 to 4 μg/g meat), high-medium ($n = 11$; 4 to 5 μg/g meat) and high ($n = 14$; >5 μg/g meat). Observing the tissue α-tocopherol levels from the dietary vitamin E treatments (Figure 1a), a clear overlap was evident due to the inter-animal variability. Therefore, grouping according to actual tissue levels of α-tocopherol would lead to more accurate results when studying effects on beef quality. Grouping the animals using this approach, the variability was reduced and significantly different values were found among groups ($P < 0.001$) (Figure 1b). All data obtained for meat quality discussed in this study used this approach.

Meat quality

Prevention of lipid oxidation by α-tocopherol results from quenching free radicals, which protects highly unsaturated fatty acids mainly found in membrane phospholipids (Faustman *et al*., 1989; Burton and Traber, 1990). In this study, there was an interaction ($P < 0.001$) for TBARS between time of ageing and tissue α-tocopherol levels in ground beef (Figure 2). TBARS from animals with high-medium and high levels of α-tocopherol did not significantly increase between days 0 and 4 of ageing, whereas the low group, followed by the low-medium group, showed higher TBARS values over time. Lower TBARS from vitamin E-supplemented beef have been reported by several authors (Eikelenboom *et al*., 2000; Gatellier *et al*., 2001; Lee *et al*., 2008) with variable effects on meat quality characteristics. A minimum of 3 to 3.5 μg of α-tocopherol/g meat has been reported to be necessary to prevent discolouration and lipid oxidation (Faustman *et al*., 1989; Arnold *et al*.; 1993, Liu *et al*., 1995). Low and low-medium vitamin E groups had α-tocopherol values <4 μg/g meat, which explains the increasing TBARS values over time. Clausen *et al*. (2009) reported that raw meat with approximately <2 mg α-tocopherol/g meat showed a very high degree of lipid oxidation measured by TBARS.

Lipid peroxidation is also known to cause damage to membrane proteins leading to a loss of muscle cell membrane integrity that can affect its ability to function as a semi-permeable barrier and may contribute to exudative losses from meat (Gray *et al*., 1996). α-Tocopherol can preserve the integrity of muscle cell membranes, reducing muscle cell disruption and this could inhibit the passage of sarcoplasmic fluid through the muscle cell membrane (Asghar *et al*., 1991). In this study, tissue levels of α-tocopherol had no effect on drip loss ($P = 0.838$) and cooking loss ($P = 0.528$; Table 3), similar to results found by den Hertog-Meischke *et al*. (1997) and Eikelenboom *et al*. (2000).

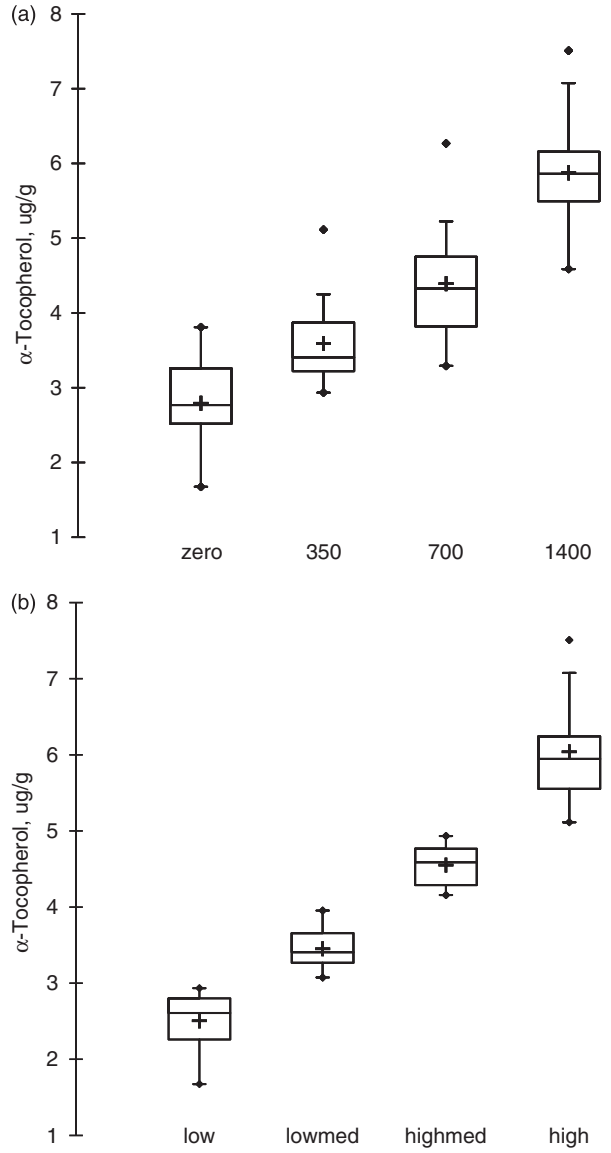

Figure 1 Intramuscular α-tocopherol levels (a) from dietary treatments (b) grouped by tissue levels. Box plot shows the smallest observation (sample minimum), lower quartile, median (+), upper quartile and the largest observation (sample maximum). Outliers (*) are also indicated when present.

A reduction in drip loss, along with an increase in cooking loss in beef steaks was reported by Mitsumoto *et al.* (1995), but the α-tocopherol concentration reported by these authors (6.7 μg/g meat) was higher than other studies. Initial colour and pH were also not affected by tissue α-tocopherol levels ($P > 0.05$; Table 3), as reported in similar studies (Arnold *et al.*, 1992; Eikelenboom *et al.*, 2000). As expected, fat content increased with α-tocopherol tissue levels ($P = 0.010$; Table 3), as α-tocopherol is a fat-soluble vitamin. The higher content of fat associated with higher α-tocopherol could have an effect on other meat quality traits, such as tenderness. In addition, the use of antioxidants such as vitamin E could enhance tenderness in beef by preventing the inactivation of the calpain system (Huff Lonergan *et al.*, 2010). However, although shear force from all groups decreased with

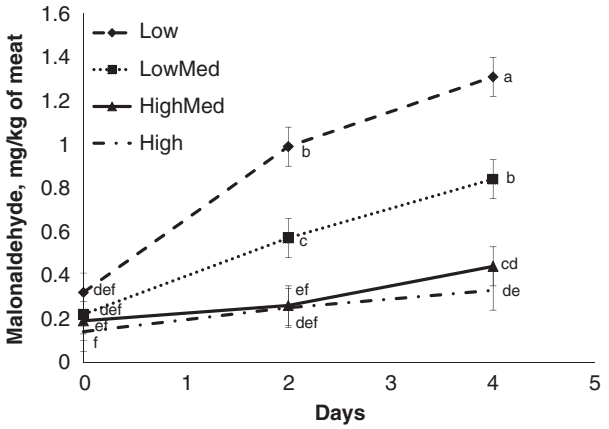

Figure 2 Thiobarbituric acid-reactive substances in ground beef (6 days of ageing) with different α-tocopherol tissue levels over 4 days of retail display ([a,b,c,d,e,f]means with different letters are significantly difference at $P < 0.05$; low: <3 μg/g meat; low-medium: 3 to 4 μg/g meat; high-medium: 4 to 5 μg/g meat; high: >5 μg/g meat).

ageing ($P < 0.001$), no effect ($P > 0.05$) of tissue α-tocopherol level was found on shear force (Figure 3).

Ageing and retail display

Vitamin E delays not only lipid oxidation in beef but also oxidation of ferrous to ferric iron in myoglobin located in sarcoplasm (Faustman and Cassens, 1990; Arnold *et al.*, 1992 and 1993). Various hypotheses have been tested to understand the mechanism of action of antioxidants and their relation to meat discolouration. Faustman *et al.* (1989), based on earlier results from Greene (1969), suggested that antioxidants inhibit oxidation by protecting the pigment from reaction with oxidation intermediates such as free radicals. Vitamin E could inhibit lipid oxidation and consequently a reaction with secondary oxidation products such as α-, β-unsaturated aldehydes (Faustman *et al.*, 1999). However, a more recent study, by O'Grady *et al.* (2001), which has been supported by Monahan *et al.* (2005), indicated that in an oxidizing muscle system the depletion of dissolved oxygen as a result of lipid oxidation is the primary cause of oxymyoglobin oxidation. The mechanistic basis of myoglobin and lipid oxidation, as well as the relation between these two processes has been extensively reviewed by Faustman *et al.* (2010). In the present study, in steaks aged 6 ($P = 0.004$) or 21 days ($P < 0.001$), and ground beef ($P < 0.001$), L^* decreased over time (Table 4). After a short ageing time (6 days), no effect ($P > 0.05$) on retail evaluation was found among samples with different tissue levels of α-tocopherol. However, there was an increase in metmyoglobin ($P < 0.001$) over time in retail display and decreases ($P < 0.001$) in the content of myoglobin and oxymyoglobin. In contrast, with meat that had been aged for 21 days, increased tissue α-tocopherol levels protected against formation of metmyoglobin over time in retail display ($P = 0.008$). The increase in metmyoglobin was smaller during retail display as α-tocopherol tissue levels increased. Oxymyoglobin levels on the last day of retail display were also higher ($P = 0.033$) in meat with a higher content

Table 3 *Effect of different tissue levels of α-tocopherol (μg/g meat) on meat quality characteristics*

	α-Tocopherol tissue level[1]					
Characteristics	Low ($n = 10$)	Low-medium ($n = 20$)	High-medium ($n = 11$)	High ($n = 14$)	s.e.	*P*-value
Initial colour (24 h)						
*L**	36.3	36.2	35.4	36.2	0.598	0.718
Chroma	24.2	23.6	23.7	23.3	0.518	0.688
Hue	24.1	23.8	23.7	23.7	0.303	0.823
pH (24 h)						
pH	5.66	5.63	5.62	5.67	0.030	0.464
Temperature	2.87	2.40	2.43	2.18	0.370	0.657
Proximate analysis (%)						
Moisture	72.8[a]	71.9[a]	72.0[ab]	71.1[b]	0.333	0.010
Fat	5.38[b]	6.38[b]	6.27[b]	7.52[a]	0.413	0.010
Protein	21.0	20.9	21.0	20.7	0.125	0.187
Cooking loss (mg/g)[2]	192	185	181	179	6.030	0.528
Water holding capacity (mg/g)						
Drip loss[3]	42.9	41.0	42.9	42.5	1.875	0.838

[a,b]Means within the same row with different superscripts are significantly different at $P < 0.05$.
[1]Low: <3 μg/g meat; low-medium: 3 to 4 μg/g meat; high-medium: 4 to 5 μg/g meat; high: >5 μg/g meat.
[2]Average values from 1, 6, 14 and 21 days of ageing.
[3]From days 6 to 10.

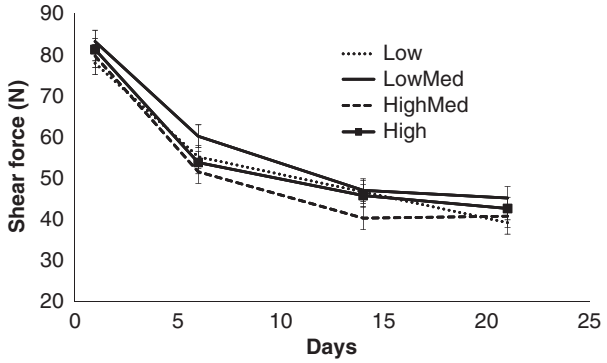

Figure 3 Shear force values of steaks with different α-tocopherol levels aged for 21 days (low: <3 μg/g meat; low-medium: 3 to 4 μg/g meat; high-medium: 4 to 5 μg/g meat; high: >5 μg/g meat).

of α-tocopherol. Consequently, chroma values of steaks with the high-medium and high levels of α-tocopherol were not affected by time in retail display, in contrast to observations for steaks with lower α-tocopherol levels ($P = 0.016$). A similar effect was found for the ground beef patties. An interaction between tissue α-tocopherol levels and days of retail display was observed for both oxymyoglobin ($P = 0.001$) and metmyoglobin ($P < 0.001$) in 21-day aged steaks; in low and low-medium α-tocopherol groups, oxymyoglobin decreased while metmyoglobin increased, whereas values for high-medium and high α-tocopherol groups did not change. In addition, the increase in hue was smaller in samples with higher tissue α-tocopherol levels ($P < 0.001$). In this study, chroma decreased over time, meaning less-intense colour in treatments with a lower level of tissue α-tocopherol. This protection with high α-tocopherol levels ($P = 0.016$) in 21-day aged beef is practically significant, as the time before boxed beef as primal cuts and manufactured/ground beef are displayed in commercial retail cases in Canada ranges from 5 to 80 days and 2 to 56 days, respectively (Gill *et al.*, 2002). Higher initial tissue α-tocopherol levels are more protective during long periods of ageing as its concentration decreases over time (Clausen *et al.*, 2009). Therefore, high tissue levels of vitamin E could lead to an improvement in colour and retail appearance of both lean and ground beef following long storage periods during distribution, which are commonly found in industry and would be particularly valuable in assuring colour stability in transoceanic shipment of chilled meat.

Dietary vitamin E recommendations

In this study, animals were fed a basal diet providing 340 IU supplemental α-tocopherol, and when >700 additional IU were supplemented per day for 120 days, this provided oxidative stability in aged samples. The level of α-tocopherol that must be supplemented in the diet to obtain oxidative stability is, however, dependant on the level naturally occurring in the diet. Given that we supplemented several levels of α-tocopherol to the diet, we were able to regress tissue α-tocopherol *v.* supplementation level. From the regression ($y = 0.0023x + 1.7695$, $R^2 = 0.78$, $P < 0.05$), where y = tissue level of α-tocopherol in μg/g and x = IU α-tocopherol added to the diet, when we set the tissue level to 0 μg/g this provided an estimate of the α-tocopherol available in the unsupplemented diet (770 IU). Consequently, the amount of α-tocopherol required for oxidative stability of aged meat in this study was 1810 IU/head per day (770 IU naturally in the diet + 340 IU in basal diet + 700 IU top dressed at feeding/head per day). Achieving this level in practice will, therefore, require knowledge of the amount and availability of vitamin E for all dietary ingredients.

Table 4 *Effect of different tissue levels of α-tocopherol (μg/g meat) on retail evaluation of aged beef and ground beef*

Toc	Low			Low-medium			High-medium			High				*P*-value		
Days of retail ageing[1]	0	2	4	0	2	4	0	2	4	0	2	4	s.e.	Toc	Day	Toc × day
Steaks (6)																
*L**	39.6	41.1	39.5	39.8	40.5	39.3	39.0	39.6	39.0	40.8	41.1	40.2	0.74	0.393	0.004	0.906
Chroma	21.2	24.1	23.8	22.0	24.4	24.6	22.0	25.2	24.9	21.3	24.4	25.0	0.58	0.469	<0.001	0.850
Hue	34.1	34.9	34.4	34.6	34.9	35.0	33.9	34.0	33.1	34.6	35.4	35.4	0.69	0.374	0.143	0.711
Metmyoglobin (%)	0.16	0.20	0.21	0.16	0.20	0.21	0.16	0.20	0.21	0.17	0.20	0.20	0.01	0.992	<0.001	0.156
Myoglobin (%)	0.09	0.07	0.08	0.08	0.06	0.07	0.08	0.06	0.07	0.08	0.06	0.07	0.01	0.282	<0.001	0.681
Oxymyoglobin (%)	0.75	0.73	0.71	0.76	0.74	0.72	0.76	0.74	0.73	0.75	0.74	0.73	0.01	0.772	<0.001	0.432
Steaks (21)																
*L**	39.4	39.3	36.6	39.7	39.0	37.6	39.3	39.3	38.5	39.1	38.7	37.0	0.78	0.819	<0.001	0.376
Chroma	26.9^{a}	26.2^{ab}	24.0^{c}	26.8^{a}	26.1^{a}	25.1^{bc}	26.3^{ab}	26.6^{a}	26.7^{a}	27.2^{a}	27.0^{a}	26.7^{a}	0.59	0.149	<0.001	0.016
Hue	35.3	36.4	37.9	34.8	35.5	36.8	34.5	35.3	35.8	34.8	35.2	35.8	0.55	0.181	<0.001	0.214
Metmyoglobin (%)	0.16^{c}	0.21^{b}	0.24^{a}	0.17^{c}	0.22^{b}	0.24^{a}	0.17^{c}	0.20^{b}	0.21^{b}	0.17^{c}	0.21^{b}	0.22^{ab}	0.01	0.442	<0.001	0.008
Myoglobin (%)	0.06	0.07	0.09	0.09	0.06	0.08	0.04	0.05	0.06	0.04	0.06	0.07	0.02	0.057	0.253	0.492
Oxymyoglobin (%)	0.78	0.72	0.67	0.73	0.72	0.69	0.79	0.75	0.73	0.78	0.73	0.72	0.02	0.033	<0.001	0.344
Ground beef (6)																
*L**	51.2	50.6	50.6	52.0	50.8	50.1	52.2	50.2	50.5	51.5	50.1	49.8	0.80	0.948	0.001	0.781
Chroma	25.1	21.6	20.3	24.4	21.8	21.2	25.5	22.8	22.2	24.7	21.9	21.7	0.70	0.112	<0.001	0.470
Hue	41.7^{f}	47.6^{cde}	53.5^{a}	41.3^{f}	48.2^{cd}	51.0^{b}	41.5^{f}	46.7^{de}	50.2^{bc}	42.1^{f}	45.5^{e}	48.0^{cd}	1.04	0.073	<0.001	<0.001
Metmyoglobin (%)	0.24^{f}	0.33^{cd}	0.42^{a}	0.24^{f}	0.32^{de}	0.38^{b}	0.23^{f}	0.31^{e}	0.36^{bc}	0.25^{f}	0.31^{e}	0.35^{cd}	0.02	0.032	<0.001	<0.001
Myoglobin (%)	0.02	0.05	0.05	0.02	0.04	0.04	0.01	0.04	0.04	0.01	0.04	0.04	0.00	0.127	<0.001	0.821
Oxymyoglobin (%)	0.74^{a}	0.62^{cd}	0.53^{f}	0.74^{a}	0.63^{bc}	0.57^{e}	0.76^{a}	0.65^{b}	0.59^{de}	0.74^{a}	0.65^{b}	0.61^{cd}	0.02	0.025	<0.001	0.001

Toc = α-tocopherol tissue level.
[a,b,c,d,e,f]Means within the same row with different superscripts are significantly different at $P < 0.05$.
[1]Low: <3 μg/g meat; low-medium: 3 to 4 μg/g meat; high-medium: 4 to 5 μg/g meat; high: >5 μg/g meat.

Conclusions

Increased tissue levels of α-tocopherol protected against retail discolouration and lipid oxidation in steaks after ageing 21 days, but had no influence in steaks after ageing only 6 days. A similar protective effect was observed in ground beef (25% fat) when prepared using lean and fat tissues aged 6 days. Shear force was not affected by tissue α-tocopherol levels. To obtain oxidative stability, this required >1040 IU supplementary vitamin E added to 770 IU estimated to be naturally available in the diet. Shear force was not affected by tissue α-tocopherol levels. Further investigations are warranted to determine whether the protective effects of vitamin E against colour deterioration after long ageing periods also protects against lipid oxidation in steaks with subsequent potential positive effects on palatability.

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References

Aldai N, Aalhus JL, Dugan MER, Robertson WM, McAllister TA, Walter LJ and McKinnon JJ 2010. Comparison of wheat- versus corn-based dried distillers' grains with solubles on meat quality of feedlot cattle. Meat Science 84, 569–577.

Arnold RN, Arp SC, Scheller KK, Williams SN and Schaefer DM 1993. Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. Journal of Animal Science 71, 105–118.

Arnold RN, Scheller KK, Arp SC, Williams SN, Buege DR and Schaefer DM 1992. Effect of long- or short-term feeding of alpha-tocopheryl acetate to Holstein and crossbred beef steers on performance, carcass characteristics, and beef color stability. Journal of Animal Science 70, 3055–3065.

Association of Official Analytical Chemists (AOAC) 1995. Official methods of analysis. AOAC, Arlington, VA, USA.

Asghar A, Gray JI, Booren AM, Gomaa EA, Abouzied MM, Miller ER and Buckley DJ 1991. Effects of supranutritional dietary vitamin E levels on subcellular deposition of α-tocopherol in the muscle and on pork quality. Journal of the Science of Food and Agriculture 57, 31–41.

Burton GW and Traber MG 1990. Vitamin E: Antioxidant activity, biokinetics, and bioavailability. Annual Review of Nutrition 10, 357–382.

Clausen I, Jakobsen M, Ertbjerg P and Madsen NT 2009. Modified atmosphere packaging affects lipid oxidation, myofibrillar fragmentation index and eating quality of beef. Packaging Technology and Science 22, 85–96.

Commission Internationale de l'Eclairage 1978. International Commission on Illumination, Colorimetry: Official Recommendations of the International Commission on Illumination. Publication CIE no. 15 (E-1.3.1). Bareau Central de la CIE, Paris, France.

den Hertog-Meischke MJA, Smulders FJM, Houben JH and Eikelenboom G 1997. The effect of dietary vitamin E supplementation on drip loss of bovine longissimus lumborum, psoas major and semitendinosus muscles. Meat Science 45, 153–160.

Dugan ME, Kramer JKG, Robertson WM, Meadus WJ, Aldai N and Rolland DC 2007. Comparing subcutaneous adipose tissue in beef and muskox with emphasis on trans 18:1 and conjugated linoleic acids. Lipids 42, 509–518.

Eikelenboom G, Hoving-Bolink AH, Kluitman I, Houben JH and Klont RE 2000. Effect of dietary vitamin E supplementation on beef colour stability. Meat Science 54, 17–22.

Faustman C and Cassens RG 1990. The biochemical basis for discoloration in fresh meat: a review. Journal of Muscle Foods 1, 217–243.

Faustman C, Chan WKM, Schaefer DM and Havens A 1998. Beef color update: the role for vitamin E. Journal of Animal Science 76, 1019–1026.

Faustman C, Liebler DC, McClure TD and Sun Q 1999. α,β-Unsaturated aldehydes accelerate oxymyoglobin oxidation. Journal of Agricultural and Food Chemistry 47, 3140–3144.

Faustman C, Sun Q, Mancini R and Suman SP 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Science 86, 86–94.

Faustman C, Cassens R, Schaefer D, Buege D, Williams S and Scheller K 1989. Improvement of pigment and lipid stability in holstein steer beef by dietary supplementation with vitamin E. Journal of Food Science 54, 858–862.

Gatellier P, Hamelin C, Durand Y and Renerre M 2001. Effect of a dietary vitamin E supplementation on colour stability and lipid oxidation of air- and modified atmosphere-packaged beef. Meat Science 59, 133–140.

Gill CO, Jones T, Rahn K, Campbell S, LeBlanc DI, Holley RA and Stark R 2002. Temperatures and ages of boxed beef packed and distributed in Canada. Meat Science 60, 401–410.

Gray JI, Gomaa EA and Buckley DJ 1996. Oxidative quality and shelf life of meats. Meat Science 43, 111–123.

Greene BE 1969. Lipid oxidation and pigment changes in raw beef. Journal of Food Science 34, 110–113.

Hewavitharana AK, Lanari MC and Becu C 2004. Simultaneous determination of vitamin E homologs in chicken meat by liquid chromatography with fluorescence detection. Journal of Chromatography A 1025, 313–317.

Houben JH, Van Dijk A and Eikelenboom G 2002. Dietary vitamin E supplementation, an ascorbic acid preparation, and packaging effects on colour stability and lipid oxidation in mince made from previously frozen lean beef. European Food Research and Technology 214, 186–191.

Huff Lonergan E, Zhang W and Lonergan SM 2010. Biochemistry of postmortem muscle – lessons on mechanisms of meat tenderization. Meat Science 86, 184–195.

Jeremiah LE and Gibson LL 2001. The influence of packaging and storage time on the retail properties and case-life of retail-ready beef. Food Research International 34, 621–631.

Katsanidis E and Addis PB 1999. Novel HPLC analysis of tocopherols, tocotrienols, and cholesterol in tissue. Free Radical Biology and Medicine 27, 1137–1140.

Krzywicki K 1979. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. Meat Science 3, 1–10.

Lee SK, Panjono SMK, Kim TS and Park YS 2008. The effects of dietary sulfur and vitamin E supplementation on the quality of beef from the *longissimus* muscle of Hanwoo bulls. Asian–Australasian Journal of Animal Sciences 21, 1059–1066.

Liu Q, Lanari MC and Schaefer DM 1995. A review of dietary vitamin E supplementation for improvement of beef quality. Journal of Animal Science 73, 3131–3140.

Liu Q, Scheller KK, Arp SC, Schaefer DM and Frigg M 1996. Color coordinates for assessment of dietary vitamin E effects on beef color stability. Journal of Animal Science 74, 106–116.

Mitsumoto M, Arnold RN, Schaefer DM and Cassens RG 1995. Dietary vitamin E supplementation shifted weight loss from drip to cooking loss in fresh beef longissimus during display. Journal of Animal Science 73, 2289–2294.

Monahan FJ, Skibsted LH and Andersen ML 2005. Mechanism of oxymyoglobin oxidation in the presence of oxidizing lipids in bovine muscle. Journal of Agricultural and Food Chemistry 53, 5734–5738.

Montgomery SP, Drouillard JS, Sindt JJ, Greenquist MA, Depenbusch BE, Good EJ, Loe ER, Sulpizio MJ, Kessen TJ and Ethington RT 2005. Effects of dried full-fat corn germ and vitamin E on growth performance and carcass characteristics of finishing cattle. Journal of Animal Science 83, 2440–2447.

Morrissey PA, Sheehy PJA, Galvin K, Kerry JP and Buckley DJ 1998. Lipid stability in meat and meat products. Meat Science 49, S73–S86.

Nielsen JH, Sørensen B, Skibsted LH and Bertelsen G 1997. Oxidation in precooked minced pork as influenced by chill storage of raw muscle. Meat Science 46, 191–197.

O'Grady MN, Monahan FJ and Brunton NP 2001. Oxymyoglobin oxidation and lipid oxidation in bovine muscle – mechanistic studies. Journal of Food Science 66, 386–392.

Realini CE, Duckett SK, Brito GW, Dalla Rizza M and De Mattos D 2004. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. Meat Science 66, 567–577.

Robbins K, Jensen J, Ryan KJ, Homco-Ryan C, McKeith FK and Brewer MS 2003. Dietary vitamin E supplementation effects on the color and sensory characteristics of enhanced beef steaks. Meat Science 64, 279–285.

Rowe LJ, Maddock KR, Lonergan SM and Huff-Lonergan E 2004a. Influence of early postmortem protein oxidation on beef quality. Journal of Animal Science 82, 785–793.

Rowe LJ, Maddock KR, Lonergan SM and Huff-Lonergan E 2004b. Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. Journal of Animal Science 82, 3254–3266.

SAS Institute 2009. SAS user's guide: statistics. SAS for windows. Release 9.2. SAS Institute Inc., Cary, NC, USA.

Secrist DS, Owens FN and Gill DR 1997. Effects of vitamin E on performance of feedlot cattle: a review. The Professional Animal Scientist 13, 47–54.