Dietary Versus Postmortem Supplementation of Vitamin E on Pigment and Lipid Stability in Ground Beef\textsuperscript{1,2}

Mitsuru Mitsumoto\textsuperscript{3}, R. N. Arnold, D. M. Schaefer, and R. G. Cassens

Muscle Biology and Meat Science Laboratory, Department of Meat and Animal Science, University of Wisconsin-Madison 53706-1181

ABSTRACT: Effects of dietary supplementation and postmortem addition of vitamin E on pigment and lipid stability in raw ground beef were examined in this study. Six Holstein steers were fed a control diet for 232 or 252 d and six Holstein steers were supplemented with 1,500 IU of vitamin E per animal daily for 232 or 252 d. Three aliquots of ground beef from each longissimus lumborum were allotted to the following postmortem treatments: no addition (NO), white mineral oil (OIL), and white mineral oil containing sufficient D-\(
\alpha\)-tocopherol to equal the mean difference of \(
\alpha\)-tocopherol concentration between beef from supplemented and control steers (OIL + E). Metmyoglobin percentages and 2-thiobarbituric acid reactive substances values were determined at d 1, 3, 5, 7, and 9 after postmortem treatment. Dietary vitamin E supplementation delayed metmyoglobin increase and highly suppressed lipid oxidation in ground beef during 9 d of display compared with the control. The postmortem addition of vitamin E (OIL + E) was slightly effective in retarding the oxidation of pigment and lipid, especially compared with the OIL treatments. Endogenous vitamin E improved pigment and lipid stability much better than exogenous vitamin E.

Key Words: Vitamin E, Beef, Pigment, Lipid, Stability

Introduction

Ground beef becomes brown and rancid more quickly than unground beef, because grinding exposes more surface to air and microbial contamination. When the color of ground beef changes from bright red (oxymyoglobin) to brown (metmyoglobin), consumers prefer not to purchase the meat. Therefore, pigment and lipid stability in ground beef are very important for both beef packers and consumers.

Various attempts have been made to reduce pigment and lipid oxidation in meats by endogenous and exogenous vitamin E treatments. Dietary vitamin E supplementation decreased pigment or lipid oxidation in poultry meat (Webb et al., 1972; Marusich et al., 1975; Bartov and Bornstein, 1977; Uebersax et al., 1978; Bartov et al., 1983), pork (Hvidsten and Astrup, 1963; Tsai et al., 1978; Buckley and Connolly, 1980), and beef (Faustman et al., 1989a,b; Mitsumoto et al., 1991a). Exogenous vitamin E treatment retarded pigment or lipid oxidation in pork (Miles et al., 1986; Whang et al., 1986) and ground beef (Benedict et al., 1975; Mitsumoto et al., 1991b). Brekke et al. (1975) reported that exogenous addition of vitamin E seems to be more than 400 times more efficient than endogenous supplementation as a procedure to improve quality and stability of rendered fowl fat. However, there are no reports regarding direct comparisons of endogenous and exogenous vitamin E effects on pigment and lipid stability in beef.

The purpose of this work was to investigate the effects of dietary vitamin E supplementation and vitamin E added after grinding on pigment and lipid stability in raw ground beef.

Materials and Methods

Dietary Vitamin E Supplementation

Longissimus lumborum (LL; Kauffman et al., 1990) from 12 Holstein steers were used in this study. Six steers were fed a control diet containing 80 IU of
\(\alpha\)-tocopherol and the other six steers were supplemented with 1,500 IU of vitamin E (DL-\(\alpha\)-tocopheryl acetate, Hoffmann-LaRoche, Nutley, NJ) per animal daily for 232 d (Slaughter Group 1) or 252 d (Slaughter Group 2). Steers were slaughtered in two groups separated by 3 wk with three steers in each treatment-slaughter group combination. Vitamin E (DL-\(\alpha\)-tocopheryl acetate) was dispersed on ground rice hulls as the carrier. Control steers received the same amount of carrier that contained no vitamin E.

Cattle were fed a 90% corn-based, 10% corn silage diet. The ranges of age and live weight at slaughter of steers were 13 to 14 mo of age and 493 to 557 kg.

Steers were slaughtered at Packerland Packing, Green Bay, WI, and the left strip loin from each steer was removed at 24 h postmortem. These subprimal cuts were then vacuum-packaged and transported to the University of Wisconsin-Madison meat laboratory and stored for an additional 6 d at 4°C.

**Postmortem Addition of Vitamin E**

Each LL muscle at 7 d after slaughter was ground three times through a 0.45-cm plate of a laboratory meat grinder at 4°C after all external fat and connective tissue were removed.

The three postmortem vitamin E treatments consisted of no addition (NO), hand-mix treatment with 1.0% white mineral oil (OIL), and hand-mix treatment with 1.0% white mineral oil containing sufficient D-\(\alpha\)-tocopherol (Sigma Chemical, St. Louis, MO) to equal the mean difference of \(\alpha\)-tocopherol concentration between supplemented LL and control LL in each slaughter group (OIL + E). The \(\alpha\)-tocopherol concentrations were measured in duplicate LL samples at 2 d after slaughter by the methods of Cort et al. (1983) and Burton et al. (1985) as modified by Arnold et al. (1993b). The \(\alpha\)-tocopherol concentrations for three treatments (NO, OIL, and OIL + E) were measured later in samples that had been stored at -20°C.

Samples of 20 g of ground meat were then shaped into miniature beef patties using the bottom half of a tissue culture dish (15 mm x 60 mm). Molding samples in this way allowed for a consistent surface area-volume ratio between samples. Each sample was placed into a 100-mL disposable weigh boat, overwrapped with polyvinylchloride film (O\(_2\) transmission was 1,000 to 1,050 mL/645 cm\(^2\) per 24 h at 23°C, Filmco Industries, Aurora, OH) and displayed under cool white fluorescent lights (2,475 lx) at 4°C for 9 d.

**Metmyoglobin Analysis**

Surface metmyoglobin percentages were determined at d 1, 3, 5, 7, and 9 by reflectance spectrophotometry (Stewart et al., 1965) using a Shimadzu UV-265 FW spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). Reflectance scanning was performed twice on different locations of a sample to obtain an average value.

**Lipid Oxidation Analysis**

2-Thiobarbituric acid reactive substances (TBARS) were measured in samples displayed for 1, 3, 5, 7, and 9 d by the method of Witte et al. (1970). The TBARS values were expressed as milligrams of malonaldehyde (MDA) per kilogram of muscle. Trichloroacetic acid solution (20% wt/vol) was used for the extraction. A Beckman DU-65 spectrophotometer (Beckman Instruments, Fullerton, CA) was used for spectrophotometric analysis. The standard calibration for malonaldehyde concentration was performed at each measurement day to reduce errors due to differences in temperature and time.

**Statistical Analyses**

A different replicate sample was used on each day for the preceding measurements. The total number of samples was 180 (two slaughter groups x two vitamin E supplemented groups x three animals x three postmortem vitamin E treatments x five measurement days) for each analysis. Data were analyzed by the GLM procedure of SAS (1985) as a split-split-plot design to account for the repeated measures aspect. Animal was designated as the main plot, muscle samples within animal as the sub-plot, and muscle samples within animal x day as the sub-sub-plot. Pairwise comparisons of means were analyzed by Scheffe's test (Snecor and Cochran, 1980).

**Results and Discussion**

Table 1 indicates \(\alpha\)-tocopherol concentrations in control and vitamin E supplemented beef and in postmortem vitamin E treatments of two slaughter groups. These results revealed that actual added \(\alpha\)-tocopherol concentration was adequate in Slaughter Group 1 (5.57 vs 6.23) but slightly lower than the target value in Slaughter Group 2 (4.30 vs 6.73). However, there was no significant interaction between slaughter group and postmortem vitamin E treatment. Least squares means for metmyoglobin percentage and TBARS values are shown in Figures 1 and 2. Relationships of three-way interactions (dietary vitamin E supplementation x postmortem vitamin E treatment x day) for these measurements are presented in Figures 3 and 4.

**Endogenous Vitamin E**

Dietary vitamin E supplementation to cattle greatly improved pigment and lipid stability in ground beef compared with the control. The average \(\alpha\)-tocopherol concentration was increased (\(P < .01\) by vitamin E supplementation in both slaughter groups (control, 1.3 mg/kg; supplemented, 6.1 mg/kg in Group 1, and control, 1.7 mg/kg; supplemented, 6.8 mg/kg in Group 2; Table 1). Vitamin E-supplemented steers had much
lower \( (P < .001) \) metmyoglobin percentages (Figure 1) and TBARS values (Figure 2) than the control steers. Dietary vitamin E supplementation showed smaller initial and final percentages of metmyoglobin from 6.8 to 40.4%; Figure 3) and much smaller values of TBARS (from .15 to .58; Figure 4) during 9 d of display than the control (metmyoglobin percentages, from 2.02 to 6.91 in Figure 3; TBARS values, from 2.02 to 6.91 in Figure 4). Greene et al. (1971) reported that consumers would reject beef containing >30% metmyoglobin. In this study 30% metmyoglobin was probably exceeded by d 2 in the control and by d 8 in vitamin E-supplemented samples

Figure 1. Least squares means for metmyoglobin percentage. a,b,c,d,e\Within main effects, means with no common letters differ significantly \( (P < .05) \). CNTRL = no vitamin E supplementation; SUPPL = vitamin E supplementation; NO = no addition; OIL = treatment with oil; OIL + E = treatment with oil and vitamin E.

Figure 2. Least squares means for 2-thiobarbituric acid reactive substances (TBARS) value. a,b,c,d,e\Within main effects, means with no common letters differ significantly \( (P < .05) \). CNTRL = no vitamin E supplementation; SUPPL = vitamin E supplementation; NO = no addition; OIL = treatment with oil; OIL + E = treatment with oil and vitamin E. MDA = malonaldehyde.

Figure 3. Relationship of dietary vitamin E supplementation \( \times \) postmortem vitamin E treatment \( \times \) day on metmyoglobin percentages. CNTRL-NO = no addition to unsupplemented beef; CNTRL-OIL = treatment with oil of unsupplemented beef; CNTRL-OIL + E = treatment with oil and vitamin E of unsupplemented beef; SUPPL-NO = no addition to vitamin E-supplemented beef; SUPPL-OIL = treatment with oil of vitamin E-supplemented beef; SUPPL-OIL + E = treatment with oil and vitamin E of vitamin E-supplemented beef.

Figure 4. Relationship of dietary vitamin E supplementation \( \times \) postmortem vitamin E treatment \( \times \) day on 2-thiobarbituric acid reactive substances [TBARS] values. MDA = malonaldehyde. CNTRL-NO = no addition to unsupplemented beef; CNTRL-OIL = treatment with oil of unsupplemented beef; CNTRL-OIL + E = treatment with oil and vitamin E of unsupplemented beef; SUPPL-NO = no addition to vitamin E-supplemented beef; SUPPL-OIL = treatment with oil of vitamin E-supplemented beef; SUPPL-OIL + E = treatment with oil and vitamin E of vitamin E-supplemented beef.
Table 1. The α-tocopherol concentrations (mg/kg of meat) in longissimus lumborum (LL) from control and vitamin E-supplemented steers and in samples obtained following vitamin E addition to ground beef (values represent the means of three analyses performed in duplicate)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Vitamin E-supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slaughter Group 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOd</td>
<td>1.33a</td>
<td>6.10a</td>
</tr>
<tr>
<td>OIL</td>
<td>1.33 (1.33)</td>
<td>6.10 (6.00)</td>
</tr>
<tr>
<td>OIL + E ( + 4.77)</td>
<td>6.10 (5.57)</td>
<td>10.87 (10.60)</td>
</tr>
<tr>
<td>Slaughter Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>1.69a</td>
<td>6.80a</td>
</tr>
<tr>
<td>OIL</td>
<td>1.69 (1.17)</td>
<td>6.80 (6.67)</td>
</tr>
<tr>
<td>OIL + E ( + 5.11)</td>
<td>6.80 (4.30)</td>
<td>11.91 (10.10)</td>
</tr>
</tbody>
</table>

Notes:
- aSamples were analyzed 2 d after slaughter.
- bTarget values were calculated from measured concentrations at 2 d after slaughter plus added concentrations.
- cActual values in parentheses were measured following 2 mo of frozen storage.
- dNO: no addition, OIL: treatment with 1.0% white mineral oil, OIL + E: treatment with 1.0% white mineral oil and the mean difference of α-tocopherol concentration between supplemented LL and control LL in each slaughter group.

(1,200 IU/animal·d] for 38 d) and crossbred beef steers (1,200 IU/animal·d] for 67 d) effectively stabilized pigment and lipid of longissimus steaks. Faustman et al. (1989a,b) also reported that vitamin E supplementation (370 IU/animal·d] of Holstein steers effectively stabilized meat color and lipid of the gluteus medius.

Vitamin E acts as an antioxidant by reacting with free radicals (Tappel, 1962). Greene (1969) found that more metmyoglobin reducing activity was retained in ground beef samples stored with an antioxidant. Oxidation in meat is reported to be initiated in the phospholipid-rich membranes (Buckley et al., 1989). Activated metmyoglobin initiates lipid oxidation (Kanner and Harel, 1985; Rhee et al., 1987), and lipid hydroperoxide causes pigment oxidation (Lin and Hultin, 1977). Pigment and lipid oxidation can be closely related. Monahan et al. (1990) and Asghar et al. (1991) confirmed α-tocopherol deposition in the cellular membranes of pigs fed vitamin E-supplemented diets; Arnold et al. (1993a) did this for finished cattle. Therefore, we suggest that dietary vitamin E was absorbed by steers and incorporated into cellular membranes. In this location vitamin E prevented pigment and lipid oxidation directly by reacting with free radicals and also indirectly maintained metmyoglobin reducing activity. Hence, the stabilities of beef color and lipid were improved.

Exogenous Vitamin E

Postmortem vitamin E treatment (OIL + E) was slightly effective in retarding the oxidation of pigment (Figure 1) and lipid (Figure 2), especially compared with the OIL treatment. The effectiveness of postmortem vitamin E on pigment and lipid stability of ground beef in this study was weaker than that in the former work (Mitsumoto et al., 1991b). We interpreted this as follows: the added postmortem concentration (mean target value, 4.9 mg/kg of meat; mean actual value, 3.9 mg/kg of meat) of α-tocopherol in this study was smaller than that (6 mg/kg of meat) in the former study, resulting in less stabilization of pigment and lipid.

Other workers have reported the effectiveness of vitamin E in reducing lipid oxidation of meat. Miles et al. (1986) used 200 mg of α-tocopherol/kg in restructured pork; Whang et al. (1986) used 100 mg and 200 mg of α-tocopherol/kg in ground pork; Benedict et al. (1975) used 50 mg of α-tocopherol/kg in ground beef. Brekke et al. (1975) reported that significantly less tocopherol (30 mg/kg) was necessary to be present in fowl fat for in vivo vs in vitro supplementation (37 mg/kg) to obtain similar AOM (active oxygen method) stability times (11.6 vs 11.9 h). In this study we used approximately 4 mg of α-tocopherol/kg, which was the difference of α-tocopherol concentration between supplemented and control beef, to determine the effectiveness of endogenous vs exogenous vitamin E on pigment and lipid stability in beef. Dietary vitamin E supplementation improved pigment and lipid stability much better than postmortem addition of vitamin E during 9 d of display (Figures 3 and 4). This result showed that postmortem addition of α-tocopherol to beef in amounts equivalent to those deposited in beef by dietary vitamin E supplementation was ineffective in controlling pigment and lipid oxidation. A much higher postmortem addition of
\( \alpha \)-tocopherol would be needed to obtain better stability of pigment and lipid. This result indicated that even after the muscle was minced the endogenous vitamin E incorporated into cellular membranes worked better than exogenous vitamin E simply mixed into ground meat. The stabilization of membranal lipids must be a critical step in improving the postmortem stability of pigment and lipid.

**Implications**

Dietary vitamin E supplementation retarded metmyoglobin formation and greatly suppressed lipid oxidation in ground beef displayed for 9 d compared with the control. Postmortem addition of vitamin E was slightly effective in retarding the oxidation of pigment and lipid. Endogenous vitamin E improved pigment and lipid stability much better than exogenous vitamin E did. Because the use of antioxidants in raw meat is not permitted now in most countries, dietary vitamin E supplementation would be a safer and more effective method for retarding pigment and lipid oxidation in beef.

**Literature Cited**


