Effects of Dietary Vitamin E Supplementation on Color Stability, Lipid Oxidation and Reducing Ability of Hanwoo (Korean Cattle) Beef during Retail Display*

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ABSTRACT : The effects of dietary vitamin E supplementation (control; 200 IU/head/day, 500 IU/head/day and 1,000 IU/head/day) on color stability, lipid oxidation and total reducing ability in *M. longissimus* and *M. semimembranosus* from Hanwoo (Korean cattle) steer during retail display (3±1°C, 1,200 lux) were investigated. The L^*, a^*, b^*, C^*, R630-R580 values and TRA (total reducing ability) of 1,000 IU group were significantly ($p<0.05$) higher than those of the other groups. The a^*, C^*, R630-R580 and TBARS values were significantly ($p<0.05$) higher in *M. longissimus* than in *M. semimembranosus*. The a^*, C^*, R630-R580 values and TRA for two beef muscles declined gradually during storage and the decline was more rapid in control and 500 IU groups. Hue angle and metmyoglobin (%) for two beef muscles increased ($p<0.05$) as display time increased, and 1,000 IU group had a lower rate of metmyoglobin accumulation during retail display. The TBARS values for two beef muscle were significantly ($p<0.05$) lower in 1,000 IU group than in the other groups over time. Consequently, the meat from 1,000 IU vitamin E-supplemented Hanwoo steer extended retail display life compared to the control and 500 IU/head/day. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 10 : 1529-1534)***

Key Words : Vitamin E, Color Stability, TBARS, Total Reducing Ability, Hanwoo

INTRODUCTION

The color of fresh meat is an important quality attribute which determines whether the consumer will purchase the product (Faustman and Cassens, 1990), and various approaches have been used to meet consumer expectation that an attractive bright-red color is compatible with long shelf-life and good eating quality (Hood and Mead, 1993). Fresh meat color depends on many factors, such as concentration of heminic pigments and particularly of myoglobin, the chemical state of these pigments, and the physical characteristics of the meat (Renerre, 2000). Other factors affecting meat color include age, sex, breed, diet, and pre-slaughter factors (Renerre, 1990).

Accumulation of the undesirable brown color of fresh meat is due to oxidation of oxymyoglobin to metmyoglobin (Renerre, 1987; Renerre, 1990). Discoloration in retail meats during conditions may occur as a combined function of muscle pigment oxidation and lipid oxidation occurring in membrane phospholipids (Sherbeck et al., 1995).

Vitamin E consists of α-, β-, γ- and δ-tocopherols and one of the main functions of vitamin E is to protect membrane fatty acids and cholesterol from peroxidative damages caused by the reactive free radicals (Buckley et al., 1995; Liu et al., 1995). It is now well established that vitamin E acts as a powerful lipid-soluble antioxidant in cell membranes (Morrissey et al., 1994), hence, one of the main purposes of dietary supplementation of animal diets with vitamin E is to delay lipid oxidation in muscle foods (Liu et al., 1995). Greene (1969) was one of the first investigators to report on the potential color-preserving effect of antioxidants in meat. Many experiments have indicated that vitamin E supplementation results in greater color and lipid stability in beef by delaying oxidation of phospholipids (Arnold et al., 1993; Faustman et al., 1989a). Although the relation between lipid oxidation and pigment oxidation is not fully understood, it has been shown in beef that vitamin E retards the oxidation of myoglobin, and thus the loss of attractive color (Faustman et al., 1989a).

Hanwoo (Korean cattle) is a hybrid of *Bos taurus*×*Bos zebu* which was transmitted and settled in the Korean Peninsula in BC 4,000 (Han, 1996; Rhee and Kim, 2001). The objective of this study was to determine the effects of dietary vitamin E supplementation on color stability, lipid oxidation and total reducing ability in *M. longissimus* and *M. semimembranosus* from Hanwoo beef during retail display.

MATERIALS & METHODS

Animals and diets

Hanwoo steers aged 20 months were divided into three groups (n=5/group). Control group was fed a common basal diet with a vitamin E (dl-α-tocopheryl acetate) of 200 IU/head/day for 6 months prior to slaughter. DL-α-tocopheryl acetate was obtained from Hoffman-La Roche Ltd., 4002 Basel, Switzerland. The other groups were fed a

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Table 1. Effects of dietary vitamin E supplementation on meat quality characteristics in Hanwoo beef

Diet condition	L^*	a^*	b^*	Chroma	Hue angle	MetMb[d]	R630-R580	TBARS[e]	TRA[f]
Control	41.52[b]	19.89[b]	10.88[b]	22.68[b]	28.79[a]	25.58[a]	20.36[c]	0.37[a]	0.29[b]
500 IU	41.85[b]	19.79[b]	10.48[c]	22.41[b]	28.12[b]	23.99[b]	22.10[b]	0.33[b]	0.29[b]
1,000 IU	42.28[a]	21.03[a]	11.29[a]	23.88[a]	28.19[b]	21.42[c]	23.12[a]	0.25[c]	0.33[a]
SEM[g]	0.24	0.20	0.13	0.23	0.23	0.40	0.42	0.01	0.003

[a-c] Least square mean values within the same column with different superscripts are significantly different (p<0.05).
[d] Metmyoglobin, [e] thiobarbituric acid reactive substances, [f] total reducing ability, [g] standard error of the least square mean.

supplemented concentrate diet with a vitamin E supplement of 500 and 1,000 IU/head/day for 6 months prior to slaughter. The formulation of the common basal diet consisted of approximately 33.91% corn grain, 15% wheat grain, 5.65% lupin whole, 3% wheat flour, 9.97% wheat bran, 3% rice bran raw, 3.42% corn gluten feed, 5% mixed fiber, 5% cane molasses, 8% coconut meal, 4% palm meal, 0.5% beef tallow, 0.5% salt dehydrated, 1.5% limestone and other additives. Each group was held in separate pens and each animal was fed individually.

Sample preparation

All animals were conventionally slaughtered on day of transport (lairage time<5 h) at one location. The *M. longissimus* (control group=pH 5.42; 500 IU group=pH 5.26; 1,000 IU group=pH 5.39) and *M. semimembranosus* (control group=pH 5.31; 500 IU group=pH 5.19; 1,000 IU group=pH 5.34) were removed about 48 h after slaughter. Muscles were sliced (1.2 cm thickness), then overwrapped in polyethylene wrap film (oxygen transmission rate 35,273 $cc/m^2/24$ h/atm, thickness 0.01 mm). Samples were then stored in a retail display case (SL-2001, Selim Tech Co., Korea) at 3±1°C under fluorescent lighting (1,200 lux) for 7 days.

Surface color measurement

CIE (Commission Internationale de l'Eclairage) L^* (lightness), a^* (redness), and b^* (yellowness) values for Illuminant C were measured by a color difference meter (CR-310, Minolta Co., Tokyo, Japan). A white ceramic tile with a specification of Y=93.7, x=0.3129, y=0.3194 was used to standardize the colorimeter. Also, chroma (C^*) value and hue-angle (h°) were calculated as $C^* = (a^{*2}+b^{*2})^{1/2}$, and $h° = \tan^{-1}(b^*/a^*)$, respectively (CIE, 1986). The samples of day 0 were measured at 30 min after cutting a fresh surface.

Surface metmyoglobin percent measurement

The relative content of metmyoglobin at the meat surface was measured by the method of Kryzwicki (1979) using reflectance at 473, 525, 572, and 730 nm. Reflectance readings were converted to absorbance [2-log (%reflectance)] and used in the following equation (Demos et al., 1996).

$$\text{Metmyoglobin (\%)} = 1.395 - [(A572 - A730)/(A525 - A730)] \times 100$$

Percent reflectance differences at 630 nm minus 580 nm (R630-R580) were used to indicate differences in redness (Strange et al., 1974). Reflectance at selected wavelengths was measured by a dual beam spectrophotometer (UV-2401PC, Shimadzu, Kyoto, Japan) provided with a diffuse reflectance attachment adjusted to 100% reflectance with a $BaSO_4$ block.

Total reducing ability and lipid oxidation measurement

Total reducing ability (TRA) was measured as described by Lee et al. (1979). Thiobarbituric acid reactive substances (TBARS) values were measured according to the method of Sinnhuber and Yu (1977). TBARS were expressed as milligrams of malonaldehyde per kilogram of meat.

Statistical analysis

Data were analyzed as a 3 (diet conditions) by 5 (storage days) by 2 (muscle types) factorial design using the General Linear Model (GLM). Least square means were used, and when F-values were significant, least square mean differences were compared by using PDIFF at p<0.05. No interactions were detected. The relationships between the measured variables were assessed by Pearson correlation coefficients (SAS Institute, Inc., 1993).

RESULTS AND DISCUSSION

Effects of dietary vitamin E supplementation and muscle

Effects of dietary vitamin E supplementation on meat quality characteristics in Hanwoo beef during retail display were compared (Table 1). The L^*, a^*, b^*, C^*, R630-R580 values and TRA (total reducing ability) of 1,000 IU group were significantly (p<0.05) higher than those of the other groups, but there were not significant (p>0.05) differences in L^*, a^*, C^* values and TRA between control and 500 IU groups. Hue angle and metmyoglobin (%) were higher in control group than in the other two. The TBARS (thiobarbituric acid reactive substances) value was different (p<0.05) in order of control>500 IU>1,000 IU, indicating that lipid stability of 1,000 IU group was higher.

Effects of muscle (*M. semimembranosus* and *M. longissimus*) on meat quality characteristics were compared (Table 2). The a^*, C^*, R630-R580 and TBARS values were significantly (p<0.05) higher in *M. longissimus* than in *M.*

Table 2. Effects of muscle on meat quality characteristics in Hanwoo beef

Muscle	L^*	a^*	b^*	Chroma	Hue angle	MetMb[c]	R630-R580	TBARS[d]	TRA[e]
Semimembranosus	42.24[a]	19.69[b]	11.00[a]	22.57[b]	29.30[a]	24.13[a]	21.19[b]	0.30[b]	0.32[a]
Longissimus	41.53[b]	20.79[a]	10.77[b]	23.41[a]	27.43[b]	23.19[b]	22.53[a]	0.34[a]	0.30[b]
SEM[f]	0.15	0.13	0.07	0.14	0.13	0.29	0.30	0.01	0.002

[a-b] Least square mean values within the same column with different superscripts are significantly different ($p<0.05$).
[c] Metmyoglobin, [d] thiobarbituric acid reactive substances, [e] total reducing ability, [f] standard error of the least square mean.

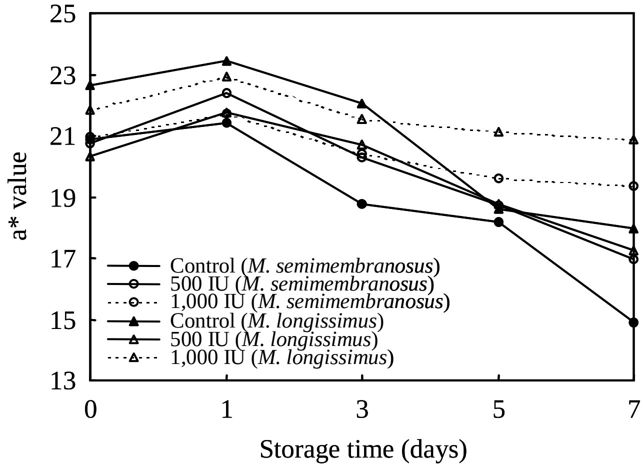

Figure 1. Effect of dietary vitamin E supplementation on a^* value of two beef muscles from Hanwoo steers during retail display (3±1°C, 1,200 lux).

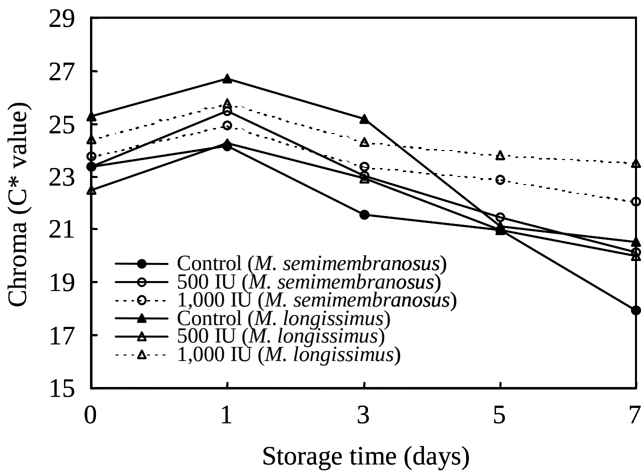

Figure 2. Effect of dietary vitamin E supplementation on chroma (C^*) value of two beef muscles from Hanwoo steers during retail display (3±1°C, 1,200 lux).

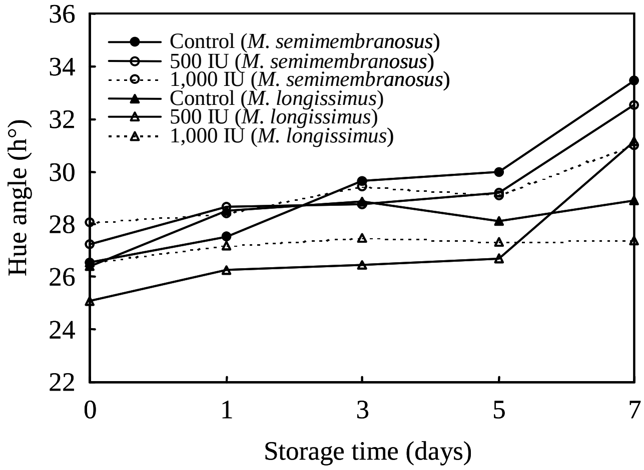

Figure 3. Effect of dietary vitamin E supplementation on hue angle of two beef muscles from Hanwoo steers during retail display (3±1°C, 1,200 lux).

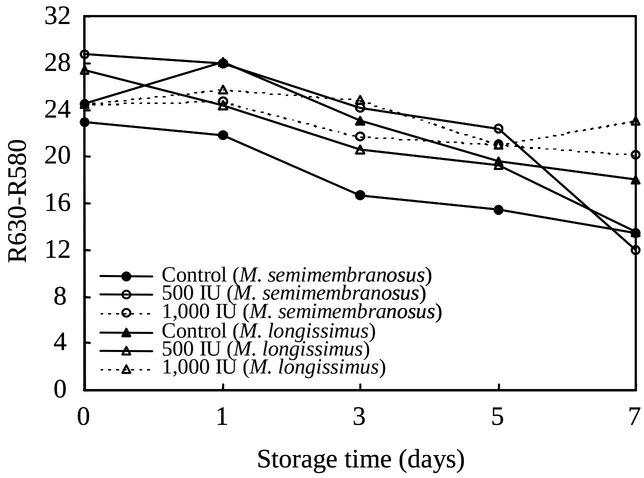

Figure 4. Effect of dietary vitamin E supplementation on R630-R580 of two beef muscles from Hanwoo steers during retail display (3±1°C, 1,200 lux).

semimembranosus. In contrast, L^*, b^*, hue angle, metmyoglobin and TRA were higher in *M. semimembranosus*.

Surface color and metmyoglobin

As a whole, the a^* (Figure 1) value increased during the first day of retail display except control group, then gradually decreased ($p<0.05$) over time. In particular, control and 500 IU groups were more accelerated compared to 1,000 IU groups. The a^* value of day 7 was significantly ($p<0.05$) higher in 1,000 IU group than in the other groups, and 1,000 IU group was not significantly ($p>0.05$) different after 3 days of storage for two beef muscles. The trend for C^* value (Figure 2) was similar to that for a^* value.

The a^* value is a measure of redness, however the a^* value alone has limited meaning (Howe et al., 1982). Hue angle specifically defines the hue of the color; in meat the larger the hue angle, the less red color. Hue angle (Figure 3) increased ($p<0.05$) as display time increased. And 1,000 IU group for two beef muscles at day 7 had lower ($p<0.05$) hue angle than those in control and 500 IU groups.

This trend of R630-R580 (Figure 4) was similar to those for a^* and C^* values. R630-R580 declined gradually during storage and the decline was more rapid in control and 500 IU groups.

Metmyoglobin of Hanwoo beef surface was influenced by dietary vitamin E level, muscle and display time (Table 3). In general, when brown metmyoglobin reaches 30-40% of total pigments on the surface of fresh retail beef, consumers make a no-purchase decision (Greene et al., 1971). The metmyoglobin (%) of day 0 (before storage) for two beef muscles was not significantly ($p>0.05$) different among 3 diet conditions. It was significantly ($p<0.05$) increased during display time for two beef muscles, but those of control and 500 IU groups at day 7 of storage were significantly ($p<0.05$) higher than 1,000 group. The 1,000 IU group had a lower rate of metmyoglobin accumulation during retail display. Arnold et al. (1993) reported that metmyoglobin on the meat surface was reduced for both 7- and 21-day-aged LL and GM following supplementation of 360 (E500) and 1,290 (E2000) IU/steer/day.

Lipid oxidation

Lipid oxidation is a major cause of deterioration in the quality of muscle foods and can directly affect many quality characteristics such as flavor, color, texture, nutritive value, and safety of the food (Buckley et al., 1995). As shown in Figure 5, thiobarbituric acid reactive substances (TBARS) value which represent fat rancidity tended to increase as display time increased. The TBARS values for two beef muscle were significantly ($p<0.05$) lower in 1,000 IU group than in the other groups over time, and it increased more rapidly in control group for *M. longissimus*. Consequently, 1,000 IU group significantly delayed lipid oxidation, whatever the muscle. Also, other investigators have reported a protective effect of dietary vitamin E against lipid oxidation in beef muscle during refrigerated display (Faustman et al., 1989b; Arnold et al., 1993). Andersen et al. (1990) provided evidence for supporting the pigment oxidation process as an initiator of lipid oxidation.

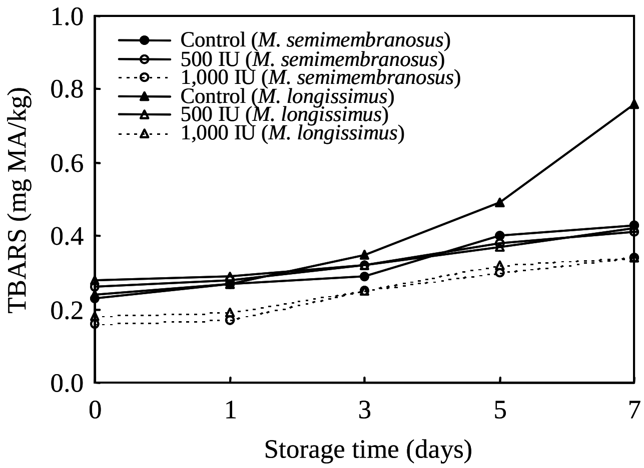

Figure 5. Effect of dietary vitamin E supplementation on TBARS (thiobarbituric acid reactive substances) of two beef muscles from Hanwoo steers during retail display (3±1°C, 1,200 lux).

Total reducing ability (TRA)

Total reducing ability (TRA) assay is not specific for metmyoglobin reducing assay or any other muscle-reducing chemistry, but it does indicate the muscle's overall reductive state (Sammel et al., 2002).Total reducing ability (Table 4) was significantly ($p<0.05$) decreased during retail

Table 3. Effect of dietary vitamin E supplementation on metmyoglobin (%) of two beef muscles from Hanwoo steers during retail display (3±1°C, 1,200 lux)

Muscle	Storage days	Diet condition			SEM^{f}
		Control	500 IU	1,000 IU	
Semimembranosus	0	19.15^{dx}	17.20^{cx}	17.23^{bx}	0.90
	1	23.10^{cx}	18.63^{cy}	18.91^{by}	0.88
	3	28.41^{bx}	25.02^{by}	22.99^{acy}	0.84
	5	30.07^{bx}	25.37^{by}	23.92^{ay}	0.88
	7	33.34^{ax}	33.54^{ax}	25.12^{ay}	1.33
	SEM^{g}	0.84	0.68	1.06	
Longissimus	0	19.85^{cx}	18.42^{ex}	18.26^{dx}	0.86
	1	21.14^{bcx}	20.36^{dx}	19.89^{cdx}	0.67
	3	25.22^{abx}	23.93^{cx}	21.14^{bcy}	0.65
	5	25.44^{abx}	26.19^{bx}	22.80^{aby}	1.23
	7	30.05^{ax}	31.22^{ax}	23.92^{ay}	1.25
	SEM^{g}	1.80	0.44	0.78	

$^{a-e}$ Least square mean values within the same column of the same muscle with different superscripts are significantly different ($p<0.05$).
^{f} Standard error of the least square mean among different diet conditions within the same storage day.
^{g} Standard error of the least square mean among different storage days within the same display condition.
$^{x-y}$ Least square mean values within the same row with different superscripts are significantly different ($p<0.05$).

Table 4. Effect of dietary vitamin E supplementation on total reducing ability (TRA) of two beef muscles from Hanwoo steers during retail display (3±1°C, 1,200 lux)

Muscle	Storage days	Diet condition			SEM^d
		Control	500 IU	1,000 IU	
Semimembranosus	0	0.39^{ax}	0.34^{ay}	0.41^{ax}	0.008
	1	0.37^{ax}	0.31^{by}	0.35^{bx}	0.006
	3	0.30^{by}	0.30^{by}	0.33^{bcx}	0.006
	5	0.27^{cy}	0.25^{cy}	0.33^{bcx}	0.011
	7	0.24^{cy}	0.24^{cy}	0.31^{cx}	0.009
	SEM^e	0.011	0.005	0.008	
Longissimus	0	0.35^{ax}	0.34^{ax}	0.35^{ax}	0.006
	1	0.29^{by}	0.31^{by}	0.34^{ax}	0.011
	3	0.27^{bcz}	0.30^{by}	0.34^{ax}	0.011
	5	0.23^{cz}	0.26^{cy}	0.29^{bx}	0.011
	7	0.23^{cy}	0.26^{cy}	0.29^{bx}	0.010
	SEM^e	0.014	0.004	0.009	

[a-c] Least square mean values within the same column of the same muscle with different superscripts are significantly different (p<0.05).
[d] Standard error of the least square mean among different vitamin E supplementation within the same storage day.
[e] Standard error of the least square mean among different storage days within the same display condition.
[x-z] Least square mean values within the same row with different superscripts are significantly different (p<0.05).

Table 5. Correlation coefficients among meat quality characteristics of Hanwoo beef

	L^*	a^*	b^*	Chroma	Hue angle	$MetMb^a$	R630-R580	$TBARS^b$	TRA^c
L^*	1.0000								
a^*	0.0499	1.0000							
b^*	0.3740^{***}	0.6338^{***}	1.0000						
Chroma	0.1262	0.9828^{***}	0.7652^{***}	1.0000					
Hue angle	0.3013^{***}	-0.5618^{***}	0.2472^{**}	-0.4064^{***}	1.0000				
$MetMb^a$	-0.0087	-0.6517^{***}	-0.3773^{***}	-0.6294^{***}	0.4265^{***}	1.0000			
R630-R580	0.0239	0.5941^{***}	0.2937^{***}	0.5615^{***}	-0.4344^{***}	-0.8397^{***}	1.0000		
$TBARS^b$	0.2423^{**}	-0.5220^{***}	-0.2752^{**}	-0.5043^{***}	0.3566^{***}	0.6496^{***}	-0.5328^{***}	1.0000	
TRA^c	-0.3722^{***}	0.3954^{***}	0.0777	0.3467^{***}	-0.4076^{***}	-0.5991^{***}	0.3751^{***}	-0.7172^{***}	1.0000

*p<0.05, ***p<0.01, ***p<0.001. [a] Metmyoglobin, [b] thiobarbituric acid reactive substances, [c] total reducing ability.

display in all of the groups, and 1,000 IU group had significantly (p<0.05) higher total reducing ability than the other groups except day 0 for *M. longissimus*. Faustman et al. (1989b) reported that the beneficial effects of vitamin E supplementation in relation to meat color and lipid stability might be linked to the protection of proteins and other biochemical metabolites involved in metmyoglobin reduction and through the slowing down of the myoglobin oxidation process.

Correlation among meat quality characteristics

The a^* value positively correlated (p<0.001) with b^*, chroma (C^*) values, R630-R580 and TRA (r=0.6338, 0.9828, 0.5941, and 0.3954 respectively) (Table 5). Also, a^* value inversely correlated (p<0.001) with hue angle, metmyoglobin and TBARS (r=-0.5618, -0.6517 and -0.5220 respectively). TBARS positively correlated (p<0.001) with metmyoglobin (r=0.6496). Faustman et al. (1992) provided evidence for a strong relationship between pigment oxidation and lipid oxidation in fresh ground veal. TRA inversely correlated with hue angle (r=-0.4076), metmyoglobin (r=-0.5991) and TBARS (r=-0.7172). Similarly, Ledward (1972) observed that aerobic reducing ability was highly inversely correlated with rate of metmyoglobin formation in beef muscles.

CONCLUSIONS

The dietary vitamin E supplementation of 1,000 IU/head/day for 6 months was effective in delaying discoloration, metmyoglobin accumulation, lipid oxidation and reduction of total reducing ability of *M. longissimus* and *M. semimembranosus* from Hanwoo steer during retail display compared to the control (200 IU/head/day) and 500 IU/head/day. Consequently, the meat from 1,000 IU vitamin E-supplemented Hanwoo steer extended retail display life.

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