Changes in Meat Color and α-Tocopherol Concentrations in Plasma and Tissues from Japanese Beef Cattle Fed by Two Methods of Vitamin E Supplementation

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ABSTRACT : The influence of dietary vitamin E supplementation on meat color and α-tocopherol concentrations in plasma, *longissimus thoracis* muscle and subcutaneous fat was investigated. Ten Japanese Black×Holstein crossbred steers were placed in three experimental groups receiving different supplements of vitamin E. Four steers (control) were fed no supplemental vitamin E. Two groups of three steers each, were supplemented with 500 mg dl-α-tocopherol acetate per animal daily for 175 days and 1,000 mg for 100 days, respectively, before slaughter. The α-tocopherol concentration in plasma increased, as vitamin E were fed, and were related to the length of time and the amount of supplement. The α-tocopherol concentrations in the muscle and the fat from the two supplemental groups did not differ significantly and were three or more times greater than those in the control group. Vitamin E supplementation did not affect the quantity of marbling of beef. Supplemental vitamin E stabilized the color of displayed beef around wavelengths of 500 nm and 640 nm. The two methods of vitamin E supplementation had similar effects on meat color. The effect of supplemental vitamin E on the color of beef with marbling was observed 2-3 days after slaughter and was followed for another two weeks. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 5 : 810-814*)

Key Words : Meat Color, Vitamin E, α-tocopherol, Japanese Cattle, Beef Cattle

INTRODUCTION

Meat color is an important factor influencing carcass grading and the consumer's purchase decision. Meat color changes over time with oxidation of oxymyoglobin to metmyoglobin. Several workers (Faustman et al., 1989a; Mitsumoto et al., 1991; Arnold et al., 1992) reported that vitamin E supplementation to steers was effective in increasing the stability of beef color. However, there have been no papers on the effect of vitamin E supplements on Japanese beef cattle that produce beef with marbling.

The present experiment was designed to examine the effects of the amount and length of feeding of vitamin E on the color of beef with high marbling from Japanese cattle by reflectance spectrophotometry, and on the α-tocopherol concentrations in plasma, muscle and fat.

MATERIALS AND METHODS

Experimental animals

Ten Japanese Black×Holstein crossbred steers averaging 363 kg body weight, raised at the Shiga Prefectural Livestock Research and Improvement Institute, were used in this study. Four steers were fed a basal diet of 80% commercial formulated feed and 20% barley without supplemental vitamin E as the control group (Group Ⅰ). Two groups, each composed of each three steers, were supplemented with 500 mg dl-α-tocopherol acetate (Kokin Chem. Co.) per animal daily for 175 days (Group Ⅱ) or 1,000 mg for 100 days (Group Ⅲ) before slaughter.

The *longissimus thoracis* at 6-7th rib was removed at 24 h post-mortem. These cuts were transported to Osaka Prefectural Agricultural and Forestry Research Center, where the marbling was evaluated, i.e., was given a Japanese beef marbling score (J. M. G. S, 1998), and the color was determined the day after, and every 2-3 days thereafter. The beef was put on a tray and displayed in a refrigerator at 4℃.

Spectrophotometry

Reflectance from the cut surface was measured with a spectrophotometer (NF-90, Nippon Densyoku Co., Ltd.). The measuring time was less than one second. Measurements from 400 to 700 nm in increments of 20 nm were collected and downloaded to a personal computer (Dynabook, Toshiba Co.) via an RS232C interface. Measurements at two sites per sample were made in triplicate and averaged. All data (10 samples, 2 sites per sample, 3 spectra per site per day, 5 days, 16 wavelength per spectrum, 1 data point per wavelength) were collected with a computer.

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α-tocopherol concentration

The α-tocopherol concentrations in plasma samples were collected from the crossbred steers during the feeding period. The number of samples during the feeding period was two for Group Ⅰ, five for Group Ⅱ, and three for Group Ⅲ. The α-tocopherol concentrations were determined for the *longissimus* muscle 2-3 days or a week after slaughter, and for subcutaneous fat which was packed and frozen immediately after slaughter. The α-tocopherol concentrations in the samples were measured by normal phase, high performance liquid chromatography with fluorescence detector.

Statistical analysis

The α-tocopherol concentrations in plasma, meat and fat were compared by one way analysis of variance at each sampling day (Tanaka and Tarumi, 1986). Using the reflectance at each wavelength, the two factorial variance analysis method (randomized method) affected by feed and time was applied. For inter-group comparison, the Tukey method was applied.

RESULTS AND DISCUSSION

α-tocopherol concentrations in plasma and tissues

α-tocopherol concentrations in plasma were increased ($p<0.01$) by feeding supplemental vitamin E (figure 1). The increases were almost linear to Day 100 for the 1,000 mg group and to Day 120 for the 500 mg group. On the final day, α-tocopherol concentrations in plasma were higher in the two groups supplemented with vitamin E than in the control group ($p<0.01$). Though the total dose of α-tocopherol received is not too different for the two treatments (100,000 mg for Ⅱ vs. 87,500 mg for Group Ⅲ), plasma concentrations do appear different (figure 1).

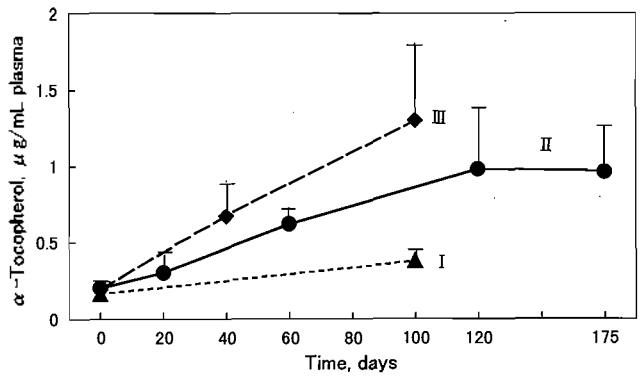

Figure 1. Effects of vitamin E on plasma α-tocopherol concentration (Control, I; Supplemental group of 500 mg vitamin E for 175 days, II; Supplemental group of 1,000 mg vitamin E for 1000d days, III)

Thus, α-tocopherol concentration in plasma is related to the supplemental method, i.e., the dosage level and time. About plasma concentration, Arnold et al. (1993a, b) reported that plasma α-tocopherol were lowest for the control, intermediate for the group supplemented with 500 IU vitamin E /day and highest for the group supplemented with 2,000 IU/day at all sampling times after the start of the experiment.

Table 1 shows the α-tocopherol concentrations in the tissues of the *longissimus* muscle and the subcutaneous fat. At the time of sampling, α-tocopherol concentrations in the beef and fat from the supplemental groups were three or more times greater than those in the control group ($p<0.01$). No significant difference was observed between the supplemental groups ($p>0.1$). In other words, vitamin E supplementations of 500 mg for 175 days and 1,000 mg for 100 days resulted in similar effects on tissue concentrations.

Table 1. Effect of supplemental vitamin E on tissue concentration of α-tocopherol (μg/g)

	Longissimus muscle				Subcutaneous fat	
	2-3 days after slaugher		5-8 days after exhibition		2-3 days after slaughter	
	MN	SD	MN	SD	MN	SD
Group Ⅰ	1.86[a]	0.42	1.21[a]	0.88	5.41[a]	0.31
Group Ⅱ	5.43[b]	1.55	5.33[b]	0.94	16.23[b]	5.57
Group Ⅲ	6.58[b]	1.78	6.13[b]	1.28	16.18[b]	5.51

[a,b] Means within a column are different superscript letter differ ($p<0.01$).

About one week after slaughter, the concentrations decreased slightly to 1.21 μg/g in Group Ⅰ, 5.33 μg/g in Group Ⅱ and 6.13 μg/g in Group Ⅲ. Concerning the relationship between dose level and time, Arnold et al. (1993b) reported that α-tocopherol accumulation in the *longissimus* muscle equilibrated with dietary vitamin E intake after 12 to 18 weeks. Liu et al. (1996b) reported that supplemental vitamin E of 2,000 IU for 42 days and 500 IU for 126 days had similar effects on the α-tocopherol concentration in *longissimus* muscle. As suggested by Arnold et al. (1992) and as shown in this experiment, α-tocopherol concentrations in tissues are related to the dose level and length of time of vitamin E supplementation.

At the start of the experiment, the concentration of α-tocopherol in *longissimus* muscle was highly correlated with that in subcutaneous fat ($r=0.91$, $p<0.01$). At the end of the feeding period, simple correlation coefficients for the relations between α-tocopherol concentration in plasma and muscle and between plasma and fat were 0.78 and 0.74,

respectively ($p<0.05$). Muscle biopsy would be more direct, but it lowers the commercial value of the meat, especially for expensive beef with marbling. Fat biopsy may be more useful to estimate the α-tocopherol concentration in muscle as an index of the effect of the supplement than blood gathering.

Spectrophotometry

The marbling scores of all the beef in this study were 4. Therefore, vitamin E supplementation did not affect the extent of marbling.

Figure 2 shows the daily changes in the reflectance spectra. The change in shape of the spectra for the control (Group I) differed from those for the supplemental groups. Spectrophotometry contains all color-related information: hue, brightness, chroma and various color systems. In all groups, a transformation with time was observed over a large part of the spectrum. Especially, the decrease at 640 nm was remarkable. The shape of the spectra became flat as time passed. The flattening means a decrease in chroma.

The statistical results (F value of variance analysis at each wavelength) are shown in figure 3. Marked effects of vitamin E and time course were found at certain points, showing little interaction. Thus, the reflectance spectrum was grouped by time in figure 4 and by vitamin E addition in figure 5.

Throughout the experimental period of display, higher F values were observed at 540, 580 and 640 nm (figure 3). The reflectance at 540 and 580 nm decreased in the order D5, D4, D3, D2, D1 as shown in figure 4. These changes in reflectance indicate an increase in yellow-green light and a decrease in red light with time. The changes were caused by a decrease in oxymyoglobin and an increase in metmyoglobin, because the absorption peaks of oxymyoglobin are 418, 544 and 588 nm and the absorption peaks of metmyoglobin are 410, 505 and 634 nm (Miller et al., 1996).

With respect to the difference in groups, the spectrophotometry (figure 5) indicated that Groups II and III, which were supplemented with vitamin E, had brighter colors, especially blue-green to green and yellow-red to red, when compared with Group I. The addition of vitamin E exerted a favorable effect on meat color and provided maximal F values at 500 and 640 nm, as shown in figures 3 and 5. The reflectance at 500 nm was highest in Group III, followed by Group II and Group I, although no significant differences were found between Groups II and III (figures 3 and 5). The reflectances at 640 nm were in the same order. These higher absorption spectra at 500 and 640 nm were considered to be associated with less metmyoglobin production in Groups II and III which was responsible for discoloration. These changes were also observed in figure 2. Vitamin E inhibited production of metmyoglobin through oxidation of oxymyoglobin to reduce the subsequent discoloration.

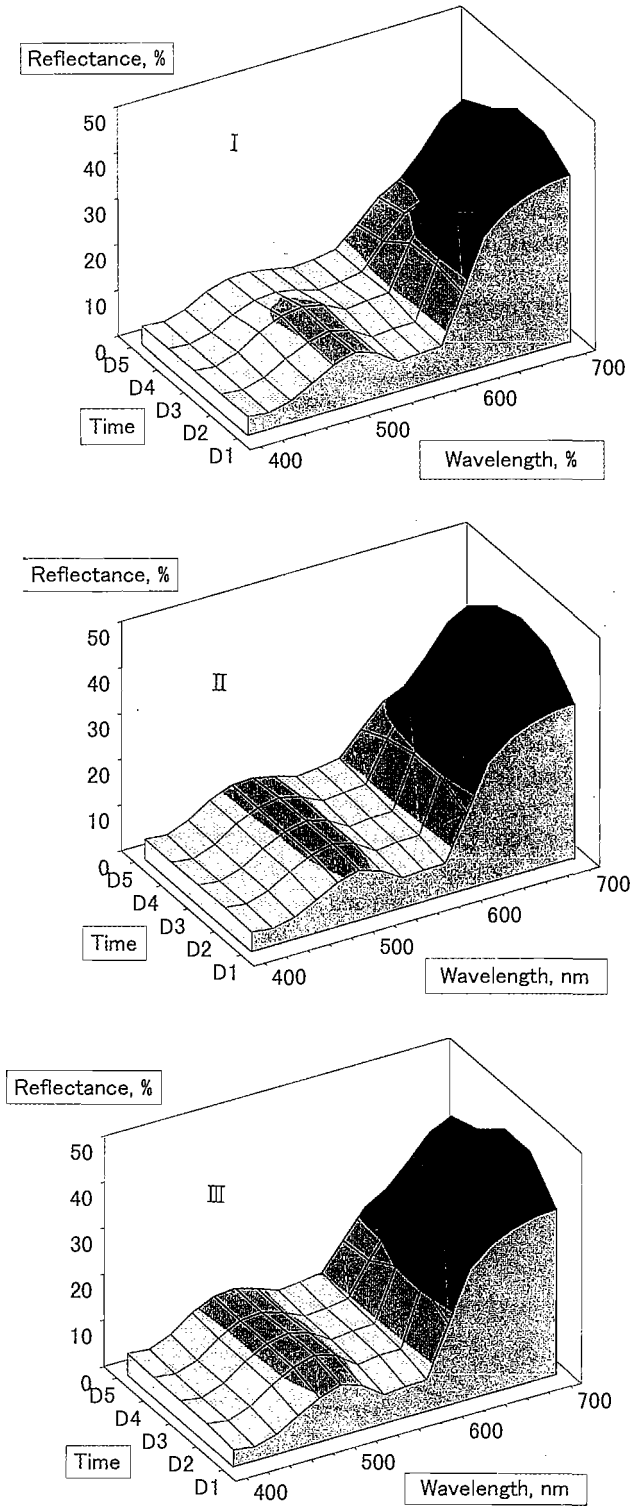

Figure 2. Reflectance spectra of beef from control and vitamin E supplemental groups during the displayes period (Control, I; Supplemental group of 500 mg vitamin E for 175 days, II; Supplemental group of 1,000 mg vitamin E for 100 days, III)

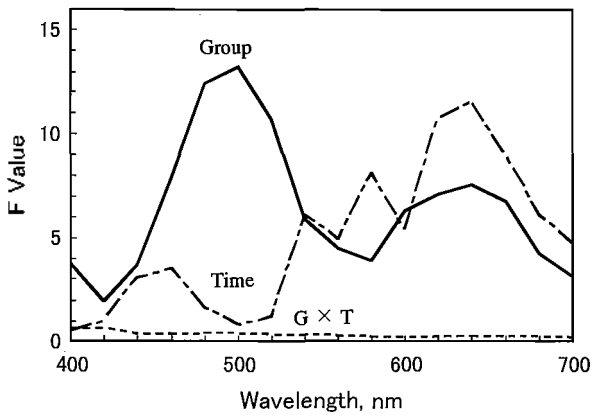

Figure 3. F value of analysis of variance for the main effects (group and time) and interaction (G×T)

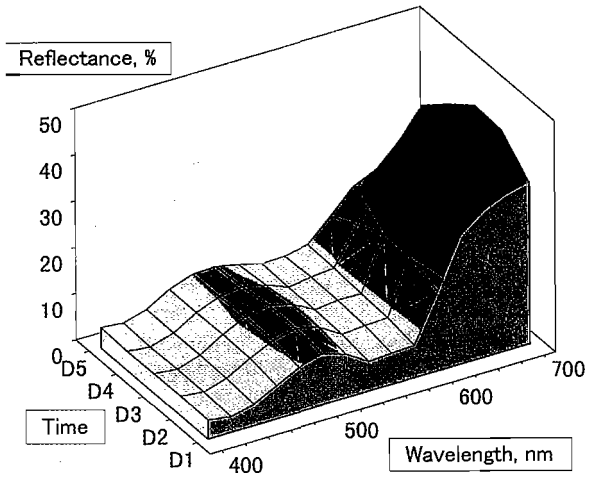

Figure 4. Changes in reflectance spectra of beef during the displayed period

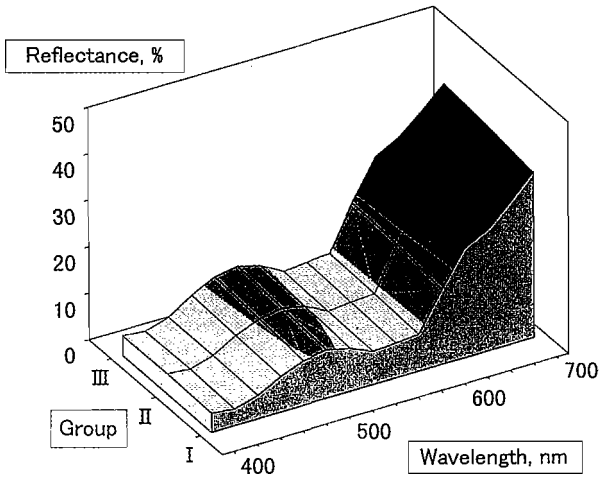

Figure 5. Difference in reflectance spectra of beef from control and vitamin E supplemental groups (Control, I; Supplemental group of 500 mg vitamin E for 175 days, II; Supplemental group of 1,000 mg vitamin E for 100 days, III)

The two dosages of vitamin E (500 mg for 175 days vs. and 1,000 mg for 100 days) produced no difference in color. When analyzing the data averages, due to the Group II spectra being higher than those of Group III, a dosage of 1,000 mg for a 175-day period is considered to be a more favorable labor-saving regimen.

Also, spectrophotometry is a useful technique for understanding changes in color. The optical device used in this experiment output a reflectance value every 20 nm wavelength from 400 to 700 nm simultaneously. Advanced devices can measure data at 1-nm intervals from 400 to 1,100 nm simultaneously.

In the present study, the spectrophotometry data showed that the effect of vitamin E was strongest around 500 and 640 nm and weakest around 420 and 580 nm. Especially, the reflectance at 500 nm was strongly affected by vitamin E but weakly affected by time. Liu et al. (1996b) observed that feeding vitamin E stabilized redness and hue and decreased yellowness. Chan et al. (1996) reported that a decrease in the a* value (redness of meat) and an increase in the hue angle (degree of change from redness to yellowness) of *longissimus lumborum* were greater in beef from crossbred steers fed no vitamin E than beef from crossbred steers fed 2,000 mg vitamin E and that there was no difference in L* and b* values between 0 and 2,000 vitamin E steaks. Faustman et al. (1989a) used "a" value (redness) and chroma $(a^2+b^2)^{0.5}$ as an index of the effect of vitamin E. Faustman et al. (1989b) and Mitsumoto et al. (1991) utilized metmyoglobin formation as the index. Lanari et al. (1995) applied a saturation index $(a^{*2}+b^{*2})^{0.5}$ and Hue angle $\tan^{-1}$ (b*/a*) as indicators of change in meat color. Our results showed that vitamin E caused the changes observed about 500 and 640 nm but little change around 420 and 580 nm.

These results agreed basically with those of Liu et al. (1996a). However, a decrease in yellowness is not caused by a change in yellow light. Although a lowering of the b* value indicates a decrease in yellowness by the colorimetric method of L* a* b*, spectrophotometry in the present study indicated that vitamin E supplementation did not affect the yellow band but stabilized blue-green components which are the complementary colors for yellow. The advantage of spectrophotometry is that it obtains information not only about myoglobin derivatives but also about color. Although researchers adopt various expressions for color, if one adopts spectrophotometry, one can recalculate various measurements-L* a* b*, saturation index and Hue angle, etc. Although the disadvantages of spectrophotometry are that it is time consuming and requires a large amount of data, development of computer technology has made this method feasible.

The concentration of α-tocopherol in tissues may

be used for an evaluative standard. In this study, the beef containing 5 μg/g or more α-tocopherol had color holding capacity. Arnold et al. (1992) observed that the number of days it took beef to discolor was positively correlated with α-tocopherol concentration in the *longissimus* muscle. Liu et al. (1996b) calculated that tissue α-tocopherol concentrations needed to be in the range 3.0 to 5.27 μg/g tissue for extended color and lipid stability. The results of this experiment are in agreement with this conclusion. This hypothesis can also apply to Japanese beef cattle with marbling.

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