Relationships between Muscle α-Tocopherol Concentrations and Metmyoglobin Percentages during Display of Six Muscles of Japanese Black Steers

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ABSTRACT : Relationships between muscle α-tocopherol concentrations and metmyoglobin percentages during display of six muscles, *m. serratus ventralis* (SV), *m. psoas major* (PM), *m. gluteus medius* (GM), *m. semimembranosus* (SM), *m. semitendinosus* (ST) and *m. longissimus lumborum* (LL), of Japanese Black steers slaughtered at 28 months of age were studied. Steers were supplemented with 0, 2,000 and 4,000 mg α-tocopheryl acetate/head/day for 28 days prior to slaughter in the VE 0, the VE 2,000 and the VE 4,000 groups, respectively. α-Tocopherol concentrations in PM, GM, SM, ST and LL of the VE 2,000 and the VE 4,000 groups were significantly ($p<0.05$) higher than those of the VE 0 group. There were no significant ($p>0.05$) differences in α-tocopherol concentrations in all muscles between the VE 2,000 group and the VE 4,000 group. The muscle α-tocopherol concentrations (μg/g meat) which can retard metmyoglobin formation in muscles were estimated to be 5.3 for SV, 4.5 for PM, 4.2 for GM, 4.0 for SM, 3.6 for ST and 3.5 for LL. The equation to predict color-shelf-life of each muscle from the α-tocopherol concentration in each muscle could be obtained. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 7 : 1014-1018)***

Key Words : Beef, α-Tocopherol, Metmyoglobin, Color-shelf-life, Japanese Black Steers

INTRODUCTION

Meat color is the main factor affecting beef product acceptability at retail points of purchase. The red color of fresh beef is due to oxymyoglobin. The discoloration of beef from red to brown during storage results from the oxidation of oxymyoglobin to metmyoglobin (Faustman and Cassens, 1990). Oxymyoglobin and cell membrane phospholipid oxidations are closely interrelated in meat (Kanner and Harel, 1985) and both are responsible for quality loss as well as shelf-life reduction. Arnold et al. (1993b) reported that dietary vitamin E supplementation to steers causes accumulation of α-tocopherol in muscle tissue and that this antioxidant delays myoglobin oxidation and prolongs the color stability of displayed beef. Similar findings were reported by Faustman et al. (1989) for Holstein steers, and Mitsumoto et al. (1998) and Irie et al. (1999) for Japanese Black steers. Arnold et al. (1993a) found that α-tocopherol level of 3.3 μg/g meat is sufficient to extend color stability in the longissimus muscle of Holstein steers. Mitsumoto et al. (1991) observed that α-tocopherol concentration over 3.5 μg/g meat appeared to retard metmyoglobin formation in the longissimus muscle of crossbred beef steers and Holstein steers. However, there have been no reports on whether there are differences in the muscle α-tocopherol concentration which can retard metmyoglobin formation in different muscles of Japanese Black steers in particular.

The purpose of this study was to examine the relationships between muscle α-tocopherol concentrations and metmyoglobin percentages during display of six muscles of Japanese Black steers and to estimate for each the muscle α-tocopherol concentration which can retard metmyoglobin formation during display.

MATERIALS AND METHODS

Animals and diets

Twelve Japanese Black steers aged 10 months (273.3± 7.3 kg) were selected at random and divided into three groups (VE 0, VE 2,000 and VE 4,000 groups) and fed both concentrate (55% flaked corn, 28% flaked barley, 10% wheat bran, 5% soybean meal and 2% vitamin-mineral mixture; 17.4 mg α-tocopherol/kg feed) and Italian ryegrass hay *ad libitum*. Steers were supplemented with 0, 2,000 and 4,000 mg α-tocopheryl acetate/head/day for 28 days prior to slaughter in the VE 0, the VE 2,000 and the VE 4,000 groups, respectively. The vitamin E used in this study was '*RovimixE-50 adsorbate*; 50% dl-α-tocopheryl acetate' (Roche Vitamins Japan K. K., Tokyo, Japan). Steers were slaughtered at the Department of Livestock and Grassland Science, National Agricultural Research Center for Western Region, Oda-shi, Shimane-ken, Japan. There were no

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Table 1. α-Tocopherol concentrations (μg/g meat) in six muscles of Japanese Black steers supplemented with 0 (VE 0), 2,000 (VE 2,000) and 4,000 (VE 4,000) mg α-tocopheryl acetate/head/day for 28 days (n=4)

Muscles [1]	VE 0	VE 2,000	VE 4,000
SV	3.65 [a, p] (±0.35)	7.01 [a, p] (±1.92)	7.03 [a, p] (±1.17)
PM	2.51 [b, q] (±0.32)	5.69 [a, p] (±1.22)	6.33 [a, pq] (±0.56)
GM	2.48 [b, q] (±0.46)	5.02 [a, p] (±1.23)	5.86 [a, pqr] (±0.41)
SM	2.09 [b, q] (±0.26)	4.27 [a, p] (±0.71)	4.11 [a, s] (±0.41)
ST	1.81 [b, q] (±0.31)	3.44 [a, p] (±0.81)	3.92 [a, s] (±0.48)
LL	2.35 [b, q] (±0.29)	3.74 [a, p] (±0.60)	4.61 [a, rs] (±0.54)

[1] SV=*m. serratus ventralis*, PM=*m. psoas major*, GM=*m. gluteus medius*, SM=*m. semimembranosus*, ST=*m. semitendinosus*, LL=*m. longissimus lumborum*.

[a, b] Means (±SE) within a row without a common superscript differ ($p<0.05$).

[p-s] Means (±SE) within a column without a common superscript differ ($p<0.05$).

significant ($p>0.05$) differences in body weight, body weight gain and feed intake between the groups. The average slaughter age and live weight of 12 steers were 28.2±0.1 months and 696±15 kg, respectively.

Muscle samples

After slaughter, carcasses were kept in a 0°C refrigerator for 48 h. Six muscles, *m. serratus ventralis* (SV), *m. psoas major* (PM), *m. gluteus medius* (GM), *m. semimembranosus* (SM), *m. semitendinosus* (ST) and *m. longissimus lumborum* (LL), were identified and removed from the left side of each carcass. Sample was excised from the midsection of each muscle. Approximately 500 g of each sample was ground twice through a 3 mm plate of a laboratory meat grinder for analyses of α-tocopherol and crude fat concentrations. The ground meats were stored in a -80°C refrigerator until required. The remainder of each muscle was vacuum-packaged and stored for an additional 6 days at 4°C for metmyoglobin analysis.

α-Tocopherol and crude fat analyses

α-Tocopherol concentration in muscles was determined by the HPLC method described by Bennink and Ono (1982). After saponification, muscle samples were extracted with hexane. In this study, the mobile phase was methanol:water (99.5:0.5) at a flow rate of 1.5 ml/min. Detection wavelengths were 296 and 325 nm for excitation and emission, respectively. α-Tocopherol standards were carried through the same procedure as described for the muscle samples. Crude fat concentration in each muscle was determined by Soxhlet extraction for 16 h with diethyl ether according to AOAC (1984).

Metmyoglobin analysis

Each muscle sample for metmyoglobin analysis was sliced into 1 cm thick steaks, and three pieces of 3 cm diameter cores were cut from these, using a template cutter. Each sample was placed in a 100 ml disposable weighing boat, over-wrapped with oxygen-permeable PVC film, and displayed under cool white fluorescent lights (1,000-1,500 lux) at 4°C for 12 days. Spectral values between 360 and 800 nm (at 5 nm intervals) of triplicate cores were measured at days 0, 3, 6, 9 and 12 of display using a KC-300 spectrophotometer (Kalnew Optical Industrial Co., Ltd., Nagoya, Japan). Percentages of surface metmyoglobin were determined by the method of Stewart et al. (1965).

Statistical analysis

The main effects of dietary α-tocopherol level and muscle upon α-tocopherol concentration and metmyoglobin percentage were analyzed by the General Linear Model procedure of SAS (SAS Institute Inc., 1985). Differences among treatment means were evaluated by the Least Significance Difference test.

RESULTS AND DISCUSSION

Table 1 shows α-tocopherol concentrations in six muscles of the VE 0, the VE 2,000 and the VE 4,000 groups. There were no significant ($p>0.05$) differences in α-tocopherol concentrations in SV between the groups. Muscle α-tocopherol concentrations in PM, GM, SM, ST and LL of the VE 2,000 and the VE 4,000 groups were significantly ($p<0.05$) higher than those of the VE 0 group. There were no significant ($p>0.05$) differences in α-tocopherol concentrations in all muscles between the VE 2,000 group and the VE 4,000 group. These results suggested that it was impossible to increase the α-tocopherol concentrations in the muscles even if supplementing with over 2,000 mg α-tocopherol to the diet. Therefore, it is not necessary to supplement with over 2,000 mg α-tocopherol to the diet of steers.

α-Tocopherol concentration in muscle of the VE 0 group was significantly ($p<0.05$) higher in SV than in the other muscles. However, there were no significant ($p>0.05$) differences in α-tocopherol concentrations between other muscles of the VE 0 group. Muramoto et al. (2003a) reported that α-tocopherol concentration in muscle of the control group was higher in LL than in SM. There were no significant ($p>0.05$) differences in α-tocopherol

Table 2. Muscle α-tocopherol concentration (μg/g meat) to maintain the metmyoglobin percentage to less than 35% in each muscle at days 0, 3, 6, 9 and 12 of display [1]

Muscles [2]	Days of display [3]				
	0	3	6	9	12
SV	<0	5.27	8.11	9.50	9.58
PM	<0	4.53	8.72	12.15	15.10
GM	<0	2.25	6.04	7.60	9.38
SM	<0	<0	3.97	5.06	5.99
ST	<0	<0	0.36	3.61	4.70
LL	<0	<0	0.59	3.45	4.66

[1] Color-shelf-life of each muscle was calculated by using each regression equation shown in Figure 1.
[2] See footnote in Table 1. [3] Under cool white fluorescent lights (1,000-1,500 lux) at 4°C.

concentrations between muscles of the VE 2,000 group. Steers in the VE 4,000 group had a significantly ($p<0.05$) higher level of α-tocopherol in SV than in SM, ST and LL. The level of α-tocopherol in PM was significantly ($p<0.05$) higher than that in SM, ST and LL. The level of α-tocopherol in GM was significantly ($p<0.05$) higher than that in SM and ST.

Lynch et al. (2000) reported that the ranking of muscle α-tocopherol concentrations in crossbred steers after supplementation followed the order: PM>GM>SV>ST> SM>LL. Liu et al. (1996) reported that α-tocopherol concentrations in muscles followed the order: GM>SM>LL. Chan et al. (1996) reported that the ranking of muscle α-tocopherol concentration in crossbred steers after supplementation followed the order: PM>GM>*m. longissimus dorsi*. The trends found in this study agree with these previous findings, except for trends regarding SV and LL. The rankings of α-tocopherol concentrations in SV and LL in this study were higher than those in other reports. Japanese Black cattle have been improved to contain a large quantity of fat in their muscles, especially the longissimus muscle. And Japanese Black steers are fattened in order to increase the quantity of fat in their muscles. In this study, the ranking ($p<0.05$) of crude fat concentration in muscle of the VE 4,000 group followed the order: SV (32.5%)>LL (19.8%)=PM (14.9%)>GM (8.0%)=ST (7.3%)=SM (6.5%). Therefore, disagreement with other reports in the rankings of muscle α-tocopherol concentrations was found in the muscles containing a large amount of fat.

Relationships between muscle α-tocopherol concentrations and surface metmyoglobin percentages of SV, PM, GM, SM, ST and LL are shown in Figure 1. Regression was used to evaluate the relationship between muscle α-tocopherol concentrations and metmyoglobin percentages. Metmyoglobin percentages of all muscles decreased with increasing α-tocopherol concentrations in muscles at days 3, 6, 9 and 12 of display. Green et al. (1971) reported that consumers would reject beef containing over 30-40% metmyoglobin. Then, in this study, 35% metmyoglobin was chosen as a threshold value to estimate color-shelf-life of each muscle. Table 2 shows the muscle α-tocopherol concentration to maintain the metmyoglobin percentage to less than 35% in each muscle at days 0, 3, 6, 9 and 12 of display calculated by using each regression equation shown in Figure 1. Though statistical analysis could not be carried out, the color-shelf-lives of all muscles appeared to increase with increasing day of display and be different between muscles. From the results shown in Table 1 and 2, the muscle α-tocopherol concentration (μg/g meat) to maintain the metmyoglobin percentage to less than 35% seemed to be 5.3 for SV, 4.5 for PM, 4.2 (2.3-6.0) for GM, 4.0 for SM, 3.6 for ST and 3.5 for LL.

Arnold et al. (1993a) found that α-tocopherol level of 3.3 μg/g meat was sufficient to extend color stability in LL of Holstein steers. Mitsumoto et al. (1991) observed that α-tocopherol concentration over 3.5 μg/g meat appeared to retard metmyoglobin formation in LL at day 16 of display of crossbred beef steers and Holstein steers. In this study, the color-shelf-life of LL containing α-tocopherol 3.5 μg/g meat was 9 day as shown in Table 2. Therefore, color-shelf-life of LL of Japanese Black steers was shorter than that of crossbred beef steers and Holstein steers. In spite of increase in α-tocopherol concentration in the longissimus muscle, the beef color stability during display became short with slaughter age (Muramoto et al., 2003b). Generally, the slaughter age of Japanese Black steers is considerably longer than that of other breeds. Japanese Black steers are fattened up to about 29 months of age in order to increase the quantity of fat in their muscles, especially the longissimus muscle. In this study, muscle samples were from steers that were slaughtered at 28 months of age. On the other hand, in the above report (Mitsumoto et al., 1991), the ranges of age at slaughter of crossbred beef steers and Holstein steers were 12-13 months of age and 18-24 months of age, respectively. Therefore, the differences in the color-shelf-life of LL containing 3.5 μg α-tocopherol/g meat seemed to be caused by the age difference rather than the breed difference.

From the data shown in Table 2, the relationships between the color-shelf-lives of muscles based on a threshold value of 35% metmyoglobin and the muscle α-tocopherol concentrations were expressed as follows:

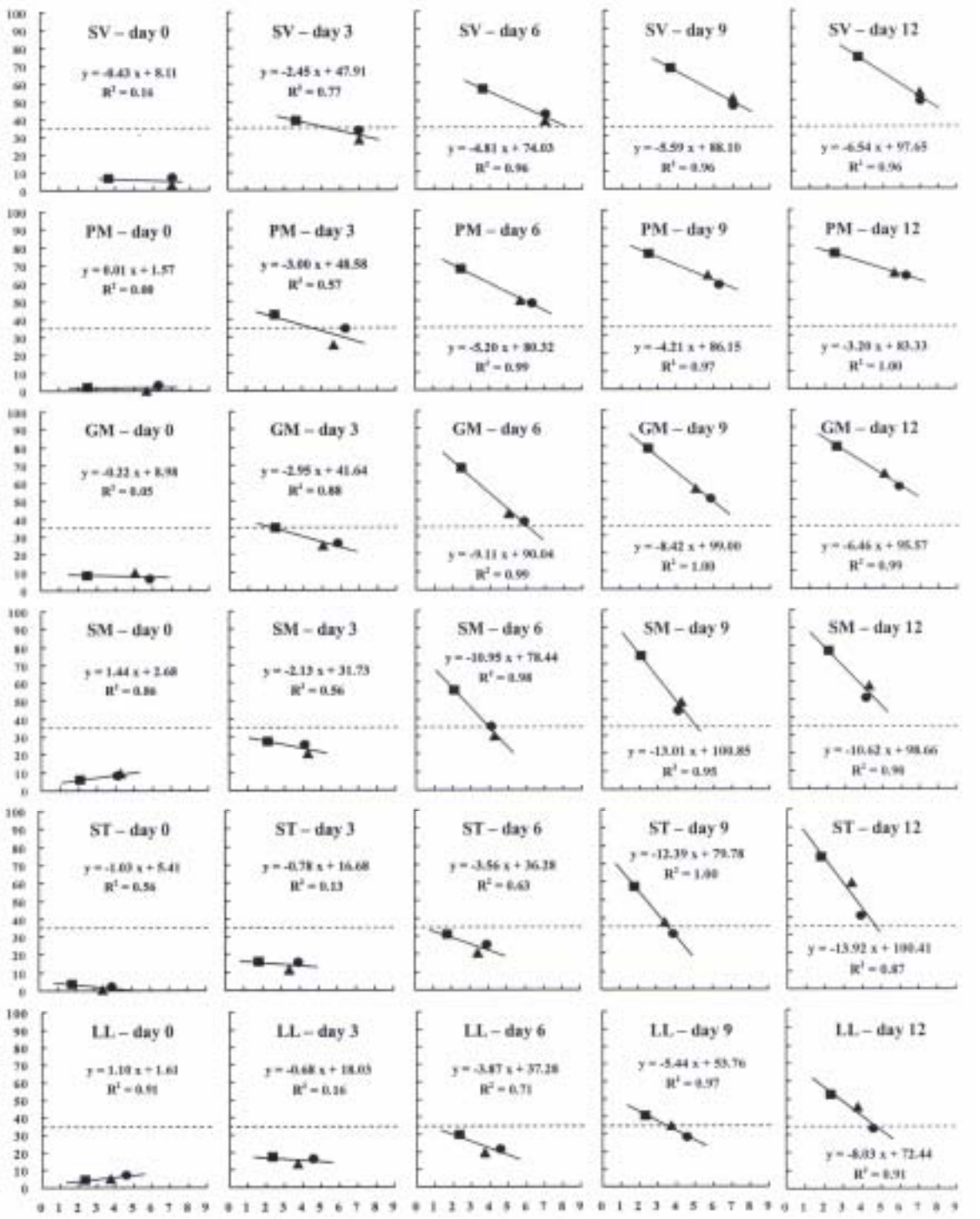

Figure 1. Relationships between muscle α-tocopherol concentrations and surface metmyoglobin percentages of six muscles, *m. serratus ventralis* (SV), *m. psoas major* (PM), *m. gluteus medius* (GM), *m. semimembranosus* (SM), *m. semitendinosus* (ST) and *m. longissimus lumborum* (LL), of Japanese Black steers supplemented with 0 (■), 2,000 (▲) and 4,000 (●) mg α-tocopheryl acetate/head/day for 28 days prior to slaughter and displayed at 4°C for 0, 3, 6, 9 and 12 days. X-axis represents the α-tocopherol concentration (μg/g meat). Y-axis indicates the metmyoglobin percentage. The dotted lines represent the 35% metmyoglobin.

SV: $Y=0.62e^{0.29X}$ ($R^2=0.95$)
PM: $Y=1.76e^{0.13X}$ ($R^2=0.99$)
GM: $Y=1.91e^{0.20X}$ ($R^2=1.00$)
SM: $Y=1.56e^{0.34X}$ ($R^2=1.00$)
ST: $Y=5.58e^{0.15X}$ ($R^2=0.97$)
LL: $Y=5.36e^{0.17X}$ ($R^2=0.99$)

where, Y=color-shelf-life (day of display), X=muscle α -tocopherol concentration (μg/g meat).

If substituting the muscle α-tocopherol concentration for X in above equation it would be possible to predict the color-shelf-life of each muscle of steers slaughtered at 28 months of age.

CONCLUSIONS

It is not necessary to supplement with over 2,000 mg α-tocopherol to the diet of steers. The muscle α-tocopherol concentrations to maintain the metmyoglobin percentages to less than 35% were different not only between the display days but also between the muscles. It is possible to predict the color-shelf-life of each muscle from the α-tocopherol concentration in each muscle.

REFERENCES

AOAC. 1984. Official Methods of Analysis. 14th edn. Association of Official Analytical Chemists, Arlington, Virginia.

Arnold, R. N., S. C. Arp, K. K. Scheller, S. N. Williams and D. M. Schaefer. 1993a. Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. J. Anim. Sci. 71:105-118.

Arnold, R. N., K. K. Scheller, S. C. Arp, S. N. Williams and D. M. Schaefer. 1993b. Dietary α-tocopheryl acetate enhances beef quality in holstein and beef breed steers. J. Food Sci. 58:28-33.

Bennink, M. R. and K. Ono. 1982. Vitamin B12, E & D content of raw and cooked beef. J. Food Sci. 47:1786-1792.

Chan, W. K. M., K. Hakkarainen, C. Faustman, D. M. Schaefer, K. K. Scheller and Q. Liu. 1996. Dietary vitamin E effect on colour stability and sensory assessment of spoilage in three beef muscles. Meat Sci. 42:387-399.

Faustman, C., R. G. Cassens, D. M. Schaefer, D. R. Buege, S. N. Williams and K. K. Scheller. 1989. Improvement of pigment and lipid stability in holstein steer beef by dietary supplementation with vitamin E. J. Food Sci. 54:858-862.

Faustman, C. and R. G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: a review. J. Muscle Foods. 1:217-243.

Green, B. E., I. Hsin and M. W. Zipser. 1971. Retardation of oxidative color changes in raw ground beef. J. Food Sci. 36:940-942.

Irie, M., K. Fujita and K. Sudou. 1999. Changes in meat color and α-tocopherol concentrations in plasma and tissues from Japanese beef cattle fed by two methods of vitamin E supplementation. Asian-Aust. J. Anim. Sci. 12:810-814.

Kanner, J. and S. Harel. 1985. Initiation of membranal lipid peroxidation by activated metmyoglobin and methemoglobin. Arch. Biochem. Biophys. 237:314-321.

Liu, Q., K. K. Scheller, S. C. Arp, D. M. Schaefer and S. N. Williams. 1996. Titration of fresh meat color stability and malondialdehyde development with Holstein steers fed vitamin E-supplemented diets. J. Anim. Sci. 74:117-126.

Lynch, A., J. P. Kerry, M. G. O'Sullivan, J. B. P. Lawlor, D. J. Buckley and P. A. Morrissey. 2000. Distribution of α-tocopherol in beef muscles following dietary α-tocopheryl acetate supplementation. Meat Sci. 56:211-214.

Mitsumoto, M., R. G. Cassens, D. M. Schaefer, R. N. Arnold and K. K. Scheller. 1991. Improvement of color and lipid stability in beef longissimus with dietary vitamin E and vitamin C dip treatment. J. Food Sci. 56:1489-1492.

Mitsumoto, M., S. Ozawa, T. Mitsuhashi and K. Koide. 1998. Effect of dietary vitamin E supplementation for one week before slaughter on drip, colour and lipid stability during display in Japanese Black steer beef. Meat Sci. 49:165-174.

Muramoto, T., N. Nakanishi, M. Shibata and K. Aikawa. 2003a. Effect of dietary β-carotene supplementation on beef color stability during display of two muscles from Japanese Black steers. Meat Sci. 63:39-42.

Muramoto, T., M. Shibata and N. Nakanishi. 2003b. Effect of slaughter age on beef color stability during display of four muscles from Japanese Black steers. Asian-Aust. J. Anim. Sci. 16:1364-1368.

SAS Institute Inc. 1985. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, North Carolina.

Stewart, M. R., M. W. Zipser and B. W. Watts. 1965. The use of reflectance spectrophotometry for the assay of raw meat pigments. J. Food Sci. 30:464-469.