

Effect of Slaughter Age on Beef Color Stability during Display of Four Muscles from Japanese Black Steers

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ABSTRACT : Effect of slaughter age (24, 28 and 38 months of age) on beef color stability during display of *m. serratus ventralis*, *m. psoas major*, *m. semitendinosus* and *m. longissimus thoracis* from Japanese Black steers was studied. Steak samples from muscles were over-wrapped with PVC film and displayed under fluorescent lights at 4°C for 12 days. Percentages of metmyoglobin of steak samples were determined at days 0, 3, 6, 9 and 12. The percentage of metmyoglobin of *m. psoas major* at day 3 of display in the 24 months group was lower ($p < 0.05$) than that in the 38 months group. The percentage of metmyoglobin of *m. semitendinosus* at day 6 of display in the 38 months group was higher ($p < 0.05$) than that in the other groups. The percentage of metmyoglobin of *m. longissimus thoracis* at day 3 of display in the 24 months group was lower ($p < 0.01$) than that in the other groups. The percentage of metmyoglobin of *m. longissimus thoracis* at day 6 ($p < 0.01$), 9 ($p < 0.01$) and 12 ($p < 0.05$) of display in the 38 months group were higher than those in the other groups. Crude fat concentration in *m. longissimus thoracis* increased ($p < 0.05$) after 28 months of age. α -Tocopherol concentration in *m. serratus ventralis* in the 38 months group was higher ($p < 0.001$) than that in the other groups. In *m. psoas major* the α -tocopherol concentration in the 38 months group was higher ($p < 0.05$) than that in the 24 months group. The α -tocopherol concentration in *m. longissimus thoracis* increased ($p < 0.001$) with age. These results suggested that in spite of increase in both the crude fat and the α -tocopherol concentrations in *m. longissimus thoracis*, the beef color stability during display became short with age. (*Asian-Aust. J. Anim. Sci.* 2003, Vol 16, No. 9 : 1364-1368)

Key Words : Beef, Color Stability, Slaughter Age, Metmyoglobin, α -tocopherol, Japanese Black Steers

INTRODUCTION

Generally, Japanese consumers prefer highly marbled beef. Therefore, the beef marbling grade is the most important factor in the evaluation of beef quality in Japan. Japanese Black cattle are famous because their longissimus muscles, the reference point of the marbling grade evaluation, tend to become highly marbled beef. In Japan, the average slaughter age of Japanese Black steers is 29.6 months of age. However, Japanese Black steers are often fattened up to and beyond 35 months of age in order to increase marbling in loin.

On the other hand, the number of Japanese consumers who prefer less marbling, or lean meat, has been increasing. In evaluating the quality of lean meat, color, firmness and texture are the most important factors. Sanders et al. (1997) reported that 58% of Japanese survey participants ($n=10,941$) identified muscle color as the most important factor in selecting beef products. The red color of fresh beef is due to oxymyoglobin. The discoloration of meat from red

to brown during storage results from the oxidation of oxymyoglobin to metmyoglobin (Faustman and Cassens, 1990). Metmyoglobin formation during storage has been delayed by α -tocopherol (Mitsumoto et al., 1998) and β -carotene (Muramoto et al., 2003) supplementation in the diet of Japanese Black steers. However, there is no report concerning the relationship between the slaughter age of Japanese Black steers and the metmyoglobin formation of beef during storage. The purpose of this study was to determine the effect of slaughter age on beef color stability during display of four muscles from Japanese Black steers.

MATERIALS AND METHODS

Animals and diets

Twelve Japanese Black steers aged 10 months (269.8 ± 9.8 kg) were selected at random and divided into three groups (24, 28 and 38 months of age groups) and fed both concentrate (55% flaked corn, 28% flaked barley, 10% wheat bran, 5% soybean meal and 2% vitamin-mineral mixture) and Italian ryegrass hay *ad libitum*. Steers were fed the same diets until the experiment was finished. Steers in the 24 months group, the 28 months group and the 38 months group were slaughtered at 23.6 ± 1.4 , 27.8 ± 0.4 and 37.8 ± 0.3 months of age, respectively. Body weight at slaughter was 567.0 ± 5.4 kg in the 24 months group, 735.5 ± 24.1 kg in the 28 months group and 830.8 ± 10.0 kg in the 38 months group. The steers were slaughtered at the

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Muscle samples

After slaughter, carcasses were kept in a 0°C refrigerator for 48 h. Four muscles, *m. serratus ventralis*, *m. psoas major*, *m. semitendinosus* and *m. longissimus thoracis*, were identified and removed from the left side of each carcass. A part of each muscle was ground twice through a 3 mm plate of a laboratory meat grinder for analyses of crude fat and α -tocopherol 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.

Crude fat and α -tocopherol analyses

The crude fat concentration in each muscle was determined by the ether extract method according to A.O.A.C. (1984). The 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.

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. The samples were 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. Percentages of surface metmyoglobin of triplicate cores were determined at days 0, 3, 6, 9 and 12 of display using the spectrophotometer (Kalnew Optical Industrial Co., Ltd., Nagoya, Japan) by the method of Stewart et al. (1965).

Statistical analysis

Data were analyzed by the General Linear Model procedure of SAS (SAS Institute Inc., 1985). Differences among treatment means were detected by the Least Significance Difference test.

RESULTS AND DISCUSSION

The effect of slaughter age on percentages of surface metmyoglobin in *m. serratus ventralis*, *m. psoas major*, *m.*

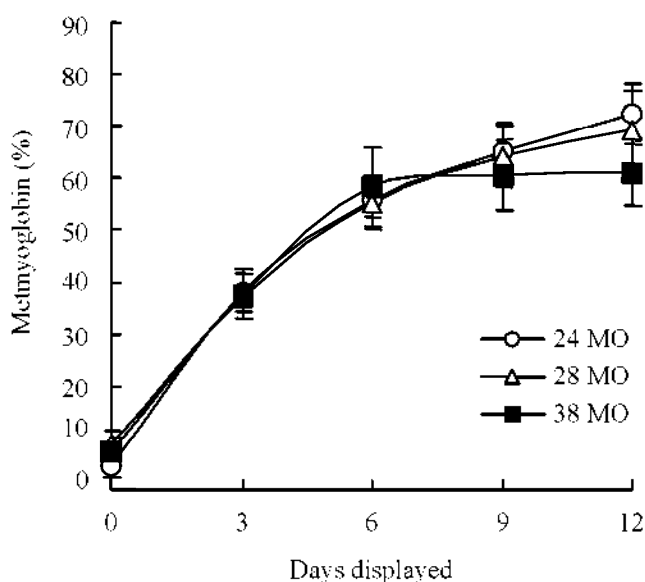


Figure 1. Percentages of metmyoglobin of *m. serratus ventralis* from Japanese Black steers slaughtered at 24 months of age (24 MO), 28 months of age (28 MO) and 38 months of age (38 MO). Each muscle sample was over-wrapped with PVC film and displayed under fluorescent light at 4°C for 12 days. Standard error bars are indicated.

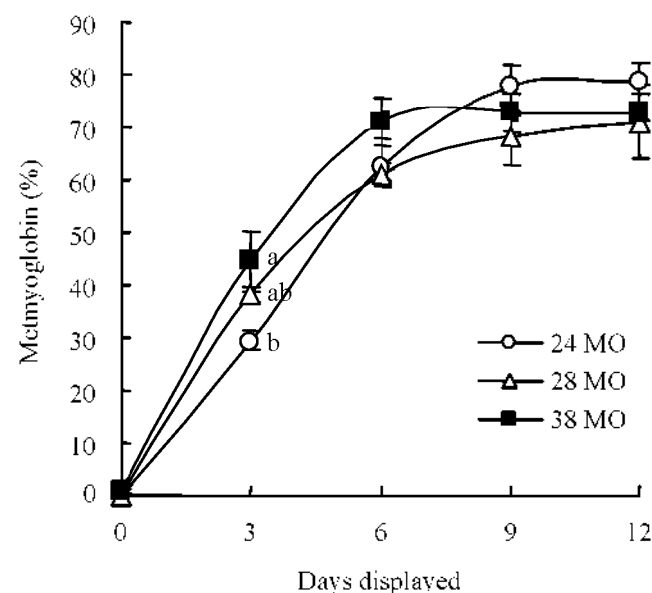


Figure 2. Percentages of metmyoglobin of *m. psoas major* from Japanese Black steers slaughtered at 24 months of age (24 MO), 28 months of age (28 MO) and 38 months of age (38 MO). Each muscle sample was over-wrapped with PVC film and displayed under fluorescent light at 4°C for 12 days. Standard error bars are indicated.

^{a,b} Values on the same display days with a different superscript letter differ ($p < 0.05$).

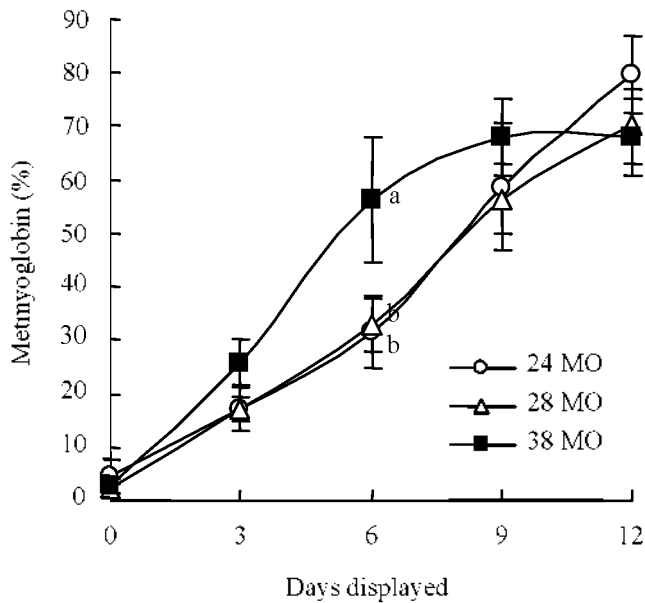


Figure 3. Percentages of metmyoglobin of *m. semitendinosus* from Japanese Black steers slaughtered at 24 months of age (24 MO), 28 months of age (28 MO) and 38 months of age (38 MO). Each muscle sample was over-wrapped with PVC film and displayed under fluorescent light at 4°C for 12 days. Standard error bars are indicated.

^{a,b} Values on the same display days with a different superscript letter differ ($p < 0.05$).

semitendinosus and *m. longissimus thoracis* are shown in Figures 1, 2, 3 and 4, respectively. The percentage of metmyoglobin of *m. serratus ventralis* during 12 days of display did not differ ($p > 0.05$) among the groups. The percentage of metmyoglobin of *m. psoas major* at day 3 of display in the 24 months group was lower ($p < 0.05$) than that in the 38 months group. At the other days of display, there was no difference ($p > 0.05$) in the percentage of metmyoglobin of *m. psoas major* among the groups. The percentage of metmyoglobin of *m. semitendinosus* at day 6 of display in the 38 months group was higher ($p < 0.05$) than that in the other groups. No effect ($p > 0.05$) of slaughter age was found on the percentage of metmyoglobin of *m. semitendinosus* at the other days of display. The percentage of metmyoglobin of *m. longissimus thoracis* at day 3 of display in the 24 months group was lower ($p < 0.01$) than that in the other groups. The percentage of metmyoglobin of *m. longissimus thoracis* at day 6 ($p < 0.01$), 9 ($p < 0.01$) and 12 ($p < 0.05$) of display in the 38 months group were higher

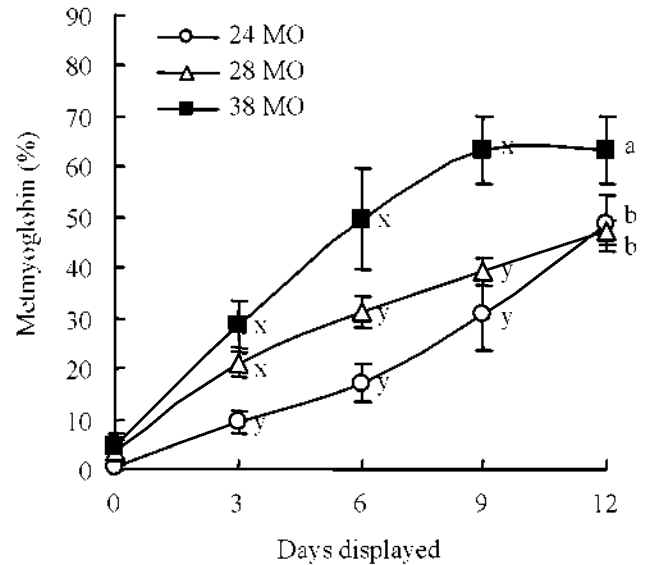


Figure 4. Percentages of metmyoglobin of *m. longissimus thoracis* from Japanese Black steers slaughtered at 24 months of age (24 MO), 28 months of age (28 MO) and 38 months of age (38 MO). Each muscle sample was over-wrapped with PVC film and displayed under fluorescent light at 4°C for 12 days. Standard error bars are indicated.

^{a,b} Values on the same display days with a different superscript letter differ ($p < 0.05$).

^{x,y} Values on the same display days with a different superscript letter differ ($p < 0.01$).

than those in the other groups. These results suggested that beef color stability of *m. psoas major*, *m. semitendinosus* and *m. longissimus thoracis* decreased with age.

Oxymyoglobin and cell membrane phospholipid oxidations are closely interrelated in meat and both are responsible for quality loss as well as shelf-life reduction (Kanner and Harel, 1985; Schaefer et al., 1995). This is supported by observations that products of both myoglobin oxidation and lipid oxidation increase during storage, and that the addition of antioxidants can result in a reduction of both of these deteriorative processes (Green, 1969; Faustman et al., 1989). Sasaki et al. (2001) reported that lipid oxidation in longissimus muscle during storage was negatively correlated with fat content. The average marbling score according to Japanese Carcass Grading Standards (JMGA, 1989) of the *m. longissimus thoracis* is 6.0. This value corresponds to approximately 17% intramuscular fat in *m. longissimus thoracis* (Cameron et al.,

Table 1. Crude fat concentrations of four muscles from Japanese Black steers slaughtered at 24, 28 and 38 months of ages (%)

	<i>M. serratus ventralis</i>		<i>M. psoas major</i>		<i>M. semitendinosus</i>		<i>M. longissimus thoracis</i>	
	MN	SE	MN	SE	MN	SE	MN	SE
24 months group	25.8	3.5	10.5	0.9	6.3	0.7	17.4 ^b	2.2
28 months group	28.5	4.2	13.9	1.8	7.7	1.0	17.4 ^b	0.9
38 months group	34.2	1.7	14.4	1.7	9.1	1.8	24.8 ^a	3.2

^{a,b} Means within a column with a different superscript letter differ ($p < 0.05$).

Table 2. α -tocopherol concentrations of four muscles from Japanese Black steers slaughtered at 24, 28 and 38 months of ages ($\mu\text{g/g}$)

	<i>M. serratus ventralis</i>		<i>M. psoas major</i>		<i>M. semitendinosus</i>		<i>M. longissimus thoracis</i>	
	MN	SE	MN	SE	MN	SE	MN	SE
24 months group	2.1 ^y	0.2	1.9 ^b	0.3	2.1	0.1	1.5 ^z	0.2
28 months group	2.8 ^y	0.2	2.1 ^{ab}	0.2	1.8	0.2	2.1 ^y	0.2
38 months group	4.4 ^x	0.4	2.9 ^a	0.4	2.1	0.2	3.2 ^x	0.2

^{ab} Means within a column with a different superscript letter differ ($p < 0.05$).

^{xz} Means within a column with a different superscript letter differ ($p < 0.001$).

1994). Table 1 shows the effect of slaughter age on crude fat concentrations of four muscles. In this study, crude fat concentration of *m. longissimus thoracis* in the 38 months group was higher ($p < 0.05$) than that in the 24 months group and the 28 months group. Therefore, the crude fat concentration in *m. longissimus thoracis* increased after 28 months of age. This result indicated that in spite of increase in crude fat concentration of *m. longissimus thoracis*, the beef color stability became short with age. There was no difference ($p > 0.05$) in crude fat concentrations among the groups of the other muscles.

Vitamin E is absorbed by animals and incorporated into cellular membranes where it performs its antioxidant function. Dietary vitamin E supplementation of steers causes accumulation of α -tocopherol in muscle tissue, which delays oxymyoglobin oxidation and prolongs the color stability of beef (Chan et al., 1995; Liu et al., 1996). In this study, α -tocopherol concentrations of four muscles from steers slaughtered at different ages were determined. Table 2 shows the effect of slaughter age on α -tocopherol concentrations of four muscles. The α -tocopherol concentration in *m. serratus ventralis* in the 38 months group was higher ($p < 0.001$) than that in the other groups. In *m. psoas major* the α -tocopherol concentration in the 38 months group was higher ($p < 0.05$) than that in the 24 months group. No difference ($p > 0.05$) was found in the α -tocopherol concentration in *m. psoas major* between the 28 months group and the other groups. There was no difference ($p > 0.05$) in the α -tocopherol concentration in *m. semitendinosus* among the 24 months group, the 28 months group and the 38 months group. On the other hand, in *m. longissimus thoracis*, the α -tocopherol concentration increased ($p < 0.001$) with age. These results suggested that in spite of increase of muscle α -tocopherol concentration, the beef color stability became short with age.

Arnold et al. (1993) found that α -tocopherol level of 3.3 $\mu\text{g/g}$ is sufficient to extend color stability in longissimus muscle. Mitsumoto et al. (1991) observed that α -tocopherol concentration over 3.5 $\mu\text{g/g}$ meat appeared to retard metmyoglobin formation in longissimus muscle in steers slaughtered at 18-24 months of age. However, the α -tocopherol levels required to retard the metmyoglobin formation in muscles, especially *m. longissimus thoracis*, may increase with age. It is necessary to clarify the

sufficient α -tocopherol levels to extend the color stability of each muscle from Japanese Black steers slaughtered at different ages.

In conclusion, 1) The beef color stability of *m. psoas major*, *m. semitendinosus* and *m. longissimus thoracis* from Japanese Black steers decreased with age. 2) In spite of increase in crude fat concentration of *m. longissimus thoracis*, beef color stability became short with age. 3) In spite of increase of muscle α -tocopherol concentration, the beef color stability became short with age.

REFERENCES

- Arnold, R. N., S. C. Arp, K. K. Scheller, S. N. Williams and D. M. Schaefer. 1993. Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *J. Anim. Sci.* 71:105-118.
- AOAC. 1984. Official Methods of Analysis. 14th edn. Association of Official Analytical Chemists, Arlington, Virginia.
- Bennink, M. R. and K. Ono. 1982. Vitamin B12, E & D content of raw and cooked beef. *J. Food Sci.* 47:1786-1792.
- Cameron, P. J., M. Zembayashi, D. K. Lunt, T. Mitsuhashi, M. Mitsumoto, S. Ozawa and S. B. Smith. 1994. Relationship between Japanese Beef Marbling Standard and intramuscular lipid in the *M. longissimus thoracis* of Japanese Black and American Wagyu cattle. *Meat Sci.* 38:361-364.
- Chan, W. K. M., K. Hakkarainen, C. Faustman, D. M. Schaefer, K. K. Scheller and Q. Liu. 1995. Color stability and microbial growth relationships in beef as affected by endogenous alpha-tocopherol. *J. Food Sci.* 60: 966-971.
- 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.
- Greene, B. E. 1969. Lipid oxidation and pigment changes in raw beef. *J. Food Sci.* 34:110-112.
- JMGA. 1989. New beef carcass grading standards. Japan Meat Grading Association, Tokyo, Japan.
- Kanner, J. and S. Harel. 1985. Initiation of membranous 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.
- 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. 2003. Effect of dietary β -carotene supplementation on beef color stability during display of two muscles from Japanese Black steers. *Meat Sci.* 63:39-42.
- Sanders, S. K., J. B. Morgan, D. M. Wulf, J. D. Tatum, S. N. Williams and G. C. Smith. 1997. Vitamin E supplementation of cattle and shelf-life of beef for the Japanese market. *J. Anim. Sci.* 75:2634-2640.
- SAS Institute Inc. 1985. *SAS User's Guide: Statistics*. SAS Institute, Inc., Cary, North Carolina.
- Sasaki, K., M. Mitsumoto and K. Kawabata. 2001. Relationship between lipid peroxidation and fat content in Japanese Black beef Longissimus muscle during storage. *Meat Sci.* 59:407-410.
- Schaefer, D. M., Q. Liu, C. Faustman and M-C. Yin. 1995. Supranutritional administration of vitamins E and C improves oxidative stability of beef. *J. Nutr.* 125:1792S-1798S.
- 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.