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Carotenoid Accumulation and Their Antioxidant Activity in Spent Laying Hens as Affected by Polarity and Feeding Period

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ABSTRACT : Since the consumption of spent laying hens as roasted skewered meat increases, the effects of various carotenoids on pigmentation and antioxidant activity were tested with 62-wk-old 250 ISA brown laying hens to improve the quality of chicken meat. In a 6-wk feeding trial, 4 carotenoids with different polarity (β-8-apo-carotenoic acid ethyl ester (ACAEE)>astaxanthin>canthaxanthin> β-carotene) at 100 mg carotenoid/kg feed were used. The more polar the carotenoids, the higher were the levels in blood. After 5-wk adaptation, the concentrations of astaxanthin, canthaxanthin, and ACAEE in blood were ~4 μg/ml. Canthaxanthin decreased significantly ($p<0.05$) the level of total blood cholesterol. Decreases in blood triglyceride by all carotenoids used were significant. ACAEE and astaxanthin tended to increase skin yellowness of thigh, breast, and wing proportionally to feeding period. In the case of polar carotenoids (ACAEE and astaxanthin), the longer the period of feeding, the higher the accumulation in skin was observed. Only astaxanthin was effective against the production of lipid peroxides in skin. Conclusively, out of the commercially available carotenoids we tested, astaxanthin is recommended for pigmentation of skin and inhibition of lipid oxidation. (**Key Words :** Pigmentation, Carotenoid, Polarity, Laying Hen, Feeding Period, Astaxanthin)

INTRODUCTION

In Japan, large quantities of roasted chicken meat (spent laying hens giving high preference of texture) are consumed. Also, the consumption of roasted skewered chicken meat increases in Korea. To promote the consumption of spent laying hens and to improve the quality of meat, the effects of various carotenoids on pigmentation and antioxidant activity were tested with 62-wk-old 250 ISA Brown laying hens.

Color of chickens greatly affects the purchasing behavior of consumers (Fletcher, 1999). Poultry accumulate carotenoids mainly in liver, skin, and shank (Allen, 1988). Since carotenoids are not produced by chickens, they must be supplied in feed for pigmentation (Dua et al., 1967). Accumulated carotenoids may increase the quality of chicken by improving flavor (Josephson, 1987), delaying oxidation and pigmenting bodies (An et al., 2004).

Carotenoids are important sources of antioxidation and pigmentation (Bertram and Vine, 2005; Higuera-Ciapara et al., 2006). They are grouped into carotenes (hydrocarbons) and xanthophylls (oxy-carotenoids), and only the latter have a coloring activity in poultry (Na et al., 2004). Pigmentation by carotenoid is important in commercial poultry industry because pigmentation level of egg yolk and broiler meat affect the product acceptability by consumers (Hernandez et al., 2001). Currently, a majority of the commercial carotenoids are synthesized via a chemical route (Ye et al., 2006).

Polarity of carotenoids affects their absorption and accumulation in chickens. Less than 1% of β-carotene, 7% of zeaxanthin, and 34% of β-apo-8-carotenoic acid ethyl ester (ACAEE) in feed accumulated in egg-yolk (polarity order: β-carotene<zeaxanthin<ACAEE) (Roche, 1988). ACAEE and canthaxanthin were absorbed 9~11- and 3~5-fold more into the blood than β-carotene (Na et al., 2004). Translocation of β-carotene from blood to skin was 2~5-fold higher than those of ACAEE and canthaxanthin (Na et

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al., 2004). The higher the polarity, the more the absorption of carotenoids into blood but the reverse was true in the case of translocation from blood to skin (An et al., 2004).

Sixty two-wk old laying hens were successfully pigmented by 6-wk trial of carotenoid feeding (Na et al., 2004). To find proper period of carotenoid feeding, the successive 6-wk trial up to 68-wk of age was performed. The corotenoids and polarity used in this study were ACAEE>astaxanthin>canthaxanthin>β-carotene (Figure 1). The results can provide information to save the unnecessary cost of carotenoid feeding. The accumulation of astaxanthin protected skin from lipid peroxide production (An et al., 2004). In this study, the carotenoids were compared for pigmentation and antioxidation of skin for chicken meat quality. Additionally, during pigmentation with carotenids, spent laying hens layed eggs and thus the pigmentation of egg yolk was also measured.

MATERIALS AND METHODS

Experimental protocol

A 6-wk feeding trial was conducted with 62-wk-old ISA Brown laying hens. Eighty laying hens were randomly allotted to 80 wire cages (29 cm×32 cm×36 cm), one bird per cage. There were five dietary treatments, 16 replicates per treatment, and one bird per replicate. A typical basal diet was formulated (Table 1) as reported previously (Na et al., 2004). The four experimental diets were prepared by adding 1 g carotenoid/kg basal diet: astaxanthin (10%, w/w; Roche, Switzerland), canthaxanthin (10%, w/w; Roche, Switzerland), ACAEE (10%, w/w; Roche, Switzerland), and β-carotene (10%, w/w; Fluka Co., Switzerland). Feed and water were provided *ad libitum*. Lighting schedule was 17 h light and 7 h dark. Room temperature was maintained at 6-12°C.

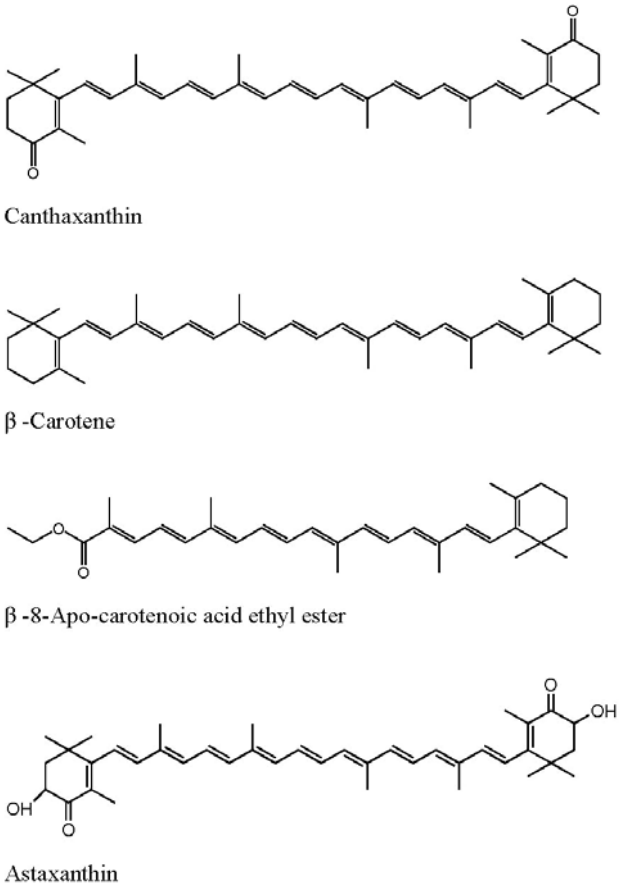

Figure 1. Carotenoids used in this study (polarity: β-8-Apo-carotenoic acid ethyl ester>astaxanthin>canthaxanthin>β-carotene).

Carotenoid analysis from blood and skin

At the termination of the feeding trial, 68-wk of age, 1 ml blood was sampled from the wing vein of all experimental birds. To 1 ml of EDTA-treated whole blood, 2 ml of dimethyl sulfoxide, 2 ml of acetone, 1 ml of petroleum ether, and 2 ml of 20% NaCl were serially added and mixed thoroughly. After centrifugation (3,000 rpm, 3 min), the upper petroleum ether layer was filtered and used for HPLC analysis. After blood sampling, these birds were slaughtered for skin sampling. Skin for carotenoid analysis was roughly cut with scissors and homogenized (Polytron PT-MR2100, Kinematica Co., Switzerland).

Twenty μl of the carotenoid extract was injected into Nucleosil column (100 Å) (MetaChem Technologies Inc.,

Table 1. Formulas and chemical composition of experimental diets for laying hens

Ingredient	Content (g/kg)
Maize	683.3-680.3[1]
Soya bean meal (CP 440 g CP/kg)	178.2
Maize gluten meal	36.0
Carotenoid	0-3[1]
Limestone	84.0
Tricalcium phosphate	9.3
DL-methionine (50%)	0.9
L-lysine·HCl	0.8
Vitamin complex[2]	5.0
Salts	2.5
Calculated composition	
Calculated ME (MJ/kg)	11.7
CP (g/kg)	160.0
Ca (g/kg)	34.0
P (g/kg)	4.0
Methionine (g/kg)	7.6
Lysine (g/kg)	3.3

[1] 0-30 g/kg of carotenoid (purity: 100 g/kg): β-8-apo-carotenoic acid ethyl ester (ACAEE), canthaxanthin, and β-carotene.

[2] Contained followings per kg of diet : vitamin A, 1,600,000 IU; vitamin D_3, 300,000 IU; vitamin E, 800 IU; vitamin K_3, 132 mg; vitamin B_2, 1,000 mg; vitamin B_{12}, 1,200 mg; niacin, 2,000 mg; pantothenate Ca, 800 mg; folic acid, 60 mg; choline chloride, 35,000 mg; DL-methionine, 6,000 mg; iron, 4,000 mg; copper, 500 mg; manganese, 12,000 mg; zinc, 9,000 mg; cobalt, 100 mg; BHT, 6,000 mg; iodide, 250 mg.

Torrance, CA, USA) of an HPLC (Younglin Instrument Co., Seoul, Korea). Carotenoids were detected by a UV-visible detector at 476 nm. Mobile system was *t*-butyl methyl ether:hexane:isopropanol:methanol = 30:65:2.5:2.5 and the flow rate was 1.5 ml/min. For the quantification of carotenoids, β-carotene (Sigma C-9750) and lutein (Sigma A-6250) were used as standards.

Colorimetric analysis

The carcasses of chicken were kept in refrigerator (4°C, 2 h). The CIE parameters (L^*, a^*, and b^* values) were determined by a chromameter (Cr 301, Minolta Co., Osaka, Japan). The colorimetric values of skin were measured after homogenization by chopping.

Five eggs were weekly collected from each treatment during the six-wk feeding period. The Roche color value of egg-yolk was measured by an egg quality meter (QCM+, Technical Services and Supplies, York, England).

Blood analyses

High density lipoprotein (HDL) cholesterol was measured by HDL ISOSPIN^{TM} (Sigma catalog # 352-2) kit, and calculated based on the following formulae: blood HDL cholestrol (mg/dl) = (Absorbance of sample-Absorbance of blank)/(Absorbance of calibrator-Absorbance of blank)×50 ×1.2.

Total cholesterol was measured by the Infinity^{TM} Cholesterol reagent (Thermo Electron, catalog # TR13521; Louisville, CO). The absorbance at 500 nm (A1) and 660 nm (A2) was used based on the calculation: ΔA = A1-A2.

Triglyceride was measured by the Infinity^{TM} Triglyceride reagent (Thermo Electron, catalog # TR22321; Louisville, CO). The absorbance at 520 nm (A1) and 660nm (A2) was used based on the calculation: ΔA = A1-A2.

Antioxidant effect of carotenoids in skin

The antioxidant activity of astaxanthin in skin was measured by measuring aldehyde formation during storage (Kosugi et al., 1989). Skin samples were incubated at 30°C with continuous lighting for 7 days. Aldehydes were detected by using 2-thiobarbituric acid (TBA) and malonaldehyde was used as a standard (An et al., 2004).

Statistical analysis

Statistical analysis was performed with SPSS 14.0. When the *F*-value was significant ($p<0.05$), post-ANOVA test was conducted by using Tukey's test.

RESULT AND DISCUSSION

Carotenoids in blood

The absorption trends of carotenoids in blood during 6-wk feeding were monitored as shown in Figure 2. At the beginning of feeding (1st wk), absorption of carotenoids were markedly affected by their polarity: the more polar, the more absorption. From the 2nd wk, the chickens were adapted and the concentrations of carotenoids were decreased. However, after 4-5th wk, the levels of carotenoids stabilized. At the end of the trial, carotenoid levels in blood were affected by its polarity but to a lesser degree (Figure 2). We previously reported that the absorption of carotenoids into blood was mainly affected by polarity (Na et al., 2004). The concentrations of carotenoids after 6-wk trial (Figure 2) were similar to the previous report (Na et al., 2004). Human also absorbed polar carotenoids (xanthophylls) more efficiently than carotenes (hydrocarbon carotenoids) (Furr and Clark, 1997).

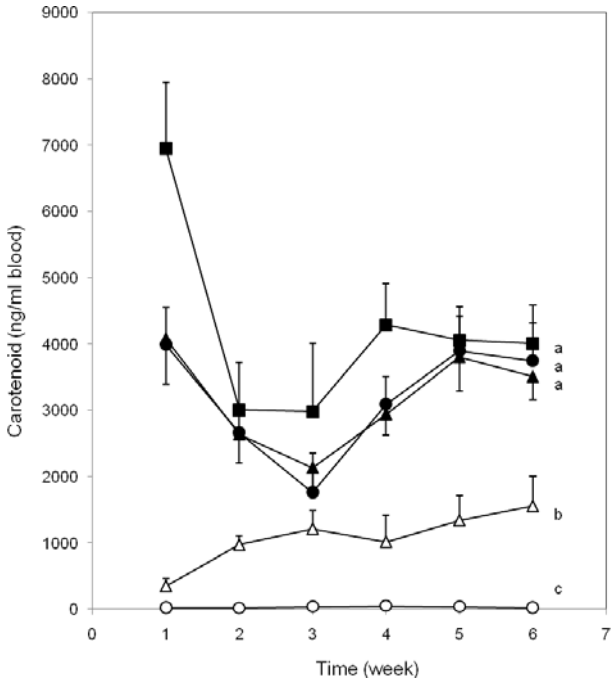

Figure 2. Concentration of carotenoids in blood. Symbols: ○, control; ●, astaxanthin; ▲, canthaxanthin; ■, ACAEE; and △, β-carotene. The control contained lutein and zeaxanthin from corn in feed. Bars indicate standard deviation.

Lipid and related factors in blood

The levels of cholesterol and triglyceride were decreased by feeding of carotenoids (Figure 3). The decreases in triglyceride by carotenoids were significant ($p<0.01$). HDL-cholesterol was increased by the feeding of xanthophylls but not by carotene (β-carotene) feeding, though the effect was not statistically significant (Figure 3). The results suggested that the cardiovascular health was improved by feeding of polar carotenoids, especially astaxanthin.

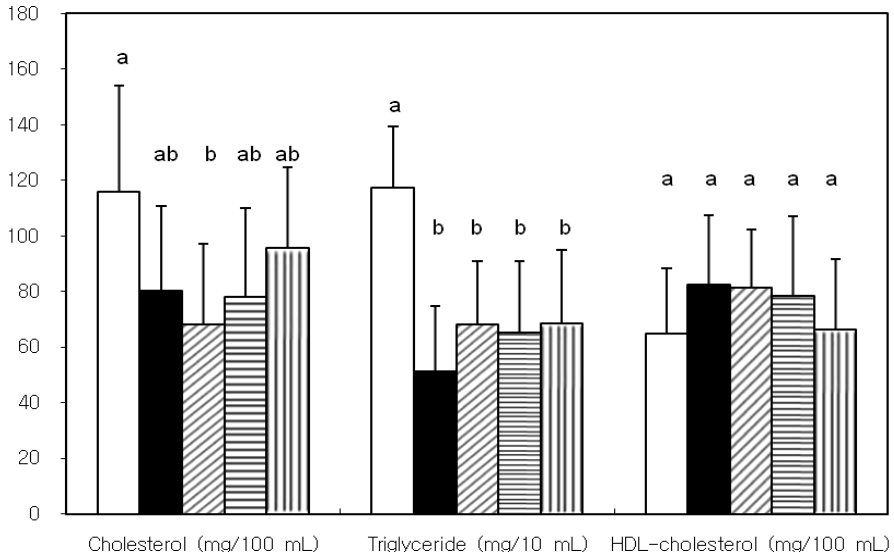

Figure 3. Triglyceride, total cholesterol, and cholesterol in high density lipoprotein after 6-wk feeding of carotenoids. Symbols: □, control; ■, astaxanthin; ▨, canthaxanthin,. ▤, ACAEE; and, β-carotene.

Pigmentation of skin by carotenoids

Accumulation of astaxanthin and ACAEE was significantly proportional to the length of feeding period (Figure 4). When chickens were fed longer than 6 wk, they might accumulate more carotenoids in skin. β-Carotene and canthaxanthin were accumulated less effectively (Figure 4).

The color changes of several skin parts were measured by the colorimetric method (CIE parameters: L^*, a^*, and b^* values). The lightness (L^*) and the redness (a^*) of skins were not affected significantly by the feeding of carotenoids (data not shown). The yellow xanthophyll (ACAEE) increased yellowness (b^*) in skin (Figure 5) because of high accumulation in skin (Figure 4) as proportional to feeding period. Interestingly astaxanthin (orange color) also increased yellowness especially in thigh skin (Figure 5a). In the cases of breast and wing skins, ACAEE was more effective on pigmentation than astaxanthin (Figure 5b and c). Therefore, astaxanthin and ACAEE showed a strong pigmentation activity. Polar carotenoids were efficiently absorbed by chickens, especially into blood, and thus caused increased pigmentation of muscle, skin and egg-yolk (Na et al., 2004). The effect of ACAEE on b^* values of skin and breast muscle was significant, proportional to concentration (George et al., 1970; Na et al., 2004).

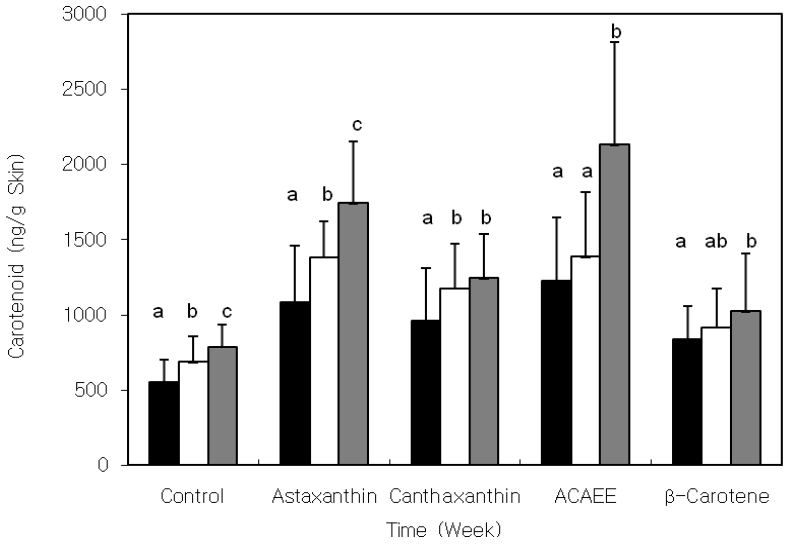

Figure 4. Accumulated carotenoid in breast skin after 6-wk feeding. Symbols: ■, 2 wk; □, 4 wk; and ▩, 6 wk.

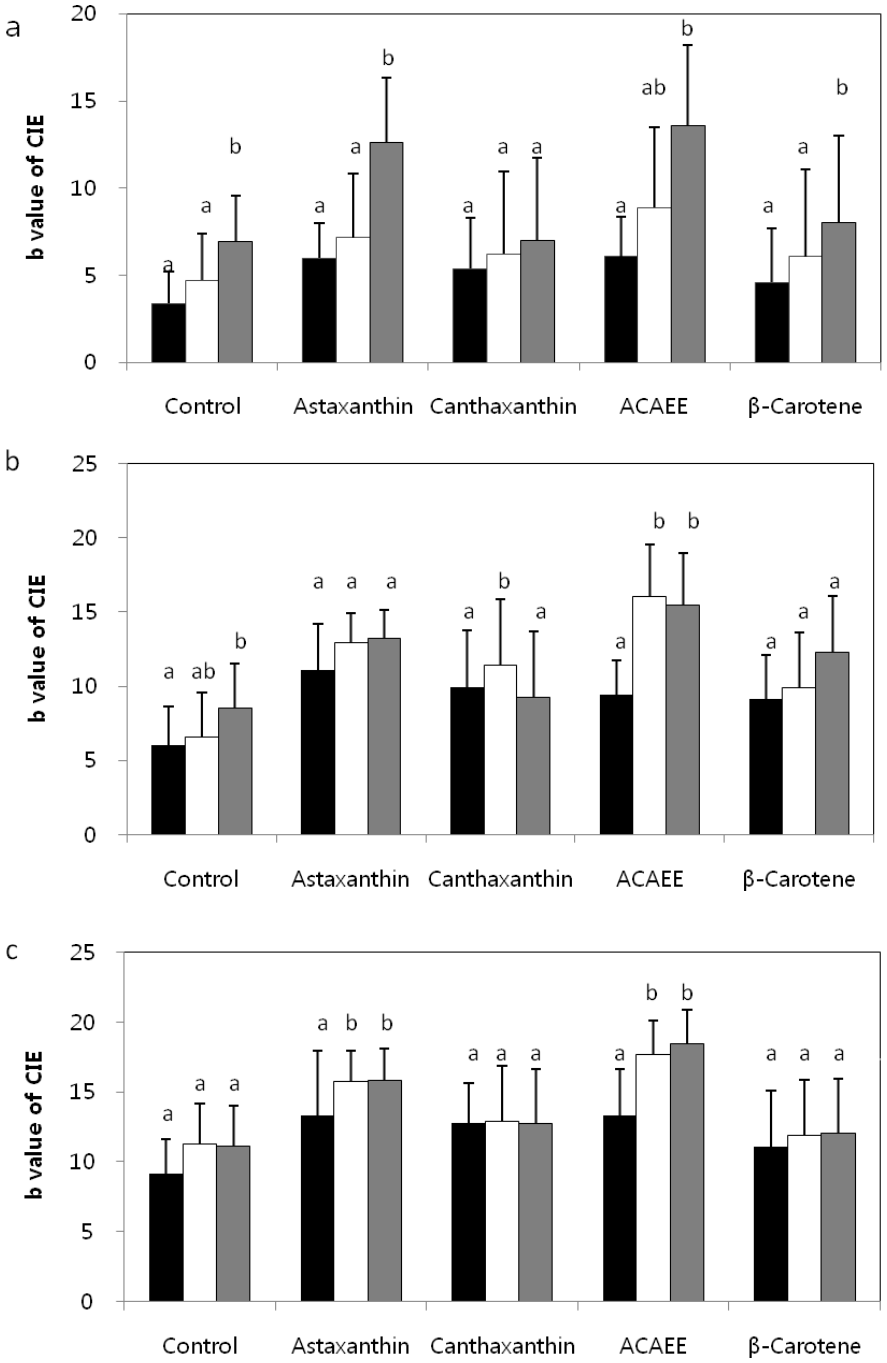

Figure 5. Pigmentation of wing, breast and thigh skin by feeding of carotenoids in laying hens. Panels: a, colorimetric b value of thigh skin; b, colorimetric b value of breast skin; and c, colorimetric b value of wing skin. Symbols: ■, 2 wk; □, 4 wk; and ▩, 6 wk.

The level of astaxanthin in the skin of broiler chicken was about 1,300 ng/g skin after 5 wk when chickens were fed at 45 mg/kg feed (An et al., 2004). The spent laying hens accumulated the similar level of astaxanthin after 4 wk at 100 mg/kg feed (Figure 4). Lutein, the major colorant of corn, was effective on the pigmentation of chicken (Marusich and Bauernfeind, 1981). Cryptoxanthin, another colorant in corn and less polar than lutein, was less effective than lutein and carotenes were least effective. Accumulation of ACAEE in chicken skin was proportional to the concentration (0-400 mg/kg feed) in feed in 3-wk trial (George et al., 1970). Canthaxanthin (0-400 mg/kg feed) also showed the similar trend in 2-wk trial (Juliusz and Hamilton, 1986).

Antioxidant effect of carotenoids

Astaxanthin and β-carotene decreased the lipid peroxidation in skin, compared to the control (Figure 6).

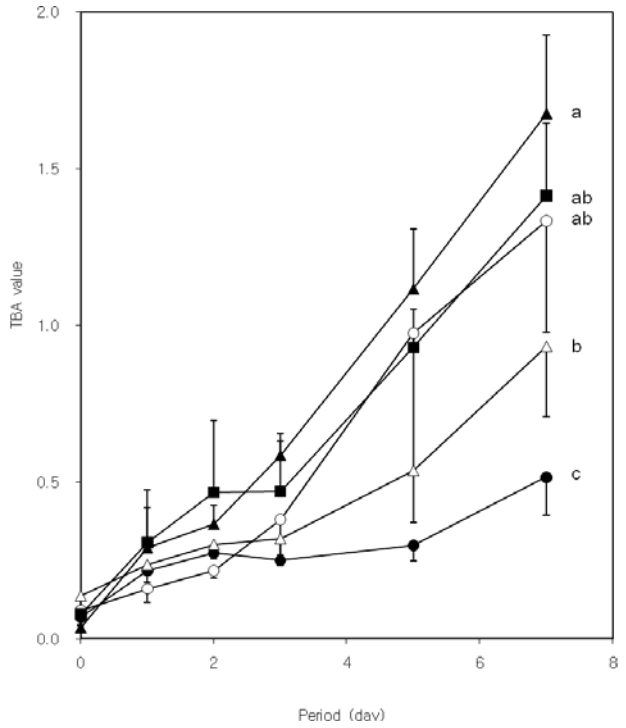

Figure 6. Antioxidant effect of carotenoids in skin of laying hens after 6-w feeding. Skin was incubated at 30℃ and malon aldehyde was used for standard. Symbols: ○, control; ●, astaxanthin; ▲, canthaxanthin; ■, ACAEE; and △, β-carotene. Bars indicate standard deviation.

Although the accumulation of β-carotene in skin was much lower than those of canthaxanthin and ACAEE, the antioxidation activity of β-carotene was significantly stronger. Astaxanthin was effectively accumulated and strongly inhibited lipid peroxidation (Figure 6). Other carotenoids, such as ACAEE, even increased lipid peroxidation (Figure 6). Therefore, when the color and antioxidation activity of skin were considered, astaxanthin is the recommended carotenoid in commercial market for the production of high quality meat.

Effect of carotenoids on color of egg yolk

During 6-wk trial of carotenoid feeding, eggs were collected and the color of egg yolk was measured. The Roche color fan score of egg-yolk were significantly increased from ~8.5 to ~15 by 1-wk feeding of xanthophylls (astaxanthin, canthaxanthin and ACAEE) (Figure 7). Feeding longer than one wk did not increase the color values of egg-yolk further. The highly preferred score by the Western people was 11-12 whereas the normal egg-yolk score was 6-7 (Roche, 1988).

In conclusion, astaxanthin is an excellent source for antioxidation and pigmentation in spent laying hens. The blood analyses suggested that the effect of astaxanthin was helpful for cardiovascular health for human.

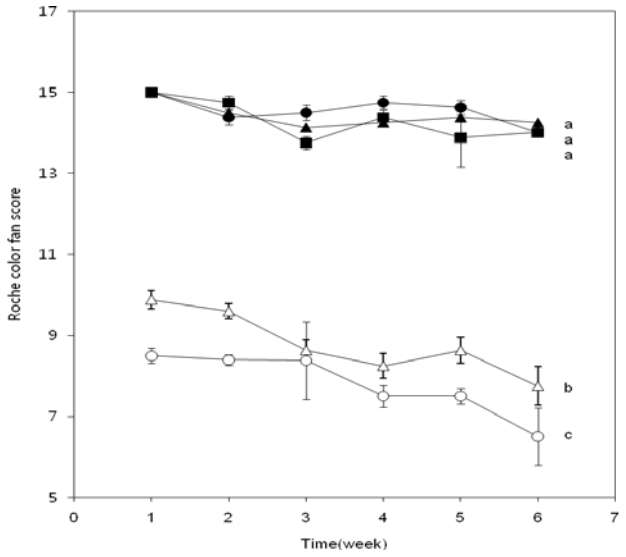

Figure 7. Changes of yolk color (Roche color fan score) by feeding of carotenoids in laying hens. Symbols: ○, control; ●, astaxanthin; ▲, canthaxanthin; ■, ACAEE; and △, β-carotene. Bars indicate standard deviation.

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