

Effect of Dietary Carotenoid on Egg Yolk Color and Singlet Oxygen Quenching Activity of Laying Hens

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The effects of dietary carotenoids on egg yolk were investigated in this study. Forty Rhode Island Red (RR) and 40 Silky Fowl (SF) hens that were 60 weeks old were used. Hens of each breed were randomly divided into four dietary groups. One group was fed a basal diet (crude protein 17%, metabolizable energy 2800 kcal/kg) only, whereas the other groups received a specific additive, namely, paprika extract, marigold petal extract, or *Paracoccus* cell powder, in addition to the same basal diet. The color and carotenoid content of egg yolk and singlet oxygen quenching activity were measured after 4 weeks. The total carotenoid content, zeaxanthin content, and singlet oxygen quenching activity in the yolk differed significantly between breeds and between diets (two-way ANOVA). The lutein content in egg yolk was affected by breed and diet, as well as by the interaction between these two factors. Regarding the Roche Yolk Color Fan values, only the effect of diet was significant. In terms of objective egg yolk color, there was a significant difference in lightness and yellowness between breeds. The total carotenoid content was higher in SF than in RR in all the groups. Likewise, the levels of zeaxanthin and lutein in the yolk were higher in SF than in RR ($P < 0.05$). The results of the present study suggest that dietary carotenoids are effective feed additives for laying hens, especially SF, to improve the color and singlet oxygen quenching activity of egg yolk.

Key words: carotenoid, hen, pigment, quenching activity, silky fowl

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Introduction

In recent years, multiple functionalities of carotenoids have been reported. In particular, it has been highlighted that zeaxanthin and lutein prevent age-related macular degeneration (Seddon *et al.*, 1994), and astaxanthin improves the amplitude of accommodation (Nagaki *et al.*, 2002). Furthermore, natural and synthetic pigments have been used to obtain specific egg yolk colors (Roberts, 2004; Santos-Bocanegra *et al.*, 2004). However, some countries, such as Sweden, do not allow the use of synthetic pigments (Roberts, 2004). Although it is well known that dietary carotenoids improve yolk color (Hinton *et al.*, 1974), other related changes in functionality

(i.e., singlet oxygen quenching activity of carotenoids) have not been fully investigated.

Silky Fowl (SF; *Gallus gallus domesticus*), which is originally from China, is an unusual chicken species with distinct characteristics: silky feathers, black-colored bones, and dark bluish skin. SF is characterized by melanin deposits on the surface of many organs, such as the skin, comb, bones, internal digestive organs, lungs, brain, and skeletal muscles, and has pigment granules in practically all tissues (Nozaki and Makita, 1998). SF may have a metabolic system different from that of general laying hens, and according to traditional Chinese medicine, SF meat is beneficial against women's diseases, and the eggs are beneficial against lung diseases (Akishinonmiya *et al.*, 1994). Notably, these traditions are likely one of the reasons that people, even those outside China, expect pharmacological effects from SF meat or eggs.

To our knowledge, there have been no reports on the effects of dietary carotenoids on egg production depending on the chicken breed. Therefore, in the present study, zeaxanthin, lutein, and astaxanthin were selected among the carotenoids added to improve the color of egg yolk, and their transfer to egg yolk as well as the singlet oxygen quenching activity

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were investigated using SF and Rhode Island Red (RR) hens.

The RR hen is a common chicken breed with a high egg-laying rate. As the metabolism of the SF hen is different from that of other chickens (Kojima *et al.*, 2014), we hypothesized that the accumulation of dietary carotenoids in egg yolks via feeding also varies.

Materials and Methods

Birds and Management

Sixty-week-old laying hens (40 RR and 40 SF) were used in this experiment, and they were placed in individual wire-floored cages. The hens were fed a basal diet (i.e., commercial formula feed: 17% crude protein, 2800 kcal/kg metabolizable energy; JA Higashinohon Kumiai Shiryou, Gunma, Japan) as a control, or any one of the following three treatments in addition to the basal diet: 30 mg/kg carotenoid (i.e., paprika extract; Ran-Red[®], Elanco Japan k.k., Tokyo, Japan; including 5 g/kg carotenoid, PA group), 60 mg/kg carotenoid (i.e., marigold petal extract; Xan-Yellow[®], Elanco Japan k.k., Tokyo, Japan; including 20 g/kg carotenoid, MA group), or 28 mg/kg carotenoid (i.e., *Paracoccus* cell powder; Panaferd-P[®], ENEOS Corporation, Tokyo, Japan; including 35 g/kg carotenoid, AX group). Carotenoids were commercially available in a powdered form. The carotenoid quantity of each treatment was selected based on the expected egg yolk color. Fletcher and Halloran (1983) reported that marigold-derived carotenoids at 60 mg/kg can be added to diets to give a Roche Yolk Color Fan (RYCF; Roche, Basel, Switzerland) score of 12.0. Therefore, 60 mg/kg marigold-derived carotenoid was used in the present study. As paprika-derived carotenoids have a higher pigmenting effect on egg yolk than that of marigolds (Fletcher and Halloran, 1983), half the amount of the marigold extract (30 mg/kg) was used in this study. Akiba *et al.* (2000) reported that an RYCF score of 12.7 was obtained by feeding a diet containing 16 mg/kg of astaxanthin derived from *Phaffia* yeast. The amount of astaxanthin used in this study was 16 mg/kg (28 mg/kg total carotenoids). There were 10 birds per treatment and five birds per replicate. The experiment lasted 28 days, and different diets and water were provided *ad libitum* during the experimental period. The experimental procedure was performed in accordance with the guidelines for animal experiments of the Tokyo Metropolitan Agriculture and Forestry Research Center (2020-1).

Measurement of Yolk Color

Eggs from days 22 to 28 were used for the measurements. Egg yolk color was measured using a spectrophotometer (CM-508b; Konica Minolta, Tokyo, Japan) and reported in the CIELAB system to calculate the values of lightness (L^*), redness (a^*), and yellowness (b^*), and the ratio of redness to yellowness (a/b). The yolk color was also measured using the RYCF.

Total Amount and Singlet Oxygen Quenching Activity of Carotenoids in Yolk

Eggs laid after 4 weeks of treatment were used for carotenoid evaluation. The yolk was weighed, and 15 mL of acetone was added to the yolk before stirring in a room at a temperature of $26 \pm 2^\circ\text{C}$ for 15 min. After collecting the su-

pernatant, 30 mL of dichloromethane-methanol (2:1, v/v) was added, and carotenoids were extracted by stirring at room temperature for 30 min (this process was repeated thrice). Hexane (100 mL) and water (100 mL) were added to each extract (100 mL), and the mixture was partitioned in a separating funnel. The upper layer (yellow) was collected and dehydrated with sodium sulfate anhydride, and the total quantity of carotenoids was assessed using an optical density of $\lambda_{\text{max}}=450\text{--}470$ nm (the following extinction coefficients, absorbance of 1% concentration, were adopted for quantification: 2400 for the control, 2072 for the PA group, 2500 for the MA group, and 2100 for the AX group; Britton *et al.*, 2004).

To examine singlet oxygen quenching activity, each extract was concentrated to dryness and subjected to silica gel column chromatography (Silica Gel 60, Kanto Chemicals, Tokyo, Japan; 10 mm i.d. by 150 mm; solvent: hexane). The silica gel column was developed in a stepwise manner with hexane (50 mL), hexane-acetone 1:1 (40 mL), and acetone (50 mL). Hexane-acetone (fraction I) and acetone (fraction II) eluents containing carotenoids were collected and concentrated to obtain yellow oils. The singlet oxygen quenching activity was examined by measuring the methylene blue-sensitized photooxidation of linoleic acid (Kobayashi and Sakamoto, 1999). To evaluate the carotenoid extracts in yolk, 80 μL of 0.025 mM methylene blue in ethanol, 100 μL of 240 mM linoleic acid in ethanol, and 140 μL ethanol with or without 40 μL extract solution (total carotenoids 50 μM , 200 μM , and 400 μM in dichloromethane, for a final total carotenoid concentration of 5 μM , 20 μM , and 40 μM , respectively) were added to micro glass vials (5.0 mL). The vials were closed tightly with a screw cap and a septum, and the mixtures were illuminated at 7000 lux and 22°C in corrugated cardboard for 3 h. Then, 100 μL of the reaction mixture was diluted to 3.0 mL with ethanol, and the absorbance at 235 nm was measured to estimate the formation of conjugated dienes (Hirayama *et al.*, 1994). The value in the absence of carotenoids was determined, and singlet oxygen quenching activity was calculated relative to this reference value. The activity corresponded to the concentration (μM) at which 50% inhibition was observed and was calculated by averaging the data from triplicate experiments.

High Performance Liquid Chromatography (HPLC) Analysis of Carotenoids in Yolk

Yolks of eggs that were laid after 4 weeks of treatment were used to analyze zeaxanthin, lutein, and astaxanthin contents. Analysis of zeaxanthin and lutein was performed as described hereafter. Firstly, 3% pyrogallol-ethanol solution (10 mL), 1% sodium chloride solution (0.5 mL), and 60% potassium hydroxide solution (1.0 mL) were mixed with each yolk sample (0.5 g) and stirred at 70°C for 30 min to saponify the oil in the sample. This mixture was then cooled, and 1% sodium chloride solution (22.5 mL) and ethyl acetate-hexane (1:9, v/v; 15 mL) were added to it before shaking it for 5 min. The upper layer of the solution was collected, and 15 mL of ethyl acetate-hexane (1:9, v/v) was added to the lower layer. The mixture was shaken for 5 min, and the upper layer was

collected; this step was repeated twice. The extracted upper layers were combined and concentrated to dryness to obtain a yellow oil. This yellow oil was dissolved in 10 mL ethanol and filtered through a 0.45- μ m Millipore filter disk (Merck KGaA, Darmstadt, Germany), and 10 μ L of the solution was subjected to HPLC (1260 Infinity II LC System, Agilent, Santa Clara, CA, USA) with a photodiode array detection (1260 Diode Array Detector HS, Agilent) to analyze carotenoid content. The column (Inertsil ODS-3V, GL Sciences, Tokyo, Japan; 4.6 mm i.d. by 150 mm) was developed with 98% methanol at a flow rate of 0.5 mL/min at 40°C. Zeaxanthin and lutein were eluted at a retention time (Rt) of 7.7 min and 6.8 min, respectively.

The analysis of astaxanthin was conducted as described hereafter. Each extract sample (1.0 g) was dissolved in 1 mL deionized water and 5 mL acetone containing 1.2 mM butylated hydroxytoluene (BHT). The mixture was stirred well after further addition of 10 mL acetone containing 1.2 mM BHT and was centrifuged at 3000 rpm for 10 min before collecting the upper layer of the solution. Thereafter, the lower layer was stirred well with 5 mL acetone containing 1.2 mM BHT and an additional 10 mL acetone containing 1.2 mM BHT was added. The mixture was centrifuged at 3000 rpm for 10 min, and the upper layer of the solution was collected. The extracted upper layers were mixed and acetone containing 1.2 mM BHT was added to obtain 50 mL of solvent. This solvent was subjected to silica gel HPLC (COSMOSIL Cholester, Nacalai Tesque, Kyoto, Japan; 4.6 mm i.d. by 250 mm) at 35°C. The mobile phase was a mixture of acetone, deionized water, and tetrahydrofuran (770:215:15, v/v) and was applied at a rate of 1.0 mL/min. Astaxanthin was eluted at Rt of 13.9 min and 14.8 min. The carotenoid content was calculated using an absorbance of 455 nm (for zeaxanthin and lutein) or 480 nm (for astaxanthin) and a calibration curve.

Statistical Analysis

Results were presented as the mean \pm standard error. The total carotenoid content in the yolk, the content of each carotenoid, egg yolk color, and singlet oxygen quenching activity were statistically analyzed using a two-way ANOVA, and the interaction between breed and diet was analyzed. Significant differences were compared using Tukey's multiple comparisons test. All statistical analyses were performed using R software (<http://www.R-project.org/>; Ihaka and Gentleman, 1996), and the significance level was set at $P < 0.05$.

Results

Table 1 shows the egg yolk color values. Diet significantly affected all items ($P < 0.001$). Breed had a significant effect on L*, b*, and a/b values ($P < 0.001$). Significant interactions between breed and diet were observed for a* and a/b values. Furthermore, the a* values and RYCF scores increased with the addition of carotenoids, i.e., the a* values and the RYCF scores of the experimental groups were significantly higher than those of the control groups ($P < 0.05$). Additionally, the L* and b* values in SF were higher than those in RR. Similarly, the accumulation of carotenoids in egg yolk was also higher in SF than in RR (Fig. 1). Specifically, significantly more carotenoids accumulated in the PA, MA, and AX groups of SF than in the control group ($P < 0.05$), while there was no significant difference among RR eggs in the different treatment groups (Fig. 1). Moreover, there were more carotenoids in the eggs of the MA group of SF than in the eggs of the PA and AX groups of RR and the eggs in both control groups ($P < 0.05$; Fig. 1). The zeaxanthin, lutein, and astaxanthin contents in the yolk increased in the PA, MA, and AX groups in both SF and RR (Fig. 2). The zeaxanthin and lutein contents recorded in both PA and MA groups were significantly higher in the yolk of SF eggs than in that of RR eggs (Fig. 2A

Table 1. Yolk color comparison

Breed ¹	Diet ²	L* value	a* value	b* value	a/b	Roche yolk color fan
RR	Cont	56.1 \pm 0.9 ^{ab}	2.46 \pm 0.32 ^d	39.0 \pm 1.5 ^{ab}	0.06 \pm 0.01 ^d	7.2 \pm 0.3 ^c
	PA	46.8 \pm 0.9 ^c	19.94 \pm 0.54 ^a	32.0 \pm 1.4 ^{bc}	0.63 \pm 0.02 ^a	14.4 \pm 0.2 ^a
	MA	53.2 \pm 0.8 ^{bc}	6.37 \pm 0.44 ^c	35.8 \pm 2.7 ^{bc}	0.18 \pm 0.01 ^c	9.8 \pm 0.2 ^b
	AX	48.8 \pm 1.3 ^{dc}	16.46 \pm 0.76 ^b	29.7 \pm 1.8 ^c	0.56 \pm 0.02 ^{ab}	14.2 \pm 0.2 ^a
SF	Cont	58.7 \pm 0.7 ^a	2.81 \pm 0.40 ^d	40.9 \pm 2.5 ^{ab}	0.07 \pm 0.01 ^d	7.3 \pm 0.2 ^c
	PA	51.0 \pm 0.6 ^{cd}	17.79 \pm 0.87 ^{ab}	36.1 \pm 2.0 ^b	0.50 \pm 0.03 ^b	14.2 \pm 0.2 ^a
	MA	56.3 \pm 0.8 ^b	6.20 \pm 0.43 ^c	46.5 \pm 2.0 ^a	0.13 \pm 0.01 ^{cd}	9.7 \pm 0.2 ^b
	AX	51.9 \pm 1.1 ^{cd}	18.12 \pm 0.74 ^{ab}	34.8 \pm 2.5 ^{bc}	0.54 \pm 0.03 ^{ab}	14.1 \pm 0.2 ^a
Variables				Probabilities		
Breed		***	NS	***	***	NS
Diet		***	***	***	***	***
Breed \times Diet		NS	*	NS	**	NS

Data are presented as mean \pm SE ($n = 12$).

^{a-e} Means within a column with different letters differ significantly ($P < 0.05$).

¹ RR = Rhode Island Red; SF = Silky Fowl.

² Cont = basal diet; PA = basal diet with 30 mg/kg carotenoid from paprika extract; MA = basal diet with 60 mg/kg carotenoid from marigold petal extract; AX = basal diet with 28 mg/kg carotenoid from *Paracoccus* cell powder.

*, **, *** and NS are $P < 0.05$, $P < 0.01$, $P < 0.001$ and no significant differences, respectively.

and B; $P<0.05$). Conversely, there was no difference in astaxanthin content between the breeds. Table 2 presents the

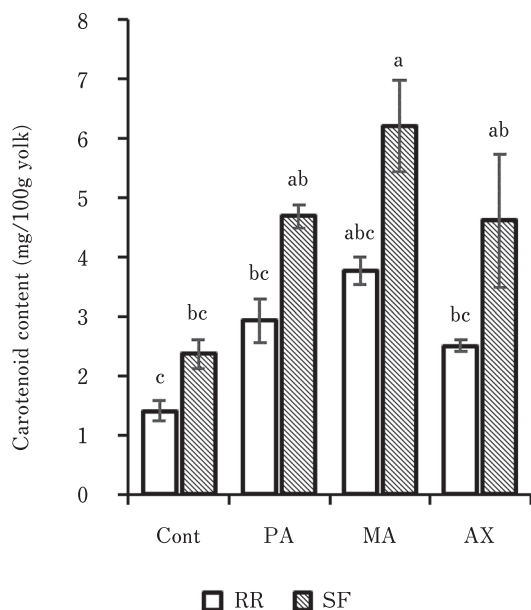


Fig. 1. **Total carotenoid content in egg yolk.** Data are presented as the mean \pm SE ($n=3$). Bars not sharing a common letter are significantly different ($P<0.05$). Cont=basal diet; PA=basal diet with 30 mg/kg carotenoid from paprika extract; MA=basal diet with 60 mg/kg carotenoid from marigold petal extract; AX=basal diet with 28 mg/kg carotenoid from *Paracoccus* cell powder. RR=Rhode Island Red; SF=Silky Fowl.

effects of breed and dietary carotenoids on egg yolk. Diet exerted significant effects on total carotenoid content, zeaxanthin content, lutein content, astaxanthin content, and singlet oxygen quenching activity ($P<0.001$). The breed had a significant effect on the total carotenoid content, zeaxanthin content, lutein content, and singlet oxygen quenching activity ($P<0.001$, $P<0.01$, $P<0.001$, and $P<0.05$, respectively). Furthermore, there was a significant interaction between the effects of breed and diet on lutein content ($P<0.01$). Fig. 3 shows the values for the singlet oxygen quenching activity of egg yolk (50% inhibitory concentration) for all the experimental groups. Notably, the RR control group significantly differed from all the other experimental groups for this parameter. No significant differences were observed among the other experimental groups. A significant difference was observed between the control and AX groups in the SF eggs.

Discussion

We investigated the effects of dietary carotenoids on the eggs of RR and SF breeds in the present study. The accumulation of total carotenoids in the yolk of SF eggs exceeded that in the yolk of RR eggs. This result suggests that dietary carotenoids are more likely to transfer to the yolks of SF eggs compared to those of RR eggs. In particular, there was a significant interaction between the effects of breed and diet on lutein content, which showed that the combination of MA dietary treatment and the SF breed resulted in a higher accumulation of lutein in the yolk. Feeding lutein to laying hens enhances hepatic SOD activity (Jang *et al.*, 2014), while feeding them with lycopene increases yolk oxidative stability (An *et al.*, 2019). The present study revealed similar findings,

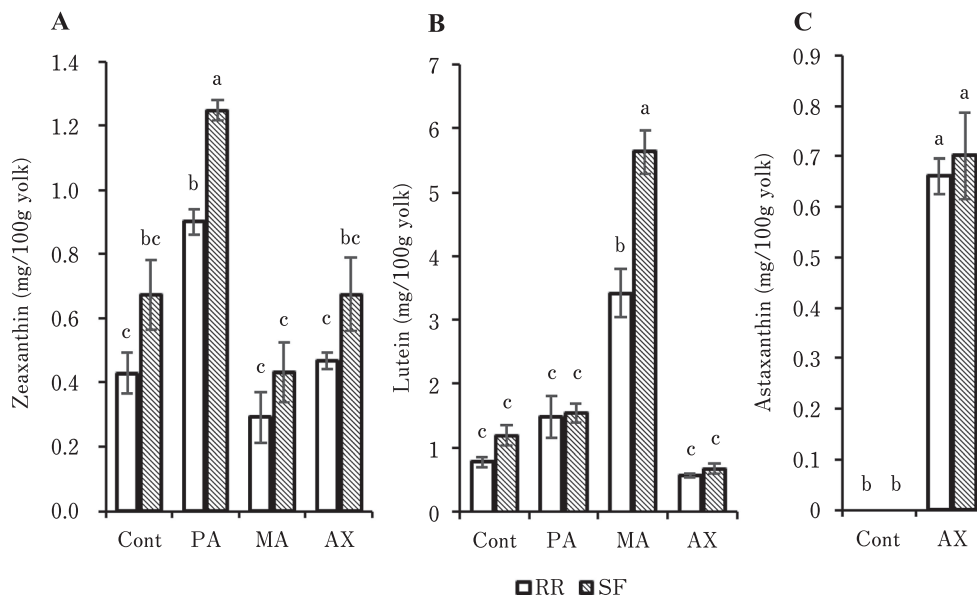


Fig. 2. **Zeaxanthin, lutein, and astaxanthin contents in egg yolk.** Data are presented as the mean \pm SE ($n=5$). Bars not sharing a common letter are significantly different ($P<0.05$). RR=Rhode Island Red; SF=Silky Fowl. Cont=basal diet; PA=basal diet with 30 mg/kg carotenoid from paprika extract; MA=basal diet with 60 mg/kg carotenoid from marigold petal extract; AX=basal diet with 28 mg/kg carotenoid from *Paracoccus* cell powder.

Table 2. Effects of breed and dietary carotenoid on total carotenoid content, each carotenoid (zeaxanthin, lutein and astaxanthin) content of egg yolk, and singlet oxygen quenching activity of egg yolk ($n=3$)

Variables	Items				
	Total carotenoid	Zeaxanthin	Lutein	Astaxanthin	Quenching activity
Breed ¹	***	**	***	NS	*
Diet	***	***	***	***	***
Breed × Diet	NS	NS	**	NS	NS

¹ Breed = Rhode Island Red and Silky Fowl.

*, **, *** and NS are $P < 0.05$, $P < 0.01$, $P < 0.001$ and no significant differences, respectively.

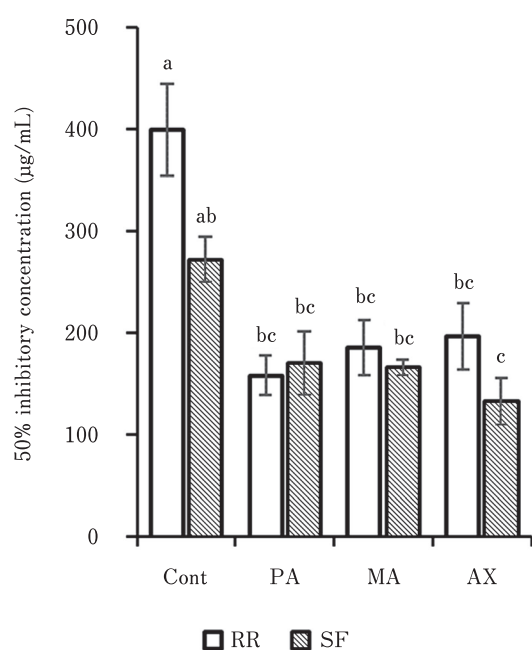


Fig. 3. Singlet oxygen quenching activity of egg yolk (IC₅₀). Data are presented as the mean \pm SE ($n=3$). Bars not sharing a common letter are significantly different ($P < 0.05$). Cont = basal diet; PA = basal diet with 30 mg/kg carotenoid from paprika extract; MA = basal diet with 60 mg/kg carotenoid from marigold petal extract; AX = basal diet with 28 mg/kg carotenoid from *Paracoccus* cell powder. RR = Rhode Island Red; SF = Silky Fowl.

although the carotenoid types were dissimilar. Dietary carotenoids enhanced the singlet oxygen quenching activity of egg yolk, and SF hens showed greater carotenoid accumulation in egg yolk than RR hens. This result was also observed in the control groups. The singlet oxygen quenching activity in the SF control group was moderately high compared to the RR control group, and it was difficult to detect differences among the SF experimental groups. *Paracoccus* cell powder contains high levels of antioxidants, carotenoids, adonirubin, and adonixanthin. Maoka *et al.* (2013) reported that adonirubin and adonixanthin had almost similar anti-

oxidant activities as those of astaxanthin. Therefore, adonirubin and adonixanthin may also have affected the singlet oxygen quenching activity in the PA groups in this study.

The RYCF scores and the a^* values increase with the accumulation of lutein in egg yolk (Islam *et al.*, 2017). The RYCF scores and the a^* values were significantly higher in the MA group than those in the control group for both SF and RR in this study ($P < 0.05$). Notably, increased lutein and zeaxanthin contents in egg yolk have been reported to improve the egg yolk color (Shin *et al.*, 2016). The RYCF scores, and the a^* and a/b values for the PA group were significantly higher ($P < 0.05$), while the L^* values were lower than that in the control group for both SF and RR. Furthermore, increasing astaxanthin content in the yolk intensifies the yolk color (Prommetta *et al.*, 2020). The RYCF scores for the AX groups of both the breeds reached a value of 14. The a^* and a/b values were also significantly higher in this group, while the L^* values were lower than that in the control group. Galobart *et al.* (2004) and Lokaewmanee *et al.* (2011) reported the a/b value as the most sensitive parameter for evaluating egg yolk color. Similar results were obtained for the RYCF scores and the a/b values in the evaluation of egg yolk color (Baiao *et al.*, 1999). However, the RYCF score seemed to evaluate yolk color more accurately than the a/b value in the present study. The a/b value of the MA group significantly differed from that of the control group for RR eggs ($P < 0.05$), while there was no such difference observed for SF eggs. The L^* and b^* values were significantly affected by both the diet and breed, which suggests that dietary carotenoids affect SF and RR differently. The lutein content of egg yolk does not influence the lightness (L^*) and yellowness (b^*) of the egg yolk (Islam *et al.*, 2017). Nevertheless, it has been reported that marigold carotenoids affect the L^* and b^* values of egg yolk (Fletcher and Halloran, 1983). The L^* value of the MA group of SF was significantly lower than that of its corresponding control group ($P < 0.05$), while there was no significant difference between the L^* values of the control and MA groups of RR. There was a significant difference between the MA groups of RR and SF, suggesting that the change in egg yolk color caused by dietary carotenoids differs depending on the breed.

The increase in the lutein and zeaxanthin contents in egg

yolk were shown to be dependent upon the feeding duration of diets containing 20% corn distillers' dried grains with solubles (Shin *et al.*, 2016). However, it has been reported that the carotenoid content in egg yolk reaches its maximum value 14 days after feeding and then decreases gradually (Islam *et al.*, 2017). We showed that the accumulation of carotenoids varied depending on the breed, which suggests that the results will differ depending on the experimental hen breed. The SF breed might have the ability to accumulate more carotenoids in the yolk with long-term feeding according to the results of this study.

In conclusion, feeding laying hens with a carotenoid-enriched diet improves egg yolk color and enhances the singlet oxygen quenching activity of egg yolk. Notably, SF hens accumulate more carotenoids (lutein and zeaxanthin) in egg yolk than RR hens. Since SF eggs contain more lipids than common chicken eggs (Ishibashi *et al.*, 2001; Kagawa, 2018), more fat-soluble carotenoids may accumulate in the eggs of this breed. Therefore, SF might be a good breed for improving egg quality through addition of carotenoid in the feed.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest.

References

- Akiba Y, Sato K, Takahashi K, Takahashi Y, Furuki A, Konashi S, Nishida H, Tsunekawa H, Hayasaka Y and Nagao H. Pigmentation of egg yolk with yeast *Phaffia rhodozyma* containing high concentration of astaxanthin in laying hens fed on a low-carotenoid diet. *Japanese Poultry Science*, 37: 77–85. 2000.
- Akishinomiya F, Kakizawa R, Roberts M and Roberts V. *Illustrated encyclopedia of European poultry*. pp. 40–41. Heibonsya. Tokyo. 1994.
- An BK, Choo WD, Kang CW, Lee J and Lee KW. Effects of dietary lycopene or tomato paste on laying performance and serum lipids in laying hens and on malondialdehyde content in egg yolk upon storage. *Journal of Poultry Science*, 56: 52–57. 2019.
- Baiao NC, Mendez J, Mateos J, Garcia M and Mateos GG. Pigmenting efficacy of several oxycarotenoids on egg yolk. *Journal of Applied Poultry Research*, 8: 472–479. 1999.
- Britton G, Liaen-Jensen S and Pfander H. *Carotenoids Handbook*. Birkhäuser Verlag. Basel. Boston. Berlin. 2004.
- Fletcher DL and Halloran HR. Egg yolk pigmentation properties of a marigold extract and paprika oleoresin in a practical type diet. *Poultry Science*, 62: 1205–1210. 1983.
- Galobart J, Sala R, Rincóón-Carruyo X, Manzanilla EG, Vilà B and Gasa J. Egg yolk color as affected by saponification of different natural pigmentation sources. *Journal of Applied Poultry Research*, 13: 328–334. 2004.
- Hinton CF, Fry JL and Harms RH. Influence of a xanthophyll-free pullet grower diet on subsequent egg yolk pigmentation. *Poultry Science*, 53: 223–226. 1974.
- Hirayama O, Nakamura K, Hamada S and Kobayashi Y. Singlet oxygen quenching ability of naturally occurring carotenoids. *Lipids*, 29: 149–150. 1994.
- Ihaka R and Gentleman R. R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, 5: 299–314. 1996.
- Ishibashi G, Kim Sangae and Kikunaga S. The value as food and screening for the nutritional effects of the silky. *Japan Integrated Study of Dietary Habits*, 12: 148–153. 2001.
- Islam KMS, Khalil M, Männer K, Raila J, Rawel H, Zentek J and Schweigert FJ. Lutein specific relationships among some spectrophotometric and colorimetric parameters of chicken egg yolk. *Journal of Poultry Science*, 54: 271–277. 2017.
- Jang I, Ko Y, Kang S, Kim S, Song M, Cho K, Ham J and Sohn S. Effects of dietary lutein sources on lutein-enriched egg production and hepatic antioxidant system in laying hens. *Journal of Poultry Science*, 51: 58–65. 2014.
- Kagawa A. *Standard tables of food composition in Japan (Seventh Revised Edition)*. pp. 186–187. Kagawa Education Institute of Nutrition. Tokyo. 2018.
- Kobayashi M and Sakamoto Y. Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*. *Biotechnology Letters*, 21: 265–269. 1999.
- Kojima S, Saegusa H and Sakata M. Histidine-containing dipeptide concentration and antioxidant effects of meat extracts from silky fowl: comparison with meat-type chicken breast and thigh meats. *Food Science and Technology Research*, 20: 621–628. 2014.
- Lokaewmanee K, Yamauchi K, Komori T and Saito K. Enhancement of yolk color in raw and boiled egg yolk with lutein from marigold flower meal and marigold flower extract. *Journal of Poultry Science*, 48: 25–32. 2011.
- Maoka T, Yasui H, Ohmori A, Tokuda H, Suzuki N, Osawa A, Shindo K and Ishibashi T. Anti-oxidative, anti-tumor-promoting, and anti-carcinogenic activities of adonirubin and adonixanthin. *Journal of Oleo Science*, 62: 181–186. 2013.
- Nagaki Y, Hayasaka S, Yamada T, Hayasaka Y, Sanada M and Uomori T. Effects of astaxanthin on accommodation, critical flicker fusion, and pattern visual evoked potential in visual display terminal workers. *Journal of Traditional Medicines*, 19: 170–173. 2002.
- Nozaki A and Makita T. The surface color measurement of major tissues of Silky Fowls and White Leghorns. *Journal of Veterinary Medical Science*, 60: 489–493. 1998.
- Prommetta K, Attamangkune S and Ruangpanit Y. Krill meal enhances antioxidant levels and n-3 fatty acid content of egg yolk from laying hens fed a low-pigment diet. *Journal of Poultry Science*, 57: 192–199. 2020.
- Roberts JR. Factors affecting egg internal quality and egg shell quality in laying hens. *Journal of Poultry Science*, 41: 161–177. 2004.
- Santos-Bocanegra E, Ospina-Osorio X and Oviedo-Rondon EO. Evaluation of xanthophylls extracted from *tagetes erectus* (Marigold Flower) and *capsicum Sp.* (Red Pepper Paprika) as a Pigment for Egg-yolks compare with synthetic pigments. *International Journal of Poultry Science*, 3: 685–689. 2004.
- Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT, Yannuzzi LA and Willett W. Dietary carotenoids, vitamin A, C, and E, and advanced age-related macular degeneration. *Journal of the American Medical Association*, 272: 1413–1420. 1994.
- Shin HS, Kim JW, Kim JH, Lee DG, Lee S and Kil DY. Effect of feeding duration of diets containing corn distillers dried grains with solubles on productive performance, egg quality, and lutein and zeaxanthin concentrations of egg yolk in laying hens. *Poultry Science*, 95: 2366–2371. 2016.