Short Paper

The Accumulation of Pigments from Paprika in the Integument of Goldfish *Carassius auratus*

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The skin and fin colors of fish are mainly due to the presence of melanin, pteridine, guanine and carotenoid. Of them, especially carotenoid is a primary factor of the color of fishes. As far as we know, animals cannot synthesize carotenoids *de novo*, and are, therefore, dependent upon dietary carotenoids.¹⁾ Hence carotenoid is commonly used as the pigmentary feedstuff for goldfish, carp, salmon, sea bream, yellowtail, shrimp, lobster, etc.²⁾ There are both synthetically produced β , β -carotene, canthaxanthin and astaxanthin, and natural materials such as carrot, palm oil, marigold, *Dunaliella*, *Spirulina*, krill and crustacean-meals.²⁾ Recently, the capsanthin-containing paprika is attracting much attention in aquacultural circles. Therefore we investigated the effect of paprika on the pigmentation of goldfish *Carassius auratus*.

Goldfishes (mean weight, 12.0 g) were divided into three groups, control, Biolet 100 (extract from paprika, capsanthin 40 ppm/kg diet) and Biolet 300 (capsanthin 120 ppm/kg diet) with ten goldfishes in each group. The water tanks were set in a place having direct sunlight (A) (illumination: 1200–15000 lux.) and indirect sunlight (B) (550–700 lux.). The control diet was composed of 25.5% wheat flour, 10.0% tapioca starch, 25.0% soybean meal, 5.0% corn gluten meal, 30.4% fish meal, 2.0% soybean oil, 1.5% NaCl, 0.4% vitamin mixture and 0.2% mineral mixture. The experimental diets, Biolet 100 and 300, were prepared by adding 2.0% and 6.0% Biolet instead of wheat flour to the control diet, respectively. Diets were fed at a rate of about 1.5–2.5% body weight three times a day for 27 weeks. After feeding experiments, the integument of goldfish was removed. According to the methods previously reported,³⁾ the contents of individual carotenoids in the integument were determined. Identification and structural elucidation of each carotenoid were carried out by means of visible light absorption spectra (VIS), mass spectra (MS), circular dichroism spectra (CD) and ¹H-NMR spectra, enzymatic hydrolysis with lipase, co-thin layer chromatography (co-TLC) and co-high performance liquid chromatography (co-HPLC) with authentic samples and chemical reactions by our routine procedures.³⁾

The contents of individual carotenoids in the integument of the goldfish after feeding experiments are shown in Table 1. Carotenoids from paprika were accumulated in

Table 1. The contents of individual carotenoids in the integument of the goldfish after feeding experiments

Group* ¹ Place	(mg/individual)					
	Control A* ²	B* ³	Biolet 100 A	B	Biolet 300 A	B
Total contents	0.51	0.27	0.93	0.49	1.44	0.65
Lutein A	0.05	0.03	+* ⁵	+	+	+
Lutein B	0.10	0.05	+	+	+	+
(3R, 3'R)-Zeaxanthin	0.02	0.01	0.03	0.02	0.04	0.02
4-Ketolutein A (Fritschellaxanthin)	0.02	0.01	+	+	0.01	+
4-Ketolutein B (α -Doradexanthin)	0.04	0.03	0.04	0.02	0.03	0.02
(3S, 3'R)-4-Ketozeaxanthin (β -Doradexanthin)	0.03	0.02	0.03	0.01	0.04	0.01
(3S, 3'S)-Astaxanthin	0.17	0.09	0.19	0.13	0.20	0.10
Capsanthin	—* ⁴	—	0.09	0.05	0.19	0.09
Compound (1) (4-Ketocapsanthin)	—	—	0.44	0.23	0.87	0.39
Unidentified carotenoids	0.08	0.03	0.11	0.03	0.06	0.02

*¹ N=10 for each group.*² The direct sunlight place.*³ The indirect sunlight place.*⁴ Symbol: —, not detected.*⁵ Symbol: +, trace.

all administered groups. The accumulation of carotenoids per individual goldfish bred at place (A) was approximately 2.0 times higher than that of place (B) and increased in proportion to the dose of paprika. Lutein A, lutein B, zeaxanthin, 4-ketolutein B(α -doradexanthin), 4-ketozeaxanthin(β -doradexanthin) and astaxanthin have hitherto been known as principal carotenoids in the goldfish.⁴⁾ It is well established that these ketocarotenoids are formed from zeaxanthin or lutein A *via* the following oxidative metabolic pathways.^{4,5)} (i) zeaxanthin $\rightarrow$ β , β -carotene-triol $\rightarrow$ 4-ketozeaxanthin $\rightarrow$ astaxanthin, (ii) lutein A $\rightarrow$ lutein B $\rightarrow$ β , ϵ -carotene-triol $\rightarrow$ 4-ketolutein B. In the goldfish fed paprika, capsanthin, astaxanthin and unknown carotenoid (1) were found as major carotenoids (Table 1). Especially compound (1) occupied 47–60% of the total carotenoids. Compound (1) had molecular ion *m/z* 598, compatible with C₄₀H₅₄O₄ and showed VIS; $\lambda_{\max}$ 473 nm (in ether). The characteristic ¹H-NMR (300 MHz, CDCl₃) signals at δ

Fig. 1. CD spectra of compound (1), 4-ketocapsanthin — and capsanthin --- in EPA solution at 20°C.

1.21 (3H, s), 1.32 (3H, s), 1.81 (1H, t), 1.95 (3H, s), 2.16 (1H, dd), 3.68 (1H, sbr) and 4.33 (1H, dd) indicated the presence of 3-hydroxy-4-keto-β-end group in the molecule of (1) (Fig. 1).⁶ On the other hand, the ¹H-NMR signals at δ 0.84 (3H, s), 1.21 (3H, s), 1.37 (3H, s), 1.49 (1H, dd), 2.96 (1H, dd) and 4.52 (1H, m) were compatible with 3',5'-(*trans*)-3'-hydroxy-6'-keto-κ-end group (Fig. 1).⁶ Although the CD spectra of (1) showed ca. 20 nm bathochromic shift owing to the presence of a keto group in the C-4 position, it was similar to that of capsanthin obtained from the paprika as shown in Fig. 1. This fact indicated that (1) has the same chiralities at C-3, C-3' and C-5' as those of capsanthin.⁷ It was determined to be (3S, 3'S, 5'R)-3, 3'-dihydroxy-β, κ-carotene-4, 6'-dione (4-ketocapsanthin) based on these evidences described above. On the other hand, it seemed that an increase of astaxanthin was due to the oxidative metabolism of zeaxanthin^{4,5} contained in paprika.

Morimoto *et al.*⁸ reported that paprika had the effect of pigmentation of fancy red carp and that capsanthin and its metabolic product were the most responsible for the pigmentation of fancy red carp. Our results in the case of goldfish were also generally in accordance with theirs. The metabolic product from capsanthin was determined to be 4-ketocapsanthin in this experiment. Therefore we drew the following conclusions. (i) Paprika was effective for pig-

mentation of goldfish and fancy red carp and its effect was increased remarkably by sunlight. (ii) The goldfish and the fancy red carp accumulated capsanthin and oxidatively metabolized capsanthin to 4-ketocapsanthin.

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