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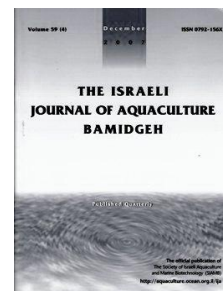
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## Effect of Astacin on Growth and Color Formation of Juvenile Red-White Ornamental Carp (*Cyprinus carpio* var. koi L)

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### Abstract

Body color is an important quality and determines the value of koi. Fish are unable to fully synthesize carotenoid pigments. Therefore, carotenoid needs to be added to their diets. This study determined the effect of astacin on the growth and body color enhancement in healthy red-white ornamental carp (*Cyprinus carpio* var. koi). Fish were fed diets containing 0 (control), 50, 100, 150, 200, or 250 mg astacin/kg diet for 8 weeks. At the end of the experiment, samples of red-white, red, and white skin, head, scales, and fin ray were collected for carotenoid content analysis and visual observation. Fish weight and specific growth rate were significantly higher in fish fed the 150, 200, and 250 mg diets than in fish fed the control while the feed conversion rates in the 200 and 250 groups were significantly lower than in the control. Carotenoid deposition in the skin, head, scale, and fin ray of fish fed diets containing astacin was significantly higher than that those fed the control. In addition, a higher astacin content resulted in a brighter body color. The highest carotenoid concentration was achieved when 250 mg astacin/kg feed was added. Astacin was mainly deposited in the skin, scales, and head.

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## Introduction

Expansion of the ornamental fish culture industry has enabled Indian producers to market locally and internationally (Jha et al., 2007). Koi, a very popular ornamental variety of common carp, has been selectively bred in Japan for centuries and is increasingly bred in China, the USA, and other countries (Balon, 1995). The body surface of koi is silvery, white as snow, and inlaid with red spots. Color plays the most important role in the overall acceptability of red-white koi, while body shape, fin shape, and size are also important in determining its market value (Paripatananont et al., 1999).

The color of fish skin is primarily dependent on chromatophores (melanophores, xanthophores, erythrophores, iridophores, leucophores, cyanophores) that contain pigments such as melanins, carotenoids (e.g., astaxanthin, canthaxanthin, lutein, zeaxanthin), pteridines, and purines (Withers, 1992). Fish exhibit different coloration patterns as a result of the dispersion or aggregation of chromatophores and the distribution of chromatophores in the skin (Withers, 1992).

Carotenoids are soluble lipid pigments that are responsible for the formation of skin color in ornamental fish (Kestemont et al., 1990; Paripatananont et al., 1999). Astaxanthin is a carotenoid well-known for its red coloring pigment (Naguib, 2000). Astacin, derived from the oxidation of astaxanthin, is a red carotenoid ketone pigment found in crustacean shells. As fish are unable to form or convert intermediary precursor pigments to carotenoids (Goodwin, 1984), pigments need to be supplied in the diet to achieve color formation. The major color-determining gene(s) in koi have been researched (Gomelsky et al., 2003), as well as the dietary supplementation of carotenoids to enhance fish color (Fey and Meyers, 1980; Gouveia et al., 1996b, 1997, 1998; Ako et al., 2000; Alagappan et al., 2004; Diler et al., 2005; Tejera et al., 2007). The purpose of this study was to determine the effect of astacin as a carotenoid source on growth and pigment formation in red-white koi.

## Materials and Methods

**Feed composition.** Six diets containing different levels of astacin (10%, Jiakangyuan Co., Ltd, Beijing) were prepared (Table 1). Ingredients were ground and passed through an 80-mesh screen. Dry ingredients were mixed with oil, and water was added until a stiff dough was achieved. Diets were extruded through a mincer with a die. The resulting strands were shadow dried, broken, sieved into pellets (2 × 3 mm), and stored in plastic bags at 4°C until use.

**Fish breeding.** Four hundred and fifty healthy juvenile red-white koi were obtained from the Xiaotangshan Tropical Fish Breeding Center of the Beijing Fisheries Research Institute and bred in our laboratory for three weeks. The initial mean body weight and length were 13.6±0.7 g and 8.83±0.5 cm. The fish were randomly distributed into six treatment

Table 1. Composition and analysis of diets for red-white koi.

	Diet (mg astacin/kg diet)					
	Control	50	100	150	200	250
<i>Ingredient (%)</i>						
Flour	32.5	32.5	32.5	32.5	32.5	32.5
Fishmeal	30	30	30	30	30	30
Soybean meal	30	30	30	30	30	30
Soybean oil	5	5	5	5	5	5
Vitamin mix <sup>1</sup>	1	1	1	1	1	1
Mineral mix <sup>2</sup>	1	1	1	1	1	1
Cellulose	0.500	0.495	0.49	0.485	0.48	0.475
Astacin	0	0.005	0.01	0.015	0.02	0.025
<i>Chemical analysis (%)</i>						
Crude protein	34.84	34.85	35.02	35.24	35.14	35.33
Crude lipid	8.17	8.42	8.21	8.55	8.31	8.26
Moisture	8.12	8.33	8.45	8.19	8.53	8.23
Crude ash	10.03	10.21	10.42	10.14	10.32	10.51
Astacin (mg/kg)	0	4.8	9.6	14.9	19.7	24.7
Total carotenoid (mg/kg)	125.56	129.92	135.01	143.42	145.02	151.53

<sup>1</sup> per kg diet: vitamin C 100 mg; thiamin hydrochloride 200 mg; riboflavin 200 mg; calcium pantothenate 1000 mg; nicotinic acid 500 mg; pyridoxine hydrochloride 12 mg; biotin 3 mg; folic acid 40 mg; vitamin B<sub>12</sub> 0.01 mg; inositol 400 mg; choline chloride 1000 mg; retinyl acetate 2000 IU; cholecalciferol 1000 IU; alpha-tocopheryl acetate 3350 mg; menadione 3600 mg. Ingredients were diluted with wheat flour to 1 kg.

<sup>2</sup> per kg diet: calcium lactate 300 g; K<sub>2</sub>PO<sub>4</sub> 241 g; CaHPO<sub>4</sub>·2H<sub>2</sub>O 126.8 g; MgSO<sub>4</sub>·7H<sub>2</sub>O 150 g; ferric citrate 15 g; ZnSO<sub>4</sub>·7H<sub>2</sub>O 2 g; MnSO<sub>4</sub>·H<sub>2</sub>O 800 mg; CuSO<sub>4</sub>·5H<sub>2</sub>O 8 g; CoCl<sub>2</sub>·6H<sub>2</sub>O 100 mg; KI 100 mg; Na<sub>2</sub>SeO<sub>3</sub> 40 mg. Ingredients were diluted with wheat flour to 1 kg.

groups with 25 fish in each of 18 triplicate 150-l tanks (70 × 56 × 37 cm). The fish were fed one type of diet twice a day (09:00 and 18:00) at 2% of their body weight for 8 weeks. Water temperature was maintained at 25±1°C, pH ranged 5.5-6, and dissolved oxygen, monitored daily, was kept above 3.0 mg/l. The natural photoperiod was used. Mortality was monitored daily. The daily exchange rate of water was 30%.

**Tissue analysis.** Skin pigmentation was determined by visual evaluation and photography at the end of the experiment. Thirty fish from each treatment group (ten randomly-sampled fish from each tank) were sacrificed. Samples of skin, head, and scales from red-white, red, and white areas were taken. The red area of the fin ray was also excised. Tissues were immediately frozen and stored at -80°C. Total carotenoid contents in the skin, head, scales, and fin ray were determined using a spectrophotometer after acetone extraction (Choubert and Storebakken, 1989) and expressed using extinction coefficients ( $E^{1\%}_{1\text{cm}}$ ) of 2100 for astacin at their respective absorption maxima in acetone solution (Gouveia et al., 1997). Total carotenoid content (mg/kg) was calculated as (absorption at maximum wavelength × volume of extract × dilution factor)/(extinction coefficient × sample wt). The relative increased amount of total carotenoid (%) was determined as 100(total carotenoid content of trial group - total carotenoid content of control group)/total carotenoid content of control group.

**Growth and feed efficiency.** All fish in each treatment group were weighed and weight gain (%) was determined as 100(final body wt - initial body wt)/initial body wt. The specific growth rate was calculated as 100(Ln final body wt - Ln initial body wt)/day. The feed conversion rate was calculated as feed given/wt gain.

**Statistical analysis.** Data are presented as means±standard deviation. Statistical analysis was performed using SPSS (Statistical Products Service and Solutions) version 13.0 for Microsoft Windows. Statistical significance was tested at a 0.05 probability level.

## Results

Visual observation showed that red areas were small in the control group and increased as the dietary astacin content increased (Fig. 1). The greatest red skin areas were obtained in fish fed the 200 mg diet.

Weight gain and specific growth rate were significantly higher in fish fed the 150, 200, or 250 mg diets while feed conversion was significantly lower in fish fed the 200 or 250 mg diets (Fig. 2). There was no mortality associated with any of the treatments.

The total carotenoid contents in the head and scale of red-white and red areas from

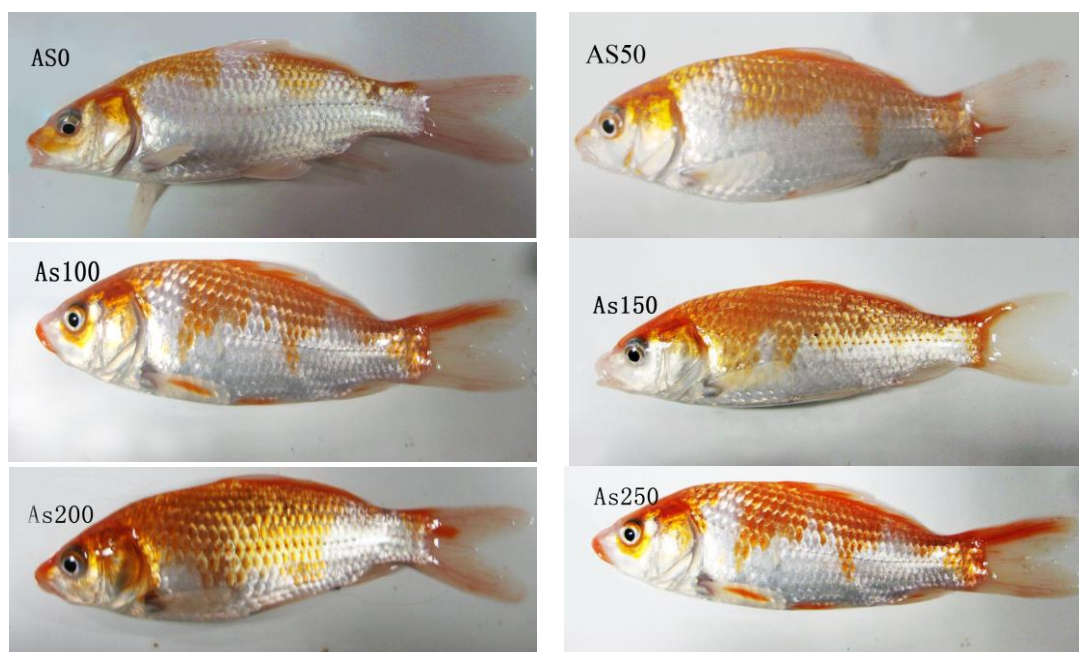


Fig. 1 Red-white koi fed diets containing 0 (AS0), 50 (AS50), 100 (AS100), 150 (AS150), 200 (AS200), or 250 (AS250) mg astacin per kg diet.

fish fed the astacin-supplemented diets were significantly higher than in the control (Fig. 3). The total carotenoid content in the head was similar in the 200 and 250 mg groups and significantly higher in red-white and red areas of the fin ray in fish fed the 150, 200, and 250 diets; no significant differences were detected among fish fed the 150, 200, and 250 diets. For skin and scales of white areas, the astacin-supplemented diets did not appear to have any effects on total carotenoid concentration. The relative increases of total carotenoids increased with the astacin dietary content (Fig. 4). The highest deposition of carotenoid was observed in the red skin, followed by the scales, red head, and red fin ray.

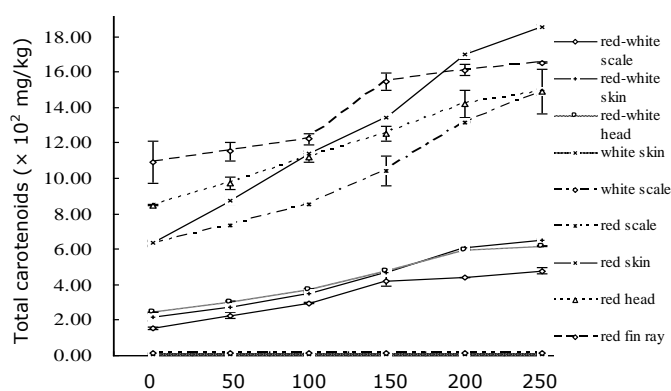


Fig. 3. Total carotenoids in skin, head, scales, and fin ray of red-white koi carp (*Cyprinus carpio*) fed diets containing 0, 50, 100, 150, 200, or 250 mg astacin per kg diet.

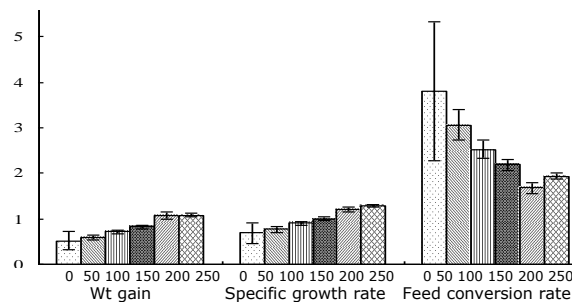


Fig. 2. Growth performance and feed utilization of red-white koi juvenile koi after eight weeks of feeding diets containing 0, 50, 100, 150, 200, or 250 mg astacin per kg diet.

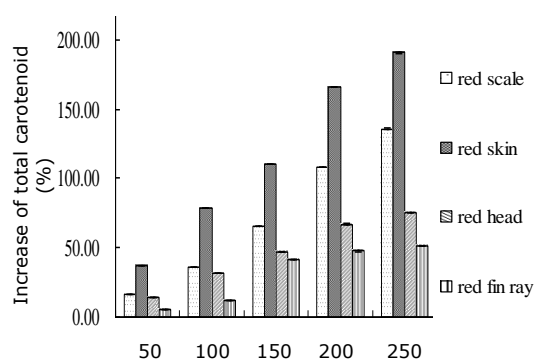


Fig. 4. Relative increase in total carotenoids in skin, head, scales, and fin ray of red koi carp (*Cyprinus carpio*) fed diets containing 0, 50, 100, 150, 200, or 250 mg astacin per kg diet.

## Discussion

Coloring and pattern formation are the key characteristics of ornamental fish. These features are important factors in determining the quality and therefore the value of any particular ornamental fish (Xiao-Hui Li et al., 2008). Carotenoids have been implicated in diverse functions, e.g., pigmentation, antioxidant activity, immunostimulation, reproduction, and intermediary metabolism (Torrissen, et al., 1989; McGraw and Ardia, 2003; Watanabe and Vassallo-Agius, 2003). Astaxanthin, e.g., provitamin A, can enhance fish growth (Pitt, 1971), as demonstrated in Atlantic salmon (Christiansen et al., 1995), kuruma prawn (Chien and Jeng, 1992), and salmonids (Torrissen, 1984). Astaxanthin diets improve skin pigmentation in goldfish (Gouveia and Rema, 2005). Freshwater microalgae (e.g., *Chlorophyta* and *Volvocales*) accumulate intracellular canthaxanthin and astaxanthin as major carotenoid pigments (Gouveia et al., 1996a). Canthaxanthin and astaxanthin have been successfully used to improve pigmentation rates of rainbow trout muscle (Gouveia et al., 1996b, 1997, 1998), seabream skin (Gouveia et al., 2002), and ornamental fish skin (Gouveia et al., 2003). Spirulina is a rich source of  $\beta$ -carotene, a type of carotenoid. Growth, gonad weight, fecundity, and coloration in goldfish fed a diet containing spirulina+300 mg vitamin E were significantly ( $p < 0.01$ ) enhanced, in comparison to other diets (James et al., 2009).

Astacin is not only an important component of aquacultural feeds (Torrissen et al., 1989) that improves muscle pigmentation in rainbow trout (Cheng et al., 2000), but is also an oxidation product of astaxanthin (Naguib, 2000). We showed in this study that dietary supplementation with astacin can improve growth and feed conversion efficiency of koi, in contrast to results showing that the growth rate of red porgy is not affected by



dietary supplementation with natural astaxanthin (Chatzifotis et al., 2005). The carotenoid deposition in the skin, head, scale, and fin ray of red-white koi fed astacin was significantly higher than in fish fed the unsupplemented control. Further, the brightness of the fish positively correlated with the astacin level. The highest carotenoid deposition (skin) was obtained in fish fed 250 mg astacin per kg diet. However, when the astacin content exceeded 200 mg/kg, carotenoid in the red fin ray did not significantly change, similar to earlier results (Xiao Xiang and Long-Bi Tang, 2001).

In summary, this study demonstrates that dietary supplementation of astacin can enhance the color and quality of red-white koi. As fish cannot fully synthesize carotenoids, dietary supplementation is necessary for cultured red-white koi to achieve reddish pigments. Optimization of the chromatophore distribution in the skin and astacin metabolism in red-white koi will be undertaken in future studies.

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