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Effects of Dietary Paprika and Lipid Levels on Growth and Skin Pigmentation of Pale Chub (*Zacco platypus*)

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ABSTRACT : Two feeding experiments were conducted to investigate the effects of dietary paprika (DP) and lipid (DL) levels on growth performance and skin pigmentation of pale chub, *Zacco platypus*. Six diets (designated as P_0L_8, P_0L_{17}, P_8L_8, P_8L_{17}, $P_{16}L_8$ and $P_{16}L_{17}$) were formulated to contain 0%, 8% and 16% paprika with 8% and 17% lipid, respectively. For the growth experiment (Exp I), three replicate groups of fish (average weight 2.6±0.2 g) were fed one of the six experimental diets for 8 weeks. At the end of the feeding period, survival was above 94% and not significantly different among dietary treatments. Weight gain, feed efficiency and protein efficiency ratio of fish fed the $P_{16}L_8$ diet were lower than for fish fed the P_0L_8 diet. The highest total carotenoid (TC) content was observed in fish fed the $P_{16}L_8$ diet. For the pigmentation experiment (Exp II), each experimental diet was fed to two replicate groups of fish (average weight 9.0±0.5 g) for 8 weeks. At the end of the feeding period, TC content of the skin was significantly affected by DP ($p<0.05$). The highest TC content of the skin was observed after 6 weeks of feeding at all dietary treatments. Astaxanthin content of the skin was not affected by DP and DL ($p>0.05$). The capxanthin and zeaxanthin contents of skin increased significantly with increasing DP, whereas the opposite trend was observed for lutein and β-cryptoxanthin contents. The skin lightness (*L** values) significantly decreased whereas the values of *a** and *b** were significantly increased in fish fed the diets containing paprika ($p<0.05$). The present results suggest that feeding a diet containing 8% paprika and 8% lipid for 6 weeks could improve skin pigmentation of pale chub without any adverse effects on growth performance. (**Key Words :** Pale Chub, *Zacco platypus*, Paprika, Lipid, Skin Pigmentation)

INTRODUCTION

Carotenoids are important pigments in birds, insects, fishes and crustaceans. In aquaculture, colors of skin and muscle are primarily indicators for quality characteristics and affect acceptance and market prices of farmed fish. Fish can not synthesize carotenoids *de novo* (Goodwin, 1984). The pigmentation of fish skin and muscle therefore relies entirely on absorption and deposition of carotenoid from feeds. However, the absorption and deposition of carotenoid are affected by several factors including their kinds and dietary concentration (Boonyaratpalin and Unprasert, 1989; Storebakken and Goswami, 1996; Booth et al., 2004; Kalinowski et al., 2005), dietary nutrients composition (Choubert and Luquet, 1983; Torrissen, 1985; Gouveia et al., 2003; Choubert et al., 2006) and feeding period (Torrissen et al., 1995). Particularly, the effects of dietary lipid source and level on the absorption and deposition of carotenoids have been extensively investigated (Torrissen, 1985; Nickell and Bromage, 1998; Barbosa et al., 1999).

To improve color of the skin and muscle of farmed fish, synthetic carotenoids are usually added in commercial feeds. However, the addition of the pigments in fish feeds has reported to increase feed production costs and reduce profitability of aquaculture operation (Torrissen et al., 1995; Buttle et al., 2001). Recent studies (Diler and Gokoglu, 2004; Ingle de la Mora et al., 2006; Sinha and Asimi, 2007; Yanar et al., 2007) therefore were conducted to search available and cheaper pigments as alternatives for the synthetic carotenoids. Paprika has been considered as a potential alternative pigment because of its high carotenoid content (Deli et al., 2001) and less expensive compared with the synthetic one. Feeding diets containing paprika was reported to improve the skin pigmentation of goldfish and koi carp (Hancz et al., 2003).

The pale chub (*Zacco platypus*) is widely distributed in Korea (Jang et al., 2003). This species has been reported as an important ornamental fish species in Korea. The aim of the present experiments were to evaluate the effects of dietary paprika and lipid levels on growth performance and skin pigmentation of juvenile pale chub.

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MATERIALS AND METHODS

Experimental diets

Ingredients and chemical composition of the experimental diets are presented in Table 1. Red paprika (*Capsicum annuum* L.) powder was purchased from a local market. Six experimental diets (designated as P_0L_8, P_0L_{17}, P_8L_8, P_8L_{17}, $P_{16}L_8$ and $P_{16}L_{17}$) were formulated to contain 0%, 8% and 16% paprika powder with 8% and 17% lipid, respectively. Anchovy meal was used as the primary protein source, squid liver oil and soybean oil as lipid sources, and wheat flour as a carbohydrate source. The dietary ingredients were thoroughly mixed with 35% distilled water and prepared using a laboratory pelleting machine. The pellets were dried at room temperature overnight and stored at -25°C until used.

Experimental fish

The pale chub (*Z. platypus*) were collected from a river in Gangwon province (Korea). They were acclimated to a laboratory recirculating tank system and fed the P_0L_8 diet for 3 weeks before being redistributed for growth and pigmentation experiments.

Growth experiment (Exp I)

Eighteen fish (average weight, 2.6±0.2 g) were randomly distributed in each rectangular plastic tank (26×34×20 cm, 35 L freshwater each) after the conditioning period. Each experimental diet was fed to three replicate groups of fish to apparent satiation twice a day (9:00 and 17:00) for 8 weeks. Freshwater at a flow rate of 3 L/min and aeration were continuously supplied in each tank. Water temperature was maintained at 23±2.5°C (mean±SD), and photoperiod was regulated by 12 h light/12 h dark using fluorescent lights during the feeding period.

Pigmentation experiment (Exp II)

Forty fish (average weight, 9.0±0.5 g) were randomly distributed in each rectangular tank (26×34×20 cm, 35 L water each) after the conditioning period. Two replicate

Table 1. Ingredients and chemical composition (% dry matter) of the experimental diets

	Diets					
	P_0L_8	P_0L_{17}	P_8L_8	P_8L_{17}	$P_{16}L_8$	$P_{16}L_{17}$
Ingredients						
Anchovy meal	55.0	55.0	55.0	55.0	55.0	55.0
Wheat flour	33.0	24.0	25.8	16.9	18.7	9.8
Squid liver oil	1.0	4.0	1.0	4.0	1.0	4.0
Soybean oil	2.0	8.0	1.2	7.1	0.3	6.2
Vitamin premix[1]	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[2]	2.0	2.0	2.0	2.0	2.0	2.0
Na-alginate	5.0	5.0	5.0	5.0	5.0	5.0
Paprika powder			8.0	8.0	16.0	16.0
Choline chloride (50%)	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition						
Crude protein	45.9	43.8	44.3	43.2	44.7	42.8
Crude lipid	8.1	17.4	7.9	17.5	7.8	17.5
Ash	10.4	10.1	10.4	10.3	9.4	10.3
Carbohydrate[3]	35.6	28.7	37.4	29.0	38.1	29.4
Gross energy (kcal/g diet)	4.7	5.2	4.6	5.1	4.3	5.0
Total carotenoids (μg/g diet)	1.2	1.2	66.6	66.9	115.5	119.3
Astaxanthin (μg/g)	0.02	0.02	0.6	1.3	2.2	2.5
Lutein (μg/g)	0.5	0.5	1.1	1.0	1.3	1.6
Capxanthin (μg/g)	0.3	0.3	11.1	10.3	14.4	19.0
Zeaxanthin (μg/g)	0.03	0.03	0.5	0.4	0.6	0.8
β-cryptoxanthin (μg/g)	0.3	0.3	1.1	0.8	1.1	1.1

[1] Vitamin premix contained the following vitamins diluted in cellulose (g/kg premix): ascorbic acid, 92.7; α-tocopheryl acetate, 14.5; thiamin, 2.1; riboflavin, 7.0; pyridoxine, 1.4; niacin, 27.8; Ca-D-pantothenate, 9.7; myo-inositol, 139.1; D-biotin, 4.2; folic acid, 0.5; p-amino benzoic acid, 13.9; K_3, 1.4; A, 0.6; D_3, 0.002; cyanocobalamin, 0.003.

[2] Mineral premix contained the following minerals (g/kg premix): $MgSO_4 \cdot 7H_2O$, 80; $NaH_2PO_4 \cdot 2H_2O$, 370; KCl, 130; Ferric citrate, 40; $ZnSO_4 \cdot 7H_2O$, 20; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.

[3] Calculated = 100-(crude protein+crude lipid+ash).

groups of fish were fed one of the experimental diets to apparent satiation twice a day (9:00 and 17:00) for 8 weeks. Freshwater at a flow rate of 3 L/min and aeration were continuously supplied to each tank. Water temperature was maintained at 20±1.0°C, and photoperiod (12 h light/12 h dark) was regulated using fluorescent lights during the feeding period.

Proximate composition and total carotenoid analysis

For growth experiment (Exp I), ten fish at the beginning and all surviving fish at the end of the feeding experiment were sampled and stored at -75°C for chemical analysis. For pigmentation experiment (Exp II), ten fish from each tank at the beginning, every 2 weeks and all surviving fish at the end of the feeding experiment were sampled for analysis of skin color, lipid content and carotenoids. Proximate composition of the experimental diets and fish tissues was determined according to standard methods (AOAC, 1995). Crude protein content was measured by Kjeldahl method using an Auto Kjeldahl System (Buchi, Flawil, Switzerland). Crude lipid content was determined by ether-extraction method. Moisture content was determined by a dry oven at 105°C for 6 h, and ash content was determined by a muffle furnace at 550°C for 4 h. Gross energy content was measured using an adiabatic bomb calorimeter (Parr, USA).

Total carotenoid (TC) content in fish tissues and the experimental diets were analyzed in two replications using a method described by Lee and Lee (2008). Samples of the experimental diets and fish tissues were extracted in 20 ml of a mixture of acetone and methanol (1:1; v:v) for 30 min. This procedure was repeated until the extract was colorless. The extracts were pooled and mixed with 20 ml petroleum ether in 250 ml separator funnels. Distilled water was added and allowed phase separation. The upper phase was collected in a balloon, vacuum dried in a rotary evaporator at 40°C. The residue was recovered in 5 ml petroleum ether and absorbance was measured at 450 nm using a spectrophotometer. TC was calculated using the extinction coefficient $E^{1\%}_{1cm}$ = 2,500 for total carotenoid in petroleum ether.

Isolation of astaxanthin, lutein, capxanthin, zeaxanthin and β-cryptoxanthin was performed with HPLC (Waters, USA) equipped with a Waters 1525 binary pump (Waters, USA) and a single manual injection with 20 μl fixed loop. Carotenoids were detected by a Water 2487 dual λ absorbance detector with an YMC^{TM} carotenoids column (3 μm, C_{18}, 250×4.6 mm, Ireland). A reversed-phase chromatography was used to separate carotenoids with mobile phases (A) and (B) composing of MeOH:methyl tertiary-butyl ether:water:triethyl amine (90:6:4:0.1, v/v/v/v) and MeOH:methyl tertiary-butyl ether:water: triethyl amine (6:90:4:0.1, v/v/v/v), respectively. Carotenoids were eluted at a rate of 0.8 ml/min as following; 0→8 min 90% A, 8→45 min 20% A, 45→50 min 20% A, 50→55 min 90% A, 55→60 min 90% A. Injection size was 20 μl, and absorbance was read at 450 nm. Carotenoids (astaxanthin, capxanthin, lutein, zeaxanthin and β-cryptoxanthin) contents were calculated by comparison with the standards supplied by Extrasynthese (France). Di-tert-butyl-4-methylphenol (BHT) was added in all solutions at a concentration of 0.01%.

Color analysis

Color of fish skin was measured at the anal fin of four fish from each tank at the beginning, every 2 weeks and the end of the feeding experiment. Three replicate measurements were taken using a chroma meter (Minolta, Japan). The color parameters were *L** values for lightness ranging from 0 for black and 100 for white, *a** values for red/green, and *b** values for yellow/blue. A standard white tile with reflectance values of *L** = 95.91, *a* = +0.09 and *b* = +2.02 was used as the reference.

Statistical analysis

Data were subjected to one- and two-way ANOVA to test the effects of dietary paprika and lipid levels on growth and pigmentation of fish. When significant differences were found in one-way ANOVA, Duncan's multiple range test (Duncan, 1955) was used to rank the groups. All statistical analyses were performed using SPSS version 12.0 (SPSS, USA) with a significance level of $p<0.05$. The values presented are mean±standard error (SE).

RESULTS AND DISCUSSION

Growth experiment (Exp I)

Growth performance and feed utilization of pale chub fed the experimental diets for 8 weeks are presented in Table 2. Survival was above 94% and not affected by dietary treatments. Daily feed intake of fish fed the $P_{16}L_8$ diet was higher than that of other diets. Weight gain, feed efficiency and protein efficiency ratio of fish fed the $P_{16}L_8$ diet were lower than those of fish fed the P_0L_8 diet. This was likely due to anti-nutritional factors such as excessive content of fiber and pungency in the diet. Büyükçapar at al. (2007) reported that excessive level of cellulose in diets containing equal to or over 6.6% red pepper impaired growth performance of rainbow trout. Ingle de la Mora et al. (2006) elucidated that pungency of dietary red chilli extract resulted in poor weight gain of fish.

Body moisture, crude lipid and ash of pale chub (Table 3) were not affected by dietary paprika (DP) and lipid (DL)

Table 2. Growth performance of pale chub fed the experimental diets for 8 weeks[1] (Exp I)

Diets	Survival (%)	DFI (%)[2]	WG (%)[3]	FE (%)[4]	PER[5]
P_0L_8	96±3.7	2.29±0.03[a]	182±31.8[b]	72±5.7[b]	1.53±0.12[b]
P_0L_{17}	100±0.0	2.24±0.01[a]	137±2.3[ab]	64±0.4[b]	1.44±0.01[b]
P_8L_8	96±1.9	2.39±0.01[a]	157±9.0[ab]	65±2.4[b]	1.43±0.05[b]
P_8L_{17}	96±3.7	2.33±0.17[a]	133±4.7[ab]	60±4.6[ab]	1.38±0.11[ab]
$P_{16}L_8$	96±1.9	2.69±0.02[b]	124±6.2[a]	50±1.6[a]	1.11±0.04[a]
$P_{16}L_{17}$	94±3.2	2.35±0.04[a]	136±17.7[ab]	60±5.3[ab]	1.37±0.12[ab]
Two-way ANOVA					
DP	p<0.6	p<0.04	p<0.3	p<0.02	p<0.04
DL	p<0.8	p<0.06	p<0.2	p<0.8	p<0.6
DP×DL	p<0.6	p<0.2	p<0.3	p<0.1	p<0.2

[1] Values (mean±SE of three replications) in each column not sharing a superscript are significantly different (p<0.05).
[2] Daily feed intake = feed intake (dry matter)×100/((initial fish wt.+final fish wt.+dead fish wt.)/2×days fed).
[3] Weight gain = (final weight-initial weight)×100/initial weight. [4] Feed efficiency = fish wet weight gain×100/feed intake (dry matter).
[5] Protein efficiency ratio = fish wet weight gain/protein intake. DP = Dietary paprika. DL = Dietary lipid.

Table 3. Proximate composition and total carotenoid content in whole body of pale chub fed the experimental diets for 8 weeks[1] (Exp I)

Diets	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)	Total carotenoids (μg/g tissue DM)
Initial	76.3	16.6	2.7	5.1	0.6
P_0L_8	68.7±1.3	17.2±0.5[ab]	11.5±0.7	2.9±0.12	0.5±0.05[a]
P_0L_{17}	67.8±0.5	15.9±0.6[a]	11.0±1.1	2.1±0.13	0.4±0.04[a]
P_8L_8	67.7±2.1	16.6±0.4[ab]	11.2±1.9	3.2±0.31	0.5±0.11[a]
P_8L_{17}	67.0±0.9	16.0±0.3[a]	11.9±0.5	2.8±0.29	0.5±0.11[a]
$P_{16}L_8$	69.1±1.3	17.5±0.3[b]	11.7±1.0	3.0±0.11	0.9±0.08[b]
$P_{16}L_{17}$	67.1±1.0	16.5±0.2[ab]	12.8±1.5	3.2±0.06	0.6±0.11[a]
Two-way ANOVA					
DP	p<0.8	p<0.3	p<0.8	p<0.9	p<0.02
DL	p<0.3	p<0.01	p<0.7	p<1.0	p<0.1
DP×DL	p<0.9	p<0.8	p<0.8	p<0.4	p<0.3

[1] Values (mean±SE of three replications) in each column not sharing a superscript are significantly different (p<0.05).
DP = Dietary paprika. DL = Dietary lipid.

levels (p>0.05). Crude protein content showed a decreasing trend with increasing DL level. The highest total carotenoid content was observed in fish fed the $P_{16}L_8$ diet. Previous studies (Torrissen, 1985; Nickell and Bromage, 1998) revealed that increasing dietary lipid level resulted in higher deposition rate of carotenoids in rainbow trout. However, we did not observe any effects of dietary lipid level on deposition of carotenoids in pale chub during the feeding period. The discrepancies may be due to fish species.

Pigmentation experiment (Exp II)

Lipid content of the skin and muscle were not affected by DP level (p<0.05), but affected by DL level (p<0.05) and increased with feeding period (Table 4). Total carotenoid (TC) content in the skin and muscle of pale chub fed the experimental diets are presented in Table 5. The TC content of the skin was significantly increased by increasing DP level (p<0.05), and a peak was observed after six weeks of feeding. The TC content in the muscle was not affected by DL level. There were no interactions between DP and DL in the muscle TC content.

Contents of astaxanthin, lutein, capxanthin, zeaxanthin and β-cryptoxanthin in the skin of pale chub fed the experimental diets for 8 weeks are presented in Table 6. Although there were no significant differences in the astaxanthin content of the skin among fish groups fed the experimental diets, but the value tended to increase by feeding the diets containing paprika. The lutein content of fish fed the diets containing paprika was lower than that of fish fed the diets without it after 4 weeks of feeding regardless of DL level. The capxanthin content of fish skin was significantly affected by DP level (p<0.05) after 2 weeks of feeding. The value in fish fed the diets without paprika supplementation was lower than that of fish fed

Table 4. Lipid content (% dry matter) of the skin and muscle of pale chub fed the experimental diets for 8 weeks[1] (Exp II)

	Feeding weeks				
	0	2	4	6	8
Skin including fin					
P_0L_8	12.7	21.4±0.0^{a}	26.7±0.4^{b}	28.3±0.2	30.4±1.7^{ab}
P_0L_{17}		23.3±2.0^{ab}	29.5±0.8^{c}	32.2±3.7	32.0±1.6^{ab}
P_8L_8		20.9±0.5^{a}	23.8±0.6^{a}	27.1±1.3	29.7±0.0^{ab}
P_8L_{17}		25.9±0.7^{b}	26.6±0.8^{b}	31.5±0.4	33.6±0.4^{b}
$P_{16}L_8$		23.2±0.8^{ab}	25.5±0.2^{ab}	26.8±0.4	27.6±0.9^{a}
$P_{16}L_{17}$		26.5±1.2^{b}	27.8±0.8^{bc}	28.6±1.4	31.7±1.5^{ab}
Two-way ANOVA					
DP		p<0.2	p<0.02	p<0.4	p<0.3
DL		p<0.01	p<0.01	p<0.06	p<0.02
DP×DL		p<0.5	p<0.9	p<0.8	p<0.6
Muscle					
P_0L_8	8.1	7.8±0.4	9.7±1.1^{abc}	10.6±1.3	10.4±0.7^{a}
P_0L_{17}		9.4±0.1	10.7±0.6^{c}	12.9±1.9	10.8±0.1^{a}
P_8L_8		8.0±0.3	8.5±0.2^{ab}	10.7±1.2	10.0±0.7^{a}
P_8L_{17}		9.9±0.5	10.1±0.4^{bc}	13.5±0.3	14.5±1.1^{c}
$P_{16}L_8$		8.1±1.2	7.9±0.2^{a}	9.7±0.1	11.5±0.5^{ab}
$P_{16}L_{17}$		9.8±0.3	11.1±0.4^{c}	11.0±1.0	14.2±1.2^{bc}
Two-way ANOVA					
DP		p<0.8	p<0.3	p<0.4	p<0.08
DL		p<0.02	p<0.005	p<0.07	p<0.008
DP×DL		p<1.0	p<0.3	p<0.9	p<0.2

[1] Values (mean±SE of replications) in each column not sharing a superscript are significantly different (p<0.05).
DP = Dietary paprika. DL = Dietary lipid.

Table 5. Total carotenoid content (μg/g DM) in the skin and muscle of pale chub fed the experimental diets for 8 weeks[1] (Exp II)

	Feeding weeks				
	0	2	4	6	8
Skin including fin					
P_0L_8	0.128	2.2±0.16^{abc}	1.9±0.23^{ab}	2.6±0.31^{a}	2.1±0.37^{ab}
P_0L_{17}		1.8±0.19^{a}	1.5±0.17^{a}	2.2±0.27^{a}	1.9±0.35^{a}
P_8L_8		1.9±0.26^{ab}	2.5±0.30^{abc}	4.1±0.19^{cd}	2.7±0.04^{ab}
P_8L_{17}		2.6±0.06^{abc}	2.6±0.52^{abc}	3.9±0.04^{bc}	2.7±0.04^{ab}
$P_{16}L_8$		3.2±0.10^{c}	3.1±0.72^{bc}	4.6±0.09^{d}	3.8±1.06^{b}
$P_{16}L_{17}$		3.1±0.71^{bc}	3.5±0.20^{c}	3.3±0.15^{b}	3.8±0.16^{b}
Two-way ANOVA					
DP		p<0.04	p<0.03	p<0.001	p<0.03
DL		p<0.8	p<0.9	p<0.007	p<1.0
DP×DL		p<0.3	p<0.7	p<0.06	p<1.0
Muscle					
P_0L_8	0.068	0.6±0.04^{b}	0.3±0.07	0.3±0.04	0.4±0.14^{a}
P_0L_{17}		0.6±0.06^{ab}	0.4±0.07	0.3±0.08	0.5±0.01^{ab}
P_8L_8		0.4±0.03^{ab}	0.4±0.00	0.3±0.03	0.7±0.03^{ab}
P_8L_{17}		0.3±0.01^{a}	0.4±0.12	0.3±0.03	0.5±0.02^{ab}
$P_{16}L_8$		0.4±0.17^{ab}	0.4±0.07	0.4±0.02	0.8±0.07^{b}
$P_{16}L_{17}$		0.3±0.07^{a}	0.5±0.01	0.3±0.08	0.6±0.13^{ab}
Two-way ANOVA					
DP		p<0.03	p<0.9	p<0.3	p<0.04
DL		p<0.4	p<0.5	p<0.8	p<0.5
DP×DL		p<1.0	p<0.5	p<0.4	p<0.5

[1] Values (mean±SE of replications) in each column not sharing a superscript are significantly different (p<0.05).
DP = Dietary paprika. DL = Dietary lipid.

Table 6. Astaxanthin, lutein, capxanthin, zeaxanthin and β-cryptoxanthin contents (% total carotenoid) in the skin of pale chub fed the experimental diets for 8 weeks[1] (Exp II)

	Feeding weeks				
	0	2	4	6	8
Astaxanthin					
P_0L_8	3.47	3.37±0.14	3.59±0.26	3.61±0.37	3.30±0.76
P_0L_{17}		3.67±0.12	3.00±0.20	3.21±0.19	3.06±0.38
P_8L_8		3.97±0.49	4.53±0.38	4.56±0.54	3.13±0.27
P_8L_{17}		4.03±0.16	4.38±0.53	4.70±0.64	3.47±0.01
$P_{16}L_8$		4.21±0.23	4.31±0.40	4.06±0.53	4.59±0.63
$P_{16}L_{17}$		3.96±0.59	4.41±0.33	4.16±0.05	4.20±0.21
Two-way ANOVA					
DP		p<0.7	p<0.06	p<0.09	p<0.07
DL		p<1.0	p<0.6	p<0.9	p<0.8
DP×DL		p<0.9	p<0.7	p<0.9	p<0.8
Lutein					
P_0L_8	38.29	38.5±6.63	41.6±0.81^{b}	40.7±1.10^{b}	39.4±1.83^{c}
P_0L_{17}		40.7±0.33	38.3±0.59^{b}	39.8±1.00^{b}	39.0±1.53^{c}
P_8L_8		31.3±1.10	26.5±2.36^{a}	23.5±2.20^{a}	29.3±1.19^{b}
P_8L_{17}		30.1±2.62	26.2±2.29^{a}	24.3±0.58^{a}	27.2±1.35^{ab}
$P_{16}L_8$		30.7±2.58	24.7±2.24^{a}	22.9±2.50^{a}	24.2±0.54^{a}
$P_{16}L_{17}$		30.2±0.74	24.9±0.12^{a}	25.0±1.54^{a}	23.9±0.53^{a}
Two-way ANOVA					
DP		p<0.06	p<0.001	p<0.001	p<0.001
DL		p<1.0	p<0.5	p<0.7	p<0.4
DP×DL		p<0.9	p<0.6	p<0.7	p<0.8
Capxanthin					
P_0L_8	27.03	31.4±6.35^{ab}	26.2±1.70^{a}	26.7±0.28^{a}	26.9±0.63^{a}
P_0L_{17}		27.6±0.74^{a}	29.0±0.66^{a}	27.8±0.54^{a}	27.7±1.61^{a}
P_8L_8		38.9±0.60^{b}	45.7±2.42^{b}	48.2±3.55^{b}	39.7±2.81^{b}
P_8L_{17}		40.8±2.93^{b}	45.6±2.63^{b}	47.8±2.44^{b}	41.8±1.75^{bc}
$P_{16}L_8$		41.4±2.74^{b}	47.8±2.54^{b}	49.2±3.73^{b}	44.7±0.61^{bc}
$P_{16}L_{17}$		41.0±0.40^{b}	47.4±0.15^{b}	45.2±2.66^{b}	46.0±0.58^{c}
Two-way ANOVA					
DP		p<0.02	p<0.001	p<0.001	p<0.001
DL		p<0.8	p<0.7	p<0.7	p<0.4
DP×DL		p<0.7	p<0.7	p<0.7	p<1.0
Zeaxanthin					
P_0L_8	4.35	4.56±1.41^{a}	4.89±0.17^{a}	5.45±0.16^{a}	5.81±0.46^{a}
P_0L_{17}		4.76±0.13^{ab}	4.49±0.21^{a}	4.84±0.11^{a}	5.45±1.61^{a}
P_8L_8		6.99±0.35^{bc}	7.46±1.01^{ab}	9.79±0.42^{b}	8.77±0.21^{ab}
P_8L_{17}		6.65±0.61^{abc}	7.02±0.68^{ab}	8.99±0.68^{b}	10.00±0.43^{b}
$P_{16}L_8$		8.17±0.22^{c}	8.68±0.87^{b}	10.66±0.59^{b}	12.06±1.58^{b}
$P_{16}L_{17}$		8.26±0.14^{c}	7.92±0.44^{ab}	10.14±0.59^{b}	10.32±0.49^{b}
Two-way ANOVA					
DP		p<0.005	p<0.004	p<0.001	p<0.003
DL		p<1.0	p<0.4	p<0.2	p<0.8
DP×DL		p<1.0	p<1.0	p<1.0	p<0.4
β-cryptoxanthin					
P_0L_8	26.87	22.1±1.83^{bc}	23.7±0.81^{b}	23.6±0.29^{b}	24.6±0.02^{c}
P_0L_{17}		23.3±0.40^{c}	25.2±0.34^{b}	24.3±0.17^{b}	24.9±1.32^{c}
P_8L_8		18.8±0.34^{ab}	15.8±1.45^{a}	14.0±1.47^{a}	19.1±1.56^{b}
P_8L_{17}		18.4±2.09^{ab}	16.8±1.55^{a}	14.2±1.91^{a}	17.6±0.82^{ab}
$P_{16}L_8$		15.5±0.62^{a}	14.5±1.57^{a}	13.2±2.36^{a}	14.5±1.06^{a}
$P_{16}L_{17}$		16.6±0.39^{a}	15.4±0.79^{a}	15.5±0.58^{a}	15.6±0.32^{ab}
Two-way ANOVA					
DP		p<0.004	p<0.001	p<0.001	p<0.001
DL		p<0.6	p<0.4	p<0.4	p<1.0
DP×DL		p<0.8	p<1.0	p<0.8	p<0.5

[1] Values (mean±SE of replications) in each column not sharing a superscript are significantly different (p<0.05).

DP = Dietary paprika. DL = Dietary lipid.

other diets. The zeaxanthin content of fish fed the diets containing paprika significantly increased during the experimental period, and the value was higher than that of fish fed the diets without paprika supplementation. The skin β-cryptoxanthin content was negatively affected by DP level ($p<0.05$), but not by DL level throughout the feeding period.

In this study, the skin pigmentation of pale chub was improved by dietary paprika supplementation. Red pepper, because of its high concentration of carotenoid, has been extensively investigated as a natural pigment source for fish (Halver, 1989; Diler and Gokoglu, 2004; Ingle de la Mora et al., 2006; Büyükçapar et al., 2007; Yanar et al., 2007). Halver (1989) reported that an increase in fish pigmentation can be obtained by feeding diets containing 0.5% to 10% red pepper after 4 to 6 weeks. Pigmentation of fish generally is affected by various factors including carotenoid sources, chemical structure and concentration, dietary lipid, fish species and environmental condition (Kang and Ha, 1994; Guillaume et al., 2001; Baker et al., 2002; Diler and Gokoglu, 2004; Ingle de la Mora et al., 2006; Hynes et al., 2009). Hynes et al. (2009) reported that concentration of carotenoid in muscle of Atlantic salmon significantly affected by its source and dietary concentration. A positive relationship between feed dose and deposition of carotenoid in muscle of Atlantic salmon was observed by Baker et al. (2002). Diler and Gokuglu (2004) reported that carotenoid concentration in rainbow trout significantly increased by feeding the diets containing red pepper and astaxanthin.

Paprika, prior to incorporating into experimental diets,

Table 7. *L**, *a**, and *b** values of the anal fin of fish fed the experimental diets for 8 weeks[1] (Exp II)

	Feeding weeks				
	0	2	4	6	8
*L** value					
P_0L_8	72.5±5.68	85±1.3	84±1.1^{b}	85±0.2^{b}	85±0.5^{c}
P_0L_{17}		85±0.2	84±0.1^{b}	84±1.4^{b}	85±0.8^{c}
P_8L_8		83±0.3	79±0.9^{a}	80±0.3^{a}	77±0.4^{a}
P_8L_{17}		83±0.1	82±0.4^{ab}	81±0.5^{a}	81±1.5^{b}
$P_{16}L_8$		84±1.0	80±1.5^{ab}	81±1.1^{a}	79±0.5^{ab}
$P_{16}L_{17}$		83±0.3	81±2.4^{ab}	82±0.1^{ab}	79±0.9^{ab}
Two-way ANOVA					
DP		p<0.05	p<0.05	p<0.01	p<0.001
DL		p<0.9	p<0.4	p<0.3	p<0.1
DP×DL		p<0.6	p<0.4	p<0.4	p<0.08
*a** value					
P_0L_8	-0.4±0.12	-0.85±0.05^{a}	-0.71±0.02^{a}	-0.92±0.22^{a}	-0.38±0.28^{a}
P_0L_{17}		-0.85±0.05^{a}	-0.62±0.05^{a}	-0.45±0.28^{a}	-0.40±0.21^{a}
P_8L_8		1.80±0.10^{b}	2.71±0.36^{b}	5.55±0.23^{c}	8.05±0.63^{c}
P_8L_{17}		1.40±0.50^{ab}	1.89±1.12^{ab}	3.16±0.97^{b}	4.26±1.16^{b}
$P_{16}L_8$		0.45±0.35^{a}	2.46±0.67^{b}	4.13±0.93^{bc}	6.54±0.33^{c}
$P_{16}L_{17}$		1.65±0.25^{ab}	3.36±1.23^{b}	3.22±0.49^{b}	6.34±0.11^{c}
Two-way ANOVA					
DP		p<0.001	p<0.01	p<0.001	p<0.001
DL		p<0.3	p<1.0	p<0.2	p<0.03
DP×DL		p<0.07	p<0.6	p<0.2	p<0.03
*b** value					
P_0L_8	1.7±2.71	4.90±1.20^{a}	4.49±0.66^{ab}	4.72±0.40^{a}	4.35±0.47^{a}
P_0L_{17}		6.20±0.10^{a}	3.87±0.25^{a}	4.40±1.08^{a}	3.63±0.95^{a}
P_8L_8		8.95±0.05^{b}	7.31±0.87^{b}	9.81±0.17^{c}	10.42±0.58^{c}
P_8L_{17}		8.45±0.95^{b}	6.66±0.62^{ab}	8.05±0.84^{bc}	8.21±0.16^{b}
$P_{16}L_8$		6.75±0.75^{ab}	7.30±1.22^{b}	8.39±0.52^{bc}	8.93±0.07^{bc}
$P_{16}L_{17}$		9.55±0.35^{b}	7.10±1.23^{b}	7.19±0.82^{b}	10.21±0.10^{c}
Two-way ANOVA					
DP		p<0.01	p<0.03	p<0.01	p<0.01
DL		p<0.09	p<0.5	p<0.2	p<0.3
DP×DL		p<0.2	p<1.0	p<0.6	p<0.06

[1] Values (mean±SE of replications) in each column not sharing a superscript are significantly different ($p<0.05$).
DP = Dietary paprika. DL = Dietary lipid.

was finely ground to ensure carotenoids being available for fish digestion. The differences in contents (% total carotenoids) of astaxanthin, lutein, capxanthin, zeaxanthin and β-cryptoxanthin of fish skin suggest that the absorption and deposition of the carotenoids were likely to be affected by their dietary concentration, chemical structure and bioavailability. Capxanthin, the most abundant carotenoid in paprika powder, was major carotenoids deposited in the fish skin. In addition, although contents of lutein and β-cryptoxanthin increased in the diets containing paprika, their contents in the fish skin significantly decreased at the end of the feeding trial.

*L**, *a** and *b** values of the anal fin of pale chub fed the experimental diets for 8 weeks in Exp II are shown in Table 7. Lightness (*L** values) were significantly affected by DP level ($p<0.05$), but not DL level. At the end of the feeding period, *L** value of fish fed the diets containing paprika significantly ($p<0.05$) decreased and the lowest value was observed in fish fed the P_8L_8 diet, followed by the $P_{16}L_8$ and $P_{16}L_{17}$ diets. The anal fin redness (*a** values) and yellowness (*b** values) of pale chub were significantly increased by DP level ($p<0.05$), but not DL level. The *a** value tended to increase with feeding period, except for the diets without paprika supplementation. Feeding diets containing natural carotenoid sources such as paprika, shrimp shell meal, and micro-algae was reported to improve skin pigmentation of several fish species including koi carp, gold fish, red porgy (Gouveia et al., 2003; Hancz et al., 2003; Kalinowski et al., 2007). Hancz et al. (2003) reported that the skin pigmentation of koi carp and goldfish were expected to improve after 4 weeks of feeding of the diets containing paprika as feed additive.

The present findings suggest that feeding the diet containing 8% crude lipid and 8% paprika powder for 6 weeks could improve the skin pigmentation of pale chub without any adverse effects on growth performance.

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REFERENCES

AOAC (Association of Official Analytical Chemists). 1995. Official Methods of Analysis, 16th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Barbosa, M. J., R. Morais and G. Choubert. 1999. Effect of carotenoid source and dietary lipid content on blood astaxanthin concentration in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 176:331-341.

Baker, R. T. M., A.-M. Pfeiffer, F.-J. Schöner and L. Smith-Lemmon. 2002. Pigmenting efficacy of astaxanthin and cantaxanthin in freshwater reared Atlantic salmon, *Salmo salar*. Anim. Feed Sci. Technol. 99:97-106.

Boonyaratpalin, M. and N. Unprasert. 1989. Effects of pigments from different sources on colour changes and growth of red *Oreochromis niloticus*. Aquaculture 79:375-380.

Booth, M. A., R. J. Warner-Smith, G. L. Allan and B. D. Glencross. 2004. Effects of dietary astaxanthin source and light manipulation on the skin colour of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801). Aquac. Res. 35:458-464.

Buttle, L. G., V. O. Crampton and P. D. Williams. 2001. The effect of feed pigment type on flesh pigment deposition and colour in farmed Atlantic salmon *Salmon salar* L. Aquac. Res. 32:102-111.

Büyükçapar, H. M., M. Yanar and Y. Yanar. 2007. Pigmentation of rainbow trout (*Oncorhynchus mykiss*) with carotenoids from Marigold flower (*Tagetes erecta*) and red pepper (*Capsicum annum*). Turk. J. Vet. Anim. Sci. 31:7-12.

Choubert, G. and P. Luquet. 1983. Utilization of shrimp meal for rainbow trout (*Salmo gairdneri* Rich.) pigmentation. Influence of fat content of the diet. Aquaculture 32:19-26.

Choubert, G., M. M. Mendes-Pinto and R. Morais. 2006. Pigmenting efficacy of astaxanthin fed to rainbow trout *Oncorhynchus mykiss*: Effect of dietary astaxanthin and lipid sources. Aquaculture 257:429-436.

Deli, J., P. Molnar, Z. Matus and G. Toth. 2001. Carotenoid composition in the fruit of red paprika (*Capsicum annuum var. lycopersiciforme rubrum*) during ripening; Biosynthesis of carotenoids in red paprika. J. Agric. Food Chem. 49:1517-1523.

Diler, I. and N. Gokoglu. 2004. Investigation of the sensory properties of the flesh of rainbow trout (*Oncorhynchus mykiss*) fed diets with astaxanthin, shrimp waste meal and red pepper meal. Eur. Food. Res. Technol. 219:217-222.

Duncan, D. B. 1955. Multiple-range and multiple *F* tests. Biometrics 11:1-42.

Goodwin, T. W. 1984. The Biochemistry of the Carotenoids. Vol. II. Animals, 2nd Ed. Chapman & Hall, London.

Gouveia, L., P. Rema, O. Pereira and J. Empis. 2003. Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. Aquacult. Nutr. 9:123-129.

Guillaume, J., S. Kaushik, P. Bergot and R. Métailler. 2001. Nutrition and feeding of fish and crustaceans. Springer Verlag, UK, 408 pp.

Halver, J. E. 1989. Fish nutrition. 2nd Ed. Academic Press, New York.

Hancz, C., I. Magyary, T. Molnar, S. Sato, P. Horn and N. Taniguchi. 2003. Evaluation of color intensity enhanced by paprika as feed additive in goldfish and koi carp using computer-assisted image analysis. Fish. Sci. 69:1158-1161.

Hynes, N., E. S. Egeland, W. Koppe, G. Baardsen and V. Kiron. 2009. Calanus oil as a natural source for flesh pigmentation in Atlantic salmon (*Salmo salar* L.). Aquacult. Nutr. 15:202-206.

Ingle de la Mora, G., J. L. Arredondo-Figueroa, J. T. Ponce-Palafox, I. delos Angeles Barriga-Sosa and J. E. Vernon-Carter. 2006. Comparison of red chilli (*Capsicum annuum*) oleoresin and astaxanthin on rainbow trout (*Oncorhynchus mykiss*) fillet pigmentation. Aquaculture 258:487-495.

Jang, M.-H., M. C. Lucas and G.-J. Joo. 2003. The fish fauna of

mountain streams in South Korean national parks and its significance to conservation of regional freshwater fish biodiversity. Biol. Conserv. 114:115-126.

Kalinowski, C. T., L. E. Robaina, H. Fernandez-Palacios, D. Schuchardt and M. S. Izquierdo. 2005. Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. Aquaculture 244:223-231.

Kalinowski, C. T., M. S. Izquierdo, D. Schuchardt and L. E. Robaina. 2007. Dietary supplementation time with shrimp shell meal on red porgy (*Pagrus pagrus*) skin colour and carotenoid concentration. Aquaculture 272:451-457.

Kang, D.-S. and B.-S. Ha. 1994. Metabolism of dietary carotenoids and effects to intensify the body color of cultured sea bass. Bull. Kor. Fish Soc. 27:272-281.

Lee, C.-R. and S.-M. Lee. 2008. Effect of dietary supplementation of pigment sources on pigmentation of the round tailed paradise fish *Macropodus chinensis* and pale chub *Zacco platypus*. J. Aquacult. 21:213-217.

Nickell, D. C. and N. R. Bromage. 1998. The effect of dietary lipid level on variation of flesh pigmentation in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 161:237-251.

Sinha, A. and O. A. Asimi. 2007. China rose (*Hibicus rosasinensis*) petals: a patent natural carotenoid source for goldfish (*Carassius auratus* L.). Aquac. Res. 38:1123-1128.

Storebakken, T. and U. C. Goswami. 1996. Plasma carotenoid concentration indicates the availability of dietary astaxanthin for Atlantic salmon, *Salmo salar*. Aquaculture 146:147-153.

Torrissen, O. J. 1985. Pigmentation of salmonids: factors affecting carotenoid deposition in rainbow trout (*Salmo gairdneri*). Aquaculture 43:133-142.

Torrissen, O. J., R. Christiansen, G. Struknæs and R. Estermann. 1995. Astanxanthin deposition in the flesh of Atlantic salmon, *Salmo salar* L., in relation to dietary astaxanthin concentration and feeding period. Aquacult. Nutr. 1:77-84.

Yanar, Y., H. Büyükçapar, M. Yanar and M. Göcer. 2007. Effect of carotenoids from red pepper and marigold flower on pigmentation, sensory properties and fatty acid composition of rainbow trout. Food Chem. 100:326-330.