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Research Note

Effects of Dietary Inclusion of Astaxanthin on Growth, Muscle Pigmentation and Antioxidant Capacity of Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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ABSTRACT: This study was designed to investigate the effects of dietary astaxanthin levels on growth performance, feed utilization, muscle pigmentation, and antioxidant capacity in juvenile rainbow trout. Four experimental diets were formulated to contain 0, 50, 75, and 100 mg/kg astaxanthin (designed as AX0, AX50, AX75, and AX100). Each diet was fed to triplicate groups of fish (18.5 g/fish) for 10 weeks. Growth performance and muscle composition of fish were not affected by dietary astaxanthin levels. Total carotenoid concentration in the muscle of fish fed the AX50 diet was higher than that of fish fed the AX0 diet, but no significant differences were observed between these fish and those fed the AX75 and AX100 diets. Muscle astaxanthin content increased with increased astaxanthin in the diet. Deposition of astaxanthin in the flesh resulted in a decrease in lightness and an increase in redness and yellowness. The fillets from trout fed the AX75 diet had significantly lower lightness than trout fed the AX50 and AX100 diets. Fish fed the AX50 and AX75 diets showed significantly lower catalase activity than those fed the control diet. Total antioxidant status increased significantly in all astaxanthin supplemented groups when compared to the control group. Superoxide dismutase activity was significantly decreased in fish fed the AX50 diet compared to fish fed the AX0 diet. These findings suggest that while fillet pigmentation increased with increasing dietary astaxanthin concentration, indices of fish antioxidant capacity may not be affected in a dose dependent manner.

Keywords: rainbow trout, carophyll pink, growth, muscle pigmentation, antioxidant capacity

INTRODUCTION

The typical pink to deep red color of the flesh and skin of wild salmonids is a common characteristic that distinguishes this group from other fish species, and the degree of flesh pigmentation is one of the most important quality criteria dictating their market value. However, the flesh color of cultured salmonids may be inferior to that of their wild counterparts due to their diets or husbandry purlieus. Indeed, it is well-know that fish, like other animals, cannot synthesize their own coloring pigments *de novo*, and must obtain these pigments from their diet. Accordingly, pigmentation of cultured salmonids has been achieved with inclusion of various synthetic carotenoids (β-carotene, canthaxanthin, zeaxanthin, and astaxanthin) and/or natural sources (yeast, bacteria, algae, higher plants, and crustacean meal) (1,2) in their diets. Today, however, nearly all carotenoids pigment supplementation to salmon and trout feeds is from Carophyll pink™, which is gelatin-encapsulated astaxanthin, the pigment found in crustaceans and wild salmonids. In recent years, however, more attention has been given to the other important biological functions of carotenoids, other than their role in muscle pigmentation in fish. Proposed functions include growth enhancement (3), improvement of broodstock performance (4-8), antioxidant status (9, 10), and immune function (9,11,12) as well as increase resistance to disease (13,14). Several of the above effects of carotenoid pigments on biological systems are directly linked to their antioxidant properties and very high scavenging affinity for toxic oxygen radicals, which are related to their molecular structure (15,16).

In order to combat oxidative stress, living organisms have developed complex defense mechanisms which involve enzymatic and non-enzymatic antioxidants of endogenous and exogenous origin that are usually effective in neutralizing harmful effects of reactive oxygen species (ROS) (17). Generally, an antioxidant defense system

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plays a key role in inactivation of ROS such as superoxide radicals ($O_2^{\cdot -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), and lipid peroxides, and thereby controls oxidative stress as well as redox signaling in living organisms, including fish. If not tightly controlled by antioxidant systems, increased cellular levels of ROS may lead to increased oxidative stress that can result in significant damage to cell membranes and macromolecules including lipids, proteins, and nucleic acids. Among the non-enzymatic defenses, particular attention is paid to carotenoids such as astaxanthin. Owing to its structure, astaxanthin exerts antioxidant action by quenching singlet oxygen and scavenging free radicals to terminate chain reactions, and thereby preventing oxidative damage to cellular macromolecules (18,19). Accordingly, it was observed that dietary red yeast, *Phaffia rhodozyma*, which is rich in astaxanthin, and synthetic astaxanthin decrease peroxide levels and transaminase activities in the serum of healthy rainbow trout (20). Similarly, Nakano et al. (21) also revealed that supplementation of fingerling rainbow trout diet with red yeast, *P. rhodozyma*, decreased the susceptibility of the liver to lipid peroxidation and strengthened the protective ability of the liver against oxidative stress. Despite its importance, however, little work has been conducted on unraveling the relationship between dietary carotenoids and antioxidant status in salmonids such as rainbow trout (21) and Atlantic salmon, *Salmo salar* (9). Furthermore, to the best of our knowledge, there has not been a study that investigates the effects of different dietary levels of Carophyll pink™, as the major commercially available source of dietary astaxanthin, on the antioxidant status of these fish species in terms of plasma antioxidant enzymes and/or plasma, and liver radical scavenging activities. Therefore, the aim of this study was to evaluate the effects of dietary levels of astaxanthin on the growth performance, feed utilization, muscle pigmentation, and antioxidant status of juvenile rainbow trout.

MATERIALS AND METHODS

Experimental diets

Ingredient and proximate composition of the experimental diets are presented in Table 1. Four isonitrogenous and isocaloric experimental diets were formulated to contain 0% (AX0), 0.05% (AX50), 0.075% (AX75), and 0.1% (AX100) Carophyll pink™ (DSM Nutritional Products Ltd., Basel, Switzerland) containing 10% astaxanthin. The diet without supplementation of astaxanthin was considered as the control diet. Fish meal and dehulled soybean meal were used as dietary protein sources and fish oil and linseed oil were used as lipid sources. All ingredients were thoroughly mixed with 30% distilled water and pellets were prepared using a laboratory moist pelleting machine. The pellets were dried at room temperature for 48 h and grounded into desirable particle sizes. All diets were stored at −25°C until used.

Table 1. Ingredients and chemical composition of the experimental diets

	Diets			
	AX0	AX50	AX75	AX100
Ingredients (%)				
Fish meal	49.0	49.0	49.0	49.0
Dehulled soybean meal	25.0	25.0	25.0	25.0
Carophyll pink (10% astaxanthin)[1)]	0.0	0.05	0.075	0.1
Wheat flour	10.0	10.0	10.0	10.0
Potato-starch	5.0	5.0	5.0	5.0
Fish oil	4.0	4.0	4.0	4.0
Linseed oil	4.0	4.0	4.0	4.0
Cellulose	0.1	0.05	0.025	0.0
Vitamin premix[2)]	1.2	1.2	1.2	1.2
Mineral premix[3)]	1.0	1.0	1.0	1.0
Vitamin C (50%)	0.3	0.3	0.3	0.3
Vitamin E (25%)	0.1	0.1	0.1	0.1
Choline	0.3	0.3	0.3	0.3
Proximate composition (% dry matter)				
Crude protein	52.6	52.3	52.2	51.8
Crude lipid	13.1	12.8	13.6	14.4
Ash	11.9	12.3	15.2	12.4
Astaxanthin (ppm)		27.6	39.8	46.1

[1)]Carophyll pink™ (DSM Nutritional Products Ltd., Basel, Switzerland).
[2)]Vitamin premix contained the following amount which were diluted in cellulose (g/kg premix): DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; *p*-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.
[3)]Mineral premix contained the following ingredients (g/kg premix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; 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.

Fish and feeding trial

Juvenile rainbow trout were transported from a local hatchery (Pyeongchang, Korea) to the Gangneung-Wonju National University (Gangneung, Korea). Experimental protocols followed the guidelines of the Animal Care and Use Committee of Gangneung-Wonju National University. The fish were acclimated to the laboratory conditions for 2 weeks by feeding commercial pellets. After this conditioning period, juvenile rainbow trout (average body weight, 18.5±0.57 g) were randomly distributed into 15 rectangular tanks of 50 L capacity at a density of 10 fish per tank. Each experimental diet was fed to 3 replicate groups of fish to visual satiation twice per day (9:00 and 17:00) for 10 weeks. Uneaten feed and dead fish were

removed and weighed every day. Fresh water was supplied at a flow rate of 3 L/min in the re-circulating system and aeration was continuously provided in each tank. Photoperiod was left at the natural condition, and the average water temperature during the trial was 13.0±0.2°C. Feeding was stopped 24 h prior to weighing or blood sampling to minimize stress on fish.

Sample collection

At the end of the feeding trial, all fish in each tank were counted and bulk-weighed for calculation of growth performance, feed utilization, and survival. Blood samples were collected from individual fish, and each sample was analyzed separately. Three fish per tank (9 fish per dietary treatment) were randomly captured, anesthetized with tricaine methanesulfonate (MS-222, Sigma, St. Louis, MO, USA) (100 mg/L), and blood samples were taken from the caudal vein with heparinized syringes. Plasma samples were separated by centrifugation at 5,000 *g* for 10 min and stored at −75°C for determination of radical scavenging and antioxidant enzyme activities. Liver was dissected out from these fish and kept at −75°C for determination of radical scavenging activities. Epaxial samples of white muscle were taken from the remaining fish in each tank and kept at −75°C for determination of proximate composition, total carotenoids, and astaxanthin content after color measurements.

Chemical analyses

***Proximate composition*:** Proximate composition of the diet and muscle samples was analyzed according to standard methods (22). The moisture content was determined with a drying oven (105°C for 6 h), the crude protein content was determined using the auto Kjeldahl system (BÜCHI Labortechnik AG, Flawil, Switzerland), the crude lipid content was determined by the ether-extraction method, using a Soxhlet extractor (VELP Scientifica, Inc., Usmate, Italy), and the ash content was determined using a muffler furnace (600°C for 4 h).

***Total carotenoid analysis*:** Freeze dried flesh (200 mg) was suspended in 1 mL dimethyl sulfoxide (DMSO), and then 1 mg of 0.5 mm glass beads were added to the flesh. After shaking for 1 min, the mixture was centrifuged at 10,000 *g* for 5 min. Then, the supernatant was transferred to a clean test tube, and 2 mL of DMSO was added to the flesh. Again after shaking for 1 min, the mixture was centrifuged at 10,000 *g* for 5 min, and the absorbance was measured at 474 nm using a Infinite F200 multiwell plate reader (Tecan Schweiz AG, Zurich, Switzerland).

***Astaxanthin analysis*:** Astaxanthin concentrations were determined using an Agilent 1200 series high-performance liquid chromatography equipped with an UV-visible detector (Agilent Technologies, Santa Clara, CA, USA) and a YMC carotenoid column (250 nm×4.6 mm, 5 μm) with a guard column operating at 25°C. The mobile phase consisted of mixtures of methanol : *tert*-butyl ethyl ether : water (81:15:4, v/v/v). The solvent flow rate of 1.0 mL/min was used. A calibaration curve using an astaxanthin standard of 98% purity (Sigma) was used for the calculation of astaxanthin contents.

***Color measurement*:** Muscle color was assessed immediately post slaughter using a chromameter (mod. CR400, Minolta Co., Ltd., Osaka, Japan) equipped with a 8 mm in diameter aperture and calibrated on a white reference plate (with reflectance values of L^*=+95.91, a^*=+0.09, and b^*=+2.02) before use as described by Choubert et al. (23). Each measurement was repeated 3 times for each sample. All measurements were expressed in the colorimetric space $L^*a^*b^*$ in accordance with the recommendations of the Commission Internationale de l'Éclairage in 1976.

Radical scavenging activities

Liver samples were homogenized (Wiggen Hauser, Berlin, Germany) in 4 volumes of buffer (5 mM Tris-HCl and 35 mM glycine, pH 8.4) followed by centrifugation at 13,000 *g* for 10 min at 4°C. The resultant supernatant was then used for determination of radical scavenging activities. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined using the method described by Nanjo et al. (24) with some modifications. Briefly, 10 μL of each sample (or ethanol as the control) was added to 30 μL of DPPH (60 μM) in an ethanol solution. After mixing vigorously for 10 s, the solution was transferred to a 100 μL quartz capillary tube which was fitted to the cavity of the electron spin resonance (ESR) spectrometer (JEOL Ltd., Tokyo, Japan). After 2 min, the spin adduct was measured on the ESR spectrometer. Measurement conditions were as follows: magnetic field, 336.5±5 mT; power, 5 mW; modulation frequency, 9.41 GHz; amplitude, 1×1,000; sweep time, 30 s.

Hydroxyl radicals were generated by the iron-catalyzed Fenton Haber-Weiss reaction and were rapidly reacted with an electron spin trap of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) (25). The resultant DMPO-OH adducts were measured with an ESR spectrometer. The sample solution (20 μL) was mixed with DMPO (0.3 M, 20 μL), $FeSO_4$ (10 mM, 20 μL), and H_2O_2 (10 mM, 20 μL) in a phosphate buffer solution (pH 7.4), and was then transferred to a 100 μL quartz capillary tube. After 2.5 min, the ESR spectrum was recorded using an ESR spectrometer. Experimental conditions were as follows: magnetic field, 336.5±5 mT; power, 1 Mw; modulation frequency, 9.41 GHz; amplitude, 1×200; sweep time, 4 min.

Alkyl radicals were generated by 2,2'-azobis-(2-amidinopropane)-hydrochloride (AAPH). The phosphate buffered saline reaction mixtures (pH 7.4) containing 10

mM AAPH, 10 mM α-(4-pyridyl *N*-oxide)-*N*-*tert*-butylnitrone, and known concentrations of tested samples (100 μg/mL), were incubated at 37°C in a water bath for 30 min (26) and then transferred to a quartz capillary tube. The spin adduct was recorded using an ESR spectrometer (JEOL Ltd.). Measurement conditions were as follows: modulation frequency, 100 kHz; microwave power, 10 mW; microwave frequency, 9,441 MHz; magnetic field, 336.5±5 Mt; sweep time, 30 s.

The radical scavenging activity (RSA) was calculated as follow:

$$\text{RSA } (\%) = 1 - \frac{A}{A_0} \times 100$$

where A and A_0 were the relative peak heights of radical signals with and without sample, respectively.

Antioxidant enzymes activities

Plasma catalase (CAT) activity and total antioxidant capacity were analyzed using commercially available kits (Bioassay Systems LLC, Hayward, CA, USA) following the manufacturer's instructions.

Superoxide dismutase (SOD) activity was measured using a SOD assay kit (Sigma, 19160) according to the manufacturer's instruction. Each endpoint was monitored by measuring the absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20 min of reaction time at 37 °C. One unit of SOD activity was defined as the amount of enzyme yielding a 50% inhibition of the reaction.

Glutathione peroxidase (GPx) activity was determined using a commercial kit (Bioassay Systems LLC). In this assay, cumene hydroperoxide was used as the peroxide substrate (ROOH), and glutathione reductase (GSSG-R) and β-nicotinamide adenine dinucleotide phosphate, reduced (NADPH) were included in the reaction mixture. The change in absorbance at 340 nm due to NADPH oxidation was monitored to assess GPx activity. Briefly, 50 μL of plasma were added to 40 μL of the reaction mixture and incubated for 15 min; 10 μL of cumene hydroperoxide were then added and the optical density (OD)1 was read at 340 nm. After 5 min of incubation, the OD2 at 340 nm was read using an enzyme-linked immunosorbent assay reader. GPx activity was calculated as U/L. One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 μmol of NADPH to $NADP^+$ under the assay kit conditions per minute at 25°C.

Statistical analysis

Data were subjected to one way analysis of variance (ANOVA) to test the effects of dietary astaxanthin on growth performance, feed utilization, antioxidant activity, and chemical composition of fish. When significant differences were found, Duncan's multiple range test (27) was used. All statistical analyses were performed using SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA) with a significance level of $P<0.05$. Data are presented as mean ±standard error of 3 replications.

RESULTS

Growth performance and feed utilization of fish fed the experimental diets for 10 weeks are presented in Table 2. No significant differences were identified in survival, weight gain, specific growth rate, feed efficiency, daily feed intake, daily protein intake, and protein efficiency ratio among the groups ($P>0.05$). The results of the muscle proximate composition analysis revealed no significant differences among dietary treatments (Table 3).

Total carotenoids and astaxanthin concentrations in

Table 2. Growth performance and feed utilization of juvenile rainbow trout fed the experimental diets for 10 weeks

	Diets			
	AX0	AX50	AX75	AX100
Survival (%)	77±6.7^{NS}	83±3.3^{NS}	80±11.5^{NS}	87±6.7^{NS}
Weight gain (%)[1)]	234±12.2	229±1.3	216±4.8	213±5.5
Specific growth rate (%)[2)]	1.7±0.09	1.6±0.03	1.6±0.02	1.5±0.02
Feed efficiency (%)[3)]	109±5.5	106±5.9	112±2.7	109±10.0
Daily feed intake (%)[4)]	1.4±0.01	1.4±0.03	1.3±0.02	1.4±0.10
Daily protein intake (%)[5)]	0.7±0.01	0.7±0.01	0.7±0.01	0.7±0.05
Protein efficiency ratio[6)]	2.1±0.11	2.0±0.11	2.1±0.05	2.1±0.20

Values are the means of triplicate groups and presented as mean±SE.
^{NS}Not significant.
[1)]Weight gain=(final fish weight−initial fish weight)×100/initial fish weight.
[2)]Specific growth rate=[(ln(final fish weight)−ln(initial fish weight)]×100/days of feeding.
[3)]Feed efficiency=wet weight gain×100/feed intake.
[4)]Daily feed intake=feed intake×100/[(initial fish weight+final fish weight+dead fish weight)/2×days reared].
[5)]Daily protein intake=protein intake×100/[(initial fish weight+final fish weight+dead fish weight)/2×days reared].
[6)]Protein efficiency ratio=wet weight gain/protein intake.

Table 3. Proximate composition of the muscle of juvenile rainbow trout fed the experimental diets for 10 weeks (unit: % wet weight)

Diets	Proximate composition			
	Moisture	Crude protein	Crude lipid	Ash
AX0	76.9±0.92^{NS}	25.7±0.29^{NS}	1.9±0.38^{NS}	1.7±0.04^{NS}
AX50	76.2±0.46	27.2±1.38	2.5±0.20	1.8±0.05
AX75	76.1±0.11	24.4±2.02	3.0±0.20	1.7±0.08
AX100	76.6±0.32	22.8±1.45	2.1±0.63	1.7±0.05

Values are the means of triplicate groups and presented as mean±SE.
^{NS}Not significant.

Table 4. Total carotenoids and astaxanthin concentrations in the muscle of juvenile rainbow trout fed the experimental diets for 10 weeks (unit: μg/g)

Diets	Total carotenoids	Astaxanthin
AX0	2.1±0.61^{a}	0.1±0.04^{a}
AX50	8.9±1.92^{b}	5.0±1.33^{b}
AX75	5.3±0.95^{ab}	5.8±0.80^{b}
AX100	8.3±4.46^{ab}	6.1±2.52^{b}

Values are the means of triplicate groups and presented as mean±SE.
Values with different letters (a,b) in the same column are significantly different (P<0.05).

Table 5. Changes of chroma values of the muscle of juvenile rainbow trout fed the experimental diets for 10 weeks

Diets	Chroma values		
	L^*	a^*	b^*
AX0	54.6±1.55^{b}	2.8±0.26^{a}	6.1±0.20^{a}
AX50	48.3±1.99^{ab}	13.6±0.52^{bc}	13.0±0.52^{cd}
AX75	43.6±0.58^{a}	15.7±0.38^{c}	12.2±0.45^{bc}
AX100	45.9±2.51^{ab}	16.5±1.20^{c}	15.3±0.58^{d}

Values are the means of triplicate groups and presented as mean±SE.
Values with different letters (a-d) in the same column are significantly different (P<0.05).

Fig. 1. Muscle color of juvenile rainbow trout fed the experimental diets for 10 weeks.

rainbow trout muscle fed the experimental diets are presented in Table 4. Total carotenoid content in the muscle of fish fed the AX50 diet was significantly higher than that of fish fed the AX0 diet. Muscle astaxanthin concentration tended to increase with increasing levels of astaxanthin supplementation and was significantly higher than that of fish fed the AX0 diet.

Color parameter values of juvenile rainbow trout muscle fed the different experimental diets are shown in Table 5. Color intensity (L^*) of fish muscle fed the control (AX0) diet was significantly stronger than those fish fed the AX75 diet while it was not significantly different from those fed the AX50 and AX100 diets. The groups of fish fed the control (AX0) diet showed a weak red (a^*) and yellow (b^*) tonalities, which differed significantly from values found for groups fed the astaxanthin supplemented diets (Table 5, Fig. 1).

The results of plasma and liver radical scavenging activities are provided in Table 6. The results showed that dietary supplementation of astaxanthin in the diets of rainbow trout has no significant effects on plasma and hepatic radicals scavenging activities.

Plasma antioxidant enzyme activities in juvenile rainbow trout fed the experimental diets are presented in Table 7. CAT activity was significantly higher in rainbow trout fed the AX0 diet compared with the AX50 and AX75 diets. The levels of total antioxidant status (TAS) were significantly higher in fish fed with the diet supplemented with astaxanthin. Fish fed the AX50 diet showed significantly lower SOD activity than fish fed the control diet. However, plasma GPx activity was not affected by dietary astaxanthin levels.

DISCUSSION

Dietary astaxanthin did not affect growth performance and feed utilization of juvenile rainbow trout in the current study. These results agree with earlier observations in rainbow trout (11,12,14,28,29), Atlantic salmon (30, 31), characins (*Hyphessobrycon callistus*) (10), red porgy (*Pagrus pagrus*) (2,32-34), and gilthead seabream (*Sparus aurata*) (35), fed diets supplemented with either natural or synthetic astaxanthin. However, these findings are not consistent with previous reports demonstrating that dietary astaxanthin deficiency can negatively affect growth performance, feed utilization, and survival of fish (9,36, 37). Nickell and Bromage (38) suggested that perhaps a longer supplementation period might be required to observe any positive effect of dietary astaxanthin on growth performance. Nevertheless, the effects of carotenoids on fish growth are still controversial and diverse, and further studies are needed to reveal the mechanisms behind their possible positive influence.

In the current study, muscle astaxanthin concentrations increased linearly with increasing dietary astaxan-

Table 6. Plasma and liver radical scavenging activities of juvenile rainbow trout fed the experimental diets for 10 weeks

Diets	Radical (%)		
	DPPH	Hydroxyl	Alkyl
Plasma			
AX0	60.4±0.88^{NS}	43.4±13.26^{NS}	39.3±6.50^{NS}
AX50	65.8±3.52	47.6±5.77	50.8±4.64
AX75	66.3±2.56	49.7±2.48	52.8±4.45
AX100	63.6±0.89	57.9±4.47	59.2±2.58
Liver			
AX0	60.1±2.93^{NS}	63.2±8.63^{NS}	60.8±2.72^{NS}
AX50	61.1±6.00	69.8±4.86	56.6±1.85
AX75	60.1±4.54	70.1±4.44	64.3±4.51
AX100	64.1±3.41	64.7±3.55	68.5±6.20

Values are the means of triplicate groups and presented as mean±SE.
^{NS}Not significant.

Table 7. Plasma antioxidant enzyme activities of juvenile rainbow trout fed the experimental diets for 10 weeks

Diets	Antioxidant enzyme activities[1)]			
	CAT (U/L)	TAS (U/mL)	SOD (U/mL)	GPx (U/L)
AX0	1.3±0.32^{b}	279.4±2.04^{a}	74.5±4.82^{b}	2.3±0.26^{NS}
AX50	0.5±0.12^{a}	296.3±3.75^{b}	68.0±2.35^{a}	1.8±0.21
AX75	0.6±0.14^{a}	293.8±4.00^{b}	72.3±11.76^{ab}	1.4±0.26
AX100	1.0±0.16^{ab}	297.6±4.80^{b}	70.5±6.38^{ab}	1.3±0.40

Values are the means of triplicate groups and presented as mean±SE.
Values with different letters (a,b) in the same column are significantly different (P<0.05).
^{NS}Not significant.
[1)]CAT, catalase; TAS, total antioxidant status; SOD, superoxide dismutase; GPx, glutathione peroxidase.

thin inclusion. Fish fed the AX100 diet showed the highest muscle astaxanthin concentration, which was not significantly different from that of fish fed the AX50 and AX75 diets. The overall muscle astaxanthin concentrations were higher than those reported by Choubert and Storebakken (39) and Kurnia et al. (40) in cultured rainbow trout and Schiedt et al. (41) in wild trout, but lower than those reported by Barbosa et al. (42) and Choubert et al. (43,44) for cultured rainbow trout. The discrepancies between the results reported by various authors may be ascribed to different experimental and environmental conditions. Indeed, it has been demonstrated that the carotenoid absorption and deposition in rainbow trout is strongly affected by several factors, some of which being the nature of carotenoids used, the dietary carotenoid concentration, the fish size or physiological state and stage of sexual maturation (45,46). Moreover, these results are consistent with previous findings indicating that the carotenoid concentration in the flesh of rainbow trout does not increase when the dietary pigment concentration is increased above 50 mg/kg (46). The lack of response to higher dietary doses than 50 mg/kg has been attributed to the reduction of carotenoid digestibility at higher inclusion levels (46,47).

In the current study, dietary astaxanthin supplementation increased the pigmentation of the rainbow trout muscle and caused an increase in chroma and a reduction in lightness after 10 weeks of feeding. The increment in the average values of a^* and b^* and a reduction of L^* in the muscle of salmonids with increasing total dietary carotenoid concentration and feeding period is well documented in previous literature (48). These results also confirmed earlier observations on fish flesh pigmentation and supported the notion that the L^* values of fillets were inversely correlated with their carotenoid contents, whereas their a^* and b^* values were directly correlated with carotenoid contents (49). It has been demonstrated that increasing carotenoid concentrations in the fish fillets led to increased a^* and b^*, and decreased L^* (48).

In addition to its effect on flesh pigmentation, one of the most important properties of astaxanthin is its antioxidant properties, which have been reported to surpass those of other carotenoids or even those of α-tocopherol (50). This has been demonstrated in both *in vivo* and *in vitro* systems, as well as in studies using membrane models (51-53). However, in the present study, plasma and liver radical scavenging activities were not affected by the dietary treatments when hydroxyl radical scavenging was assessed by the ESR technique. This is in contrast with our earlier observations (54) where liver and plasma radical scavenging activities were significantly affected by dietary astaxanthin inclusion in the diets of juvenile olive flounder.

Effective destruction of ROS also requires the action of several antioxidant enzymes in addition to non-enzymatic antioxidant compounds. The enzymatic antioxidant system involves a wide range of enzymes including SOD, CAT, and GPx. SOD is involved in the conversion of the $O_2^{\cdot -}$ to H_2O_2, which may in turn be toxic to the cells. CAT and GPx then detoxify H_2O_2 by catalyzing its conversion to water. Although the activities of antioxidant enzymes have been found to be inversely related to the dietary carotenoid content, information regarding its potential beneficial effects in the oxidative status of fish and underlying mechanisms are still scarce. Lygren et al. (55) indicated that high levels of fat-soluble antioxidants, such as astaxanthin, in postsmolt Atlantic salmon diet might reduce the need for endogenous antioxidant enzymes, such as CAT and SOD, protection against H_2O_2 and $O_2^{\cdot -}$, respectively. This is consistent with several previous studies showing a decrease in antioxidant enzyme activities in plasma or in body tissues of both ornamental (10) and farmed fish (54) as dietary astaxanthin increased. Thus, the higher activity of SOD in rainbow trout fed with the AX0 diet and the lower levels of CAT and GPx in fish fed with the astaxanthin-supple-

mented diets, in the current study, may indicate a decreasing need to detoxify the active oxygen species. Apparently, decreasing the activities of antioxidant enzymes are related to the antioxidants and free radical scavenging properties of dietary astaxanthin that effectively neutralize free radicals before they cause an induction of endogenous antioxidant enzymes activity as a compensatory response. Hence, these findings may suggest that dietary administration of synthetic astaxanthin could improve the antioxidant capacity of juvenile rainbow trout. This suggestion is further supported by the results of the current study where the TAS values, representing the enzymatic and non-enzymatic antioxidant activities, in all supplemented groups were significantly higher than that of the untreated control group. Further studies are required to clarify the exact underlying mechanism through which astaxanthin influences the antioxidant status of fish.

In conclusion, in addition to specifically improving pigmentation, dietary astaxanthin has, apparently, also improved the antioxidant status in juvenile rainbow trout. Based on the results of the present study, dietary inclusion of 50 mg/kg astaxanthin in rainbow trout diet was sufficient to impart the desirable flesh coloration necessary for consumer acceptance. Our results also suggest that 50 mg astaxanthin per kg feed may be enough to enhance the antioxidant capability of juvenile rainbow trout which may exert a number of beneficial effects on human health.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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