

Research Article

Effects of dietary astaxanthin on the growth and skin and muscle pigmentation of sexually immature rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) (Teleostei: Salmonidae)

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Abstract: This study was conducted to investigate the effects of dietary synthetic astaxanthin on the growth performance and skin and muscle pigmentation of sexually immature rainbow trout. Four astaxanthin supplemented diets were prepared to contain 50, 100, 150 and 200mg astaxanthin kg⁻¹ diet. The basal diet without astaxanthin supplementation was used as a control. Juvenile rainbow trout of the similar size (20.07±1.45g) were fed with these experimental diets for 30 days. Results showed that there were no significant differences in weight gain, specific growth rate, condition factor, survival rate and feed conversion ratio among all treatments ($P>0.05$). The skin carotenoid content was significantly affected by the dietary supplementation ($P<0.05$) and the lowest carotenoid content was measured in the treatments of the control and the fish fed 50 mg astaxanthin kg⁻¹ diet ($P<0.05$). However, the fish fed 200mg astaxanthin kg⁻¹ diet demonstrated the highest carotenoid content in the skin ($P<0.05$). Although the muscle carotenoid content increased in all groups, this augmentation was significantly prominent in the fish received the higher concentration of astaxanthin through the feed. According to the obtained results, it is proposed that in sexually immature rainbow trout, astaxanthin is mainly deposited in muscle in compared to skin. Also, diet supplementation with astaxanthin is necessary for skin pigmentation in rainbow trout.

Keywords: Carotenoid, Growth, Muscle, Salmonidae, Skin.

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Introduction

The color of fish skin and muscle is a primary indicator for quality and market prices of farmed fish. Carotenoids are the principal pigments presented in a large variety of aquatic animals, including fishes. Salmonids are distinguished by their prominent muscle coloration (Goodwin 1986) as well as nuptial skin pigmentation, caused by deposition of relatively large amounts of carotenoids, viz. astaxanthin (3,3'-dihydroxy- β,β -carotene-4,4'-dione) and canthaxan-

thin (β,β -carotene-4,4'-dione). However, the former is more effectively utilized by salmonids (Foss et al. 1984, 1987; Gobantes et al. 1997; Page & Davies 2006). In general, animals are unable to biosynthesize carotenoids de novo (Davies 1985), and salmonids, like other teleosts, depend entirely on dietary origin to achieve their natural pigmentation. Fish in wild obtain their requirement from prey, but in intensive fish cultures, the necessary requirements should be met through artificial feeding.

Rainbow trout, *Oncorhynchus mykiss* is almost the exclusive cold-water species cultured in Iran, comprising more than 40% of the total farmed fishes in 2014. Although dietary supplementation with carotenoid is a feasible method in trout farming industry, in many cases the cultured rainbow trout still displays a darker color or pale hue unlike specimens produced in optimal condition.

Culture condition can influence on fish performance including the color and pigmentation, especially in the skin. One of the physiological responses of fish to culture condition stressors including high fish density, high intensity of sunlight or other stress imposed by aquaculture practices, is darkening the fish skin. Melanin, which is synthesized from tyrosine and whose main function is photoprotection, could be overproduced as a physiological response to stressors, to the detriment of fish value and price. Culture condition of rainbow trout exposed to the high intensity of light, overcrowding, as well as food with high tyrosine content, which all may promote melanogenesis. To compensate these disadvantages, carotenoid supplementation could be useful to enhance the natural skin pigmentation in the cultured population.

Besides, carotenoids, especially astaxanthin are vital for salmonid reproduction as thereof primarily deposits in muscle and in lower concentration in skin during growout (Bjerkeng et al. 1992) and mobilize to skin and ovaries at the time of sexual maturation (Torrissen & Torrissen 1985; Bjerkeng et al. 1992; Rajasingh et al. 2006). Dietary astaxanthin is found in different forms in the skin in compare to that of the muscle as the former is deposited as esterified form whereas the latter is in free form (Torrissen & Ingebrigtsen 1992; Tejera et al. 2007; Kalinowski et al. 2011).

Several studies have been evaluated the different roles of carotenoids in fish physiology including reproductive performance (Verakunpiriya et al. 1997; Ahmadi et al. 2006), innate immunity (Amar et al. 2004; Li et al. 2014), coloration and behavior (Baron et al. 2008; Yanar et al. 2016) and growth

performance (Wang et al. 2006). These studies cover a variety of fishes such as rainbow trout *Oncorhynchus mykiss* (Choubert et al. 2009), red dwarf gourami *Trichogaster lalius* (Hamilton, 1822) (Baron et al. 2008), Arctic char, *Salvelinus alpinus* (Linnaeus, 1758) (Bjerkeng et al. 2000), Atlantic salmon, *Salmo salar* Linnaeus, 1758 (Page & Davies 2006), Atlantic halibut, *Hippoglossus hippoglossus* (Linnaeus, 1758) (Bjerkeng & Berge 2000), red porgy *Pagrus pagrus* (Linnaeus, 1758) (Chatzifotis et al. 2005), discus fish *Symphysodon* spp. (Song et al. 2017), Australian snapper, *Pagrus auratus* (Linnaeus, 1758) (Doolan et al. 2009), red sea bream, *Pagrus major* (Temminck & Schlegel, 1843) (Lin et al. 1998), large yellow croaker, *Larimichthys crocea* (Richardson, 1846) (see Li et al. 2014), Atlantic cod, *Gadus morhua* Linnaeus, 1758 (Sawanboonchun et al. 2008) and ornamental carp, *Cyprinus carpio* Linnaeus, 1758 (Sun et al. 2012).

It follows from the literature survey that the pathways of carotenoid metabolism are species specific and there is no universal mobilization pattern of deposited carotenoids in different fish tissues, especially the muscle and the skin as predominant sites. So the aim of the current study was to investigate the effect of dietary astaxanthin on skin and muscle pigmentation in terms of carotenoid concentration in these tissues of rainbow trout during a 30-day period of administration of supplemented feed with this carotenoid.

Materials and Methods

Fish and facilities: The experiment was run with three hundred and seventy five rainbow trout, *Oncorhynchus mykiss* with body weight of 20.07 ± 1.45 g (mean \pm SD) and total length of 124.86 ± 5.35 mm (mean \pm SD) at a private fish farm. Fish were randomly divided into five different groups, in triplicates. Each treatment was in a 60-L fiberglass tank provided with spring water (constant temperature $17.5 \pm 1^\circ\text{C}$; pH 7.5; total hardness $175\text{mg}\cdot\text{L}^{-1}$; dissolved oxygen $8\text{mg}\cdot\text{L}^{-1}$) at a rate of $0.8\text{L}\cdot\text{min}^{-1}$. Outdoor tanks received a natural

photoperiod (26th August to 26th September). Fish were fed twice during the acclimation period (15 days), prior to introduction to the experimental diets, using a non-astaxanthin supplemented food.

Experimental procedure and sampling: A basal diet containing of approximately 37% protein and 14% fat, without added any colorings, was used in this experiment (Table 1). The prepared diet was supplemented with 50, 100, 150 and 200mg astaxanthin kg⁻¹ diet. The basal diet without astaxanthin supplementation was used as control. Astaxanthin was added to diets by weighing the precise amount of astaxanthin powder (10% astaxanthin, ASTA PLUS, SEPEHR SANAT-E-SAHAND Co., Ltd., Tabriz, Iran), mixing it with 10 mL of warm water (40°C) to make astaxanthin dispersion, and evenly spraying the solution on the prepared diet. To preserve the pigments, all diets were stored at 4°C and were protected from light throughout the experiment.

The amount of food fed to the fish was calculated based on a standard feeding chart (3.4% of the total biomass). Fish were handfed at three times during daylight hours from 8.00 to 16.00 hours. To evaluate the fish performance, three sampling was carried out, at the commencement of the experiment, after 2 weeks interval and at the end of the trial. Before each sampling time, the 1-day starved fish were anaesthetized by clove powder (Sladky et al. 2001). Skin and muscle of four individuals were sampled in each sampling time and immediately were covered with aluminium foil and stored at -20°C. The samples were taken from a constant part of the fish body between lateral line and dorsal fin.

Tissue carotenoid concentration assessment: The total carotenoid concentration in the fish skin and muscle was measured spectrophotometrically following a modification of the method described by Schiedt & Liaaen-Jensen (1995). In this procedure, samples of skin (0.1g) and muscle (0.5g) were weighed carefully and finely homogenized with scissors, separately. The proper volume of acetone, as a solvent, was added to the homogenized tissues

Table 1. Ingredients of the basal diet used to feed experimental treatments.

Ingredients	Weight (g.kg ⁻¹)
Fish meal	270
Meat meal	330
Rice meal	160
Wheat meal	90
Fish oil	35
Beet molasses	62
Soybean meal	50
Vitamin C	3

and left until no color was observed anymore. The volume of the extract was reduced to approximately 5ml through partial evaporation. The obtained acetone extracts was mixed with 5 ml of n-hexane and 2 ml of water and was shaken and allowed to diphas. Then, the upper phase was washed by n-hexane until no color was detected. After pooling, the organic epiphases evaporated and were dried. The extract was dissolved in a known amount of n-hexane. To measure carotenoid in n-hexane, the absorbance was read at 470nm, and then the concentration of carotenoid was estimated and expressed as mg kg⁻¹ using the extinction coefficient ($E_{1\%, 1cm}$) of 2100.

Calculations and statistical analysis: Growth parameters including weight gain (WG), percentage of weight gain (WG %) and feed conversion ratio (FCR) were calculated as follows (Li et al. 2009):

$$WG (g) = (\text{final body weight} - \text{initial body weight})$$

$$WG (\%) = (\text{weight gain}/\text{initial body weight}) \times 100$$

$$FCR = \text{total feed intake}/\text{weight gain}$$

$$\text{Specific growth rate (SGR)} = 100 \times (\ln W_f - \ln W_i) / (t)$$

Where ' $\ln W_f$ ' and ' $\ln W_i$ ' are the natural logarithm of the final and initial weights of the fish respectively and 't' is days between $\ln W_f$ and $\ln W_i$. Fulton's condition factors (CF) were calculated as $100 \times \text{weight} / (\text{length})^3$ (Busacker et al. 1990).

In this experiment, a complete randomized design was applied. The data were assessed for normal distribution by Shapiro-Wilk test as well as for homogeneity of the variances using Levene's F-test. All data were subjected to One-way ANOVA followed by Tukey's post hoc test to identify

Table 2. Growth parameters of rainbow trout, *Oncorhynchus mykiss* fed experimental food supplemented with different concentration of astaxanthin for a 30-day trial (mean±SD).

Parameters	Treatments (mg astaxanthin kg ⁻¹ diet)				
	Control	50	100	150	200
IBW	19.69±0.16 ^a	20.16±0.18 ^a	20.32±0.16 ^a	20.08±0.17 ^a	20.09±0.17 ^a
FBW	34.40±0.89 ^a	35.49±0.97 ^a	35.86±1.13 ^a	36.34±0.95 ^a	37.29±0.84 ^a
ITL	12.6±0.1 ^a	12.5±0.1 ^a	12.4±0.1 ^a	12.4±0.1 ^a	12.5±0.1 ^a
FTL	14.1±0.1 ^a	14.1±0.1 ^a	14.2±0.1 ^a	14.2±0.1 ^a	14.3±0.1 ^a
WG	14.63±1.01 ^a	15.29±0.44 ^a	15.62±0.75 ^a	16.30±0.90 ^a	17.19±0.42 ^a
WG (%)	74.33±5.43 ^a	75.87±2.41 ^a	76.87±3.73 ^a	81.09±3.84 ^a	85.56±2.51 ^a
SGR (%.day ⁻¹)	1.85±0.10 ^a	1.88±0.05 ^a	1.90±0.07 ^a	1.98±0.07 ^a	2.06±0.05 ^a
FCR	1.43±0.11 ^a	1.36±0.05 ^a	1.38±0.07 ^a	1.34±0.08 ^a	1.28±0.03 ^a
CF	1.23±0.02 ^a	1.26±0.02 ^a	1.25±0.02 ^a	1.27±0.02 ^a	1.27±0.02 ^a
Survival rate (%)	85.3±2.7 ^a	84.0±2.3 ^a	80.0±4.0 ^a	90.7±1.3 ^a	86.7±2.7 ^a

IBW, initial body weight; FBW, final body weight; ITL, initial total length; FTL, final total length; WG, weight gain; WG (%), percentage of weight gain; SGR, specific growth rate; FCR, feed conversion ratio; CF, condition factor. Different superscripts in a row indicate significant differences ($P<0.05$) between the treatments.

significant differences between various treatments. Kruskal-Wallis and Mann-Whitney tests were run when prerequisites of parametric tests were not met. Comparison of all growth parameters between initial and end of the experiment was evaluated by Student's t-test. ArcSin data transformation was done for data in percentage before analysis (Zar 2010). Statistical significance was tested at a 0.05 probability level. Data are presented as mean±standard deviation (S.D). All statistical analysis was run using SPSS for Windows, Version 15.0.

Results

Growth performance: Some mortalities are recorded throughout the experiment which are shown in Table 2. Survival rate was not significantly different between the treatments at the end of the study ($\chi^2=6.409$, $N=15$, $P=0.171$). There was no significant difference in different fish growth parameters including FBW (g) ($F_{4,199}=1.263$, $P=0.286$), WG (g) ($F_{4,14}=1.722$, $P=0.221$), WG (%) ($F_{4,14}=1.575$, $P=0.255$) and SGR (%.day⁻¹) ($F_{4,14}=1.440$, $P=0.291$) at the end of the 30-day trial (Table 2) ($P>0.05$). Similarly, the fish total length was significantly same when compared between different treatments ($\chi^2=2.536$, $N=200$, $P=0.638$). The value of FCR in all treatments was the same ($F_{4,14}=0.567$, $P=0.692$). The CF did not significantly vary between

various treatments ($\chi^2=3.979$, $N=200$, $P=0.409$).

The content of carotenoids in the skin: The results of this study showed that the carotenoid content in the skin of fish has minor fluctuation during the experiment only in the control and the fish fed the lowest amount of astaxanthin (50mg astaxanthin kg⁻¹ diet). In the other treatments, the skin carotenoid content increased significantly over the time, however this increase was more prominent in the fish fed the highest amount of astaxanthin (200mg astaxanthin kg⁻¹ diet) (Fig. 1). The highest coefficient of variation (CV%=64.93%) was recorded in the treatment administered with 200mg astaxanthin kg⁻¹ diet. In the fish fed 100 and 150mg astaxanthin kg⁻¹ diet, the value of CV% was obtained 39.32 and 41.67% respectively. However, the lowest amount of CV% was measured in the control (24.96%) and the fish fed 50mg astaxanthin kg⁻¹ diet (24.59%). Although the fish located in the treatment fed 50mg astaxanthin kg⁻¹ diet demonstrated significantly higher carotenoid content in the skin at the beginning of the experiment ($\chi^2=17.474$, $N=60$, $P=0.002$), showed the lowest amount at the midtrial sampling time, along with the control ($\chi^2=45.610$, $N=60$, $P=0.000$) (Table 3).

At the completion of the experiment, the carotenoid content in the skin of fish fed 200mg astaxanthin kg⁻¹ diet were significantly higher than

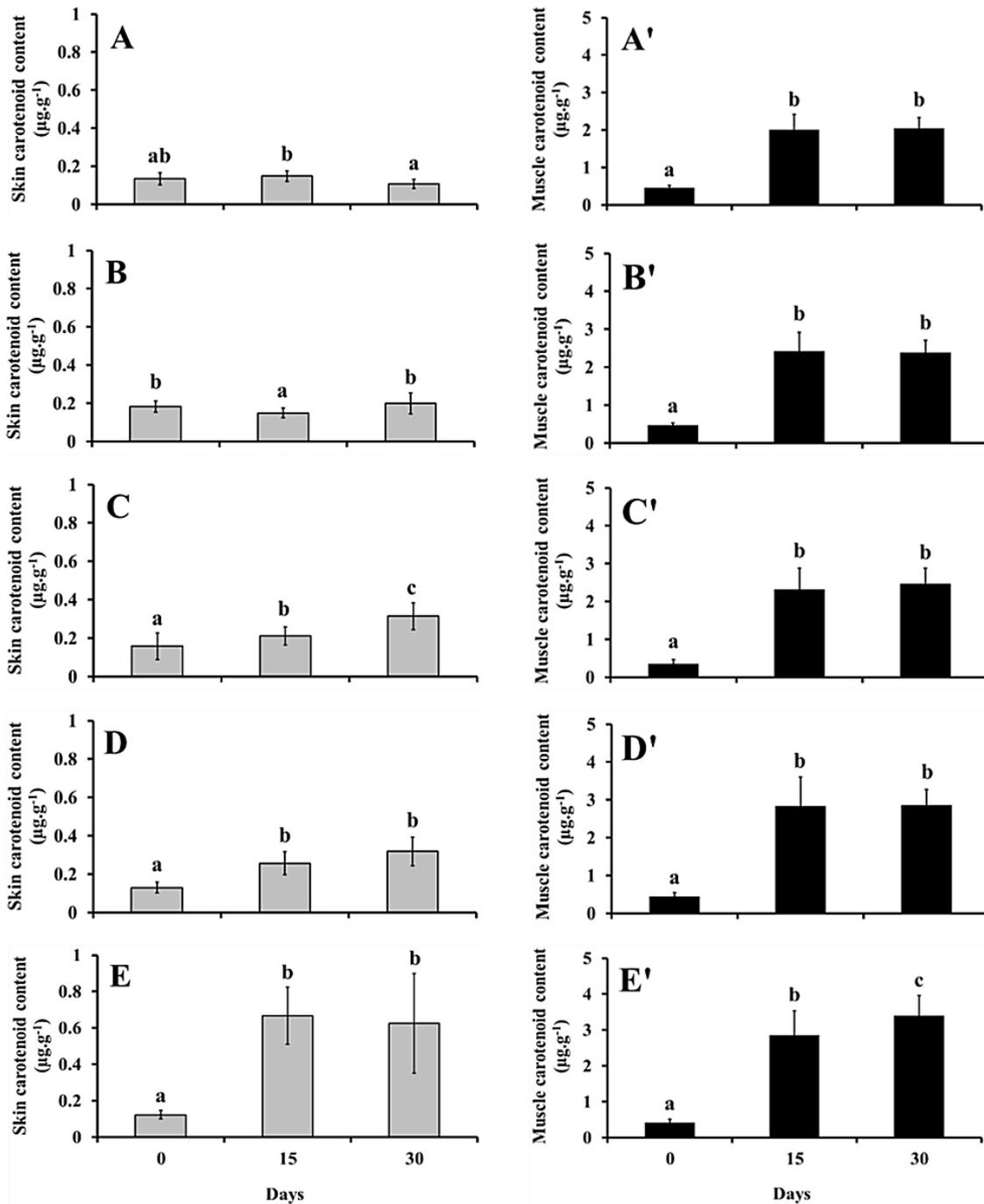


Fig.1. Carotenoid levels in rainbow trout *Oncorhynchus mykiss* skin (grey bars, n=12) and muscle tissues (black bars, n=12) after feeding on basal diet (A and A') or supplemented with synthetic astaxanthin with 50mg (B and B'), 100 mg (C and C'), 150mg (D and D') and 200mg (E and E') astaxanthin kg⁻¹ diet. Bars with vertical error bars represent means±S.D. Different letters over bars indicate significant difference (P<0.05).

that of other treatments. The lowest amount of carotenoid in the skin was measured in control, followed by fish fed 50 mg astaxanthin kg⁻¹ diet. Both fish that fed 100 and 150mg astaxanthin kg⁻¹ diet demonstrated the same carotenoid content in the

skin ($\chi^2=45.966$, N=60, $P=<0.001$).

The content of carotenoid in the muscle: In all treatments, carotenoid content in the muscle was significantly higher in the middle and at the end of the study in compare to the beginning. However, the

Table 3. Effect of the experimental food supplemented with different concentration of astaxanthin on the rainbow trout, *Oncorhynchus mykiss* skin and muscle carotenoid content (mean±S.D, n=12).

Treatments (mg/kg diet)	Total carotenoid (mg/kg muscle)			Total carotenoid (mg/kg skin)		
	Days			Days		
	0	15	30	0	15	30
Control	0.455±0.072 ^{ab}	2.008±0.416 ^a	2.041±0.295 ^a	0.134±0.032 ^a	0.148±0.029 ^a	0.107±0.025 ^a
50	0.475±0.049 ^b	2.420±0.499 ^b	2.387±0.326 ^a	0.183±0.030 ^b	0.150±0.025 ^a	0.199±0.056 ^b
100	0.361±0.108 ^a	2.329±0.559 ^{ab}	2.471±0.417 ^{ab}	0.158±0.069 ^a	0.211±0.048 ^b	0.314±0.070 ^c
150	0.449±0.103 ^{ab}	2.835±0.774 ^b	2.869±0.410 ^b	0.130±0.028 ^a	0.257±0.060 ^b	0.319±0.074 ^c
200	0.424±0.091 ^{ab}	2.849±0.683 ^b	3.397±0.557 ^c	0.123±0.023 ^a	0.667±0.156 ^c	0.625±0.274 ^d

Figures in each column with different superscript letters are statistically different ($P < 0.05$).

muscle content of carotenoid were not different significantly in these sampling times, except than the fish fed 200mg astaxanthin kg^{-1} diet which showed higher carotenoid content at the end of the study ($\chi^2=25.090$, $N=36$, $P < 0.001$). The coefficient of variances for the control, the fish fed 50, 100, 150 and 200 mg astaxanthin kg^{-1} diet were 53.56%, 55.73%, 61.22%, 71.57% and 63.02% respectively. The value of CV% was significantly higher in treatments received 100, 150 and 200mg astaxanthin kg^{-1} diet than that of the control and the fish fed 50mg astaxanthin kg^{-1} diet.

At the commencement of the experiment, the muscle carotenoid content was the same in all treatments except than the fish fed 50 mg astaxanthin kg^{-1} diet which was slightly higher than that of the fish fed 100mg astaxanthin kg^{-1} diet ($F_{4, 59}=3.093$, $P=0.023$) (Table 3). The second sampling time demonstrated significantly higher amounts of carotenoid in the muscle of fish fed 50, 150 and 200mg astaxanthin kg^{-1} diet ($\chi^2=14.929$, $N=60$, $P=0.005$), however the carotenoid content in the fish fed 100mg astaxanthin kg^{-1} diet and the control was statistically the same. At the end of the experiment, the highest amount of carotenoid was measured in the muscle of the fish fed 200mg astaxanthin kg^{-1} diet. The lowest amount was recorded in the control and the fish fed 50mg astaxanthin kg^{-1} diet. The muscle carotenoid content in the fish fed 100 and 150mg astaxanthin kg^{-1} diet was not significantly different ($P > 0.05$), however only in the latter group the muscle

carotenoid content was significantly higher than that of the control and the fish fed 50mg astaxanthin kg^{-1} diet ($F_{4,59}=19.111$, $P=0.000$).

Discussion

Red carotenoids, mainly associated with astaxanthin and cantaxanthin, are the predominant organic natural pigments of interest in aquaculture that should be provided through dietary as fishes are unable to synthesis these important compounds de novo (Matsuno 2001; Maoka 2011). It is well documented that these pigments play vital roles in a variety of different concepts of fish physiology including behavior, immunity, growth, nutrition and reproduction (Ando et al. 1990; Torrissen & Christiansen 1995; Christiansen & Torrissen 1997; Řehulka 2000; Amar et al. 2004; Ahmadi et al. 2006; Baron et al. 2008; Kalinowski et al. 2011; Yi et al. 2014). Among different kinds of carotenoids, astaxanthin is the main natural pigment responsible for the typical pink color appearance of the salmonid flesh (Schiedt et al. 1985; Storebakken & Choubert 1991; No & Storebakken 1992; Storebakken & No 1992).

The results from the present study on the skin carotenoid content demonstrated that the dietary astaxanthin supplements has significant effects on the fish skin pigmentation with increasing along with the concentration of the astaxanthin inclusion. At the end of the trial, the concentration of the skin carotenoid in all experimental fish was significantly

higher than that of the control, however this amount was noticeably higher in the fish fed 200mg astaxanthin kg^{-1} diet. These results are in agreement with the findings of other conducted experiments on salmonids (Choubert 1979; Schiedt et al. 1988; Bjerkgeng et al. 1990; Sommer et al. 1991; Bjerkgeng et al. 1992; No & Storebakken 1992; Storebakken & No 1992; Shahidi et al. 1993).

Several studies on non-salmonids including gilthead seabream *Sparus aurata* (Gomes et al. 2002), red porgy, *Pagrus pagrus* (Kalinowski et al. 2007; Tejera et al. 2007), discus *Symphysodon* spp. (Song et al. 2017), olive flounder, *Paralichthys olivaceus* (Pham et al. 2014), large yellow croaker, *Larimichthys crocea* (Yi et al. 2014), Australian snapper, *P. auratus* (now= *Chrysophrys auratus* (see Booth et al. 2004; Doolan et al. 2009), goldfish, *Carassius auratus* (see Paripatananont et al. 1999; Gouveia & Rema 2005) and channel catfish, *Ictalurus punctatus* (see Li et al. 2007) have also reported improved skin pigmentation by carotenoid inclusion in the dietary, either by supplemented synthetic astaxanthin or through natural carotenoid sources.

Although the skin carotenoid content in the fish fed 50mg astaxanthin kg^{-1} diet was significantly higher than that of the control, in both groups the value remained to some extent in a plateau situation throughout the experiment. During this time, carotenoid content in the control fish reached from 0.134mg kg^{-1} skin at the initial time to 0.107mg kg^{-1} skin after a trial of 30 days. These values are 0.183 and 0.199mg kg^{-1} skin in the fish fed 50mg astaxanthin kg^{-1} diet, respectively. The skin carotenoid content in the other two experimental fish fed with 100 and 150mg astaxanthin kg^{-1} diet was around 3 times of the value in the control while in the fish fed with the highest astaxanthin concentration the magnitude of this value was double. This phenomenon supports that astaxanthin which is the main skin pigmentation in salmonids (Hata & Hata 1975; Storebakken & No 1992; Amar et al. 2001; Maoka 2011), should be provided by food as fish are

unable to biosynthesize or bioconvert (Storebakken & No 1992; Maoka 2011) this important pigment. The basal diet may be contained other carotenoids in addition to astaxanthin like canthaxanthin but bioconversion of different kinds of pigments to astaxanthin is not proven in salmonids (Matsuno 2001; Maoka 2011).

In the present study, increased muscle carotenoid content was observed in all groups in line with increasing dietary astaxanthin inclusion. The rainbow trout fed with 150 and 200mg astaxanthin kg^{-1} diet showed significantly higher carotenoid content in the muscle in compared to the control and the fish fed with 50mg astaxanthin kg^{-1} diet. After 30 days, the muscle of fish fed with 200mg astaxanthin kg^{-1} diet contained 3.397mg kg^{-1} of total carotenoids, which is 66% more than that of the control. Foss et al. (1984) reported concentration of 2.375mg kg^{-1} of astaxanthin in muscle of rainbow trout after 4 weeks of feeding with diets supplemented with astaxanthin. Choubert & Heinrich (1993) reported that muscle carotenoid concentration of 11.8mg kg^{-1} would be obtained in rainbow trout after 4 weeks of feeding with dietary with synthetic astaxanthin inclusion. The similar improvements in the muscle carotenoid contents after feeding with supplemented carotenoids also reported in discus fish with Liu et al. (2016) and in olive flounder by Pham et al. (2014). Other studies conducted with dietary carotenoid supplementation on the improvements in the muscle pigmentation of rainbow trout (Choubert et al. 2009; Rahman et al. 2016), Arctic charr, *Salvelinus alpinus* (Hatlen et al. 1998; Bjerkgeng et al. 2000), Japanese ornamental carp, koi, *Cyprinus carpio* (Sun et al. 2012), characins, *Hyphessobrycon eques* (Steindachner, 1882) (Wang et al. 2006), olive flounder (Wang et al. 2006), red porgy (Kalinowski et al. 2007) and Atlantic salmon, *Salmo salar* (Sigurgisladottir et al. 1994; Torrissen et al. 1995) have revealed supporting results. Based on the obtained results, increased muscle carotenoid content in rainbow trout is a sign of fish ability to deposit astaxanthin in the muscle. Indeed, it has been reported that astaxanthin binds

non-specifically to hydrophobic sites on the actomyosin (Henmi et al. 1990) at the storing capacity of 0.9mg astaxanthin g⁻¹ actomyosin (Henmi et al. 1989). According to this assumption, salmonids have a high storing capacity to accumulate carotenoids in white muscle and the obtained levels in this study are not a limiting maximum level.

Retention of carotenoids in fish tissues depends on several factors including fish species, fish organs, life cycle, fish physiological condition, carotenoid types as well as environmental factors. Rainbow trout like other salmonids, deposits astaxanthin as the most prominent carotenoid and canthaxanthin to a lesser extent, in different tissues like muscle and skin (Bjerkeng et al. 1992; Řehulka 2000). This red pigment stored in fish skin in the esterified form while in the other organs like muscles deposited in the free form (Schiedt et al. 1985; Kaisuyama & Matsuno 1988; No & Storebakken 1991; Tejera et al. 2007). The muscle carotenoid content in all groups were ascending during the experimental time however this augmentation was not the same as the highest concentration of the muscle carotenoid content was in fish fed with 200mg astaxanthin kg⁻¹ diet followed by the fish fed with 150mg astaxanthin kg⁻¹ diet. Increasing the muscle carotenoid content in the experimental fish fed with dietary astaxanthin inclusion is obviously resulted as ingestion and accumulation of supplemented astaxanthin in basal diet. This result strongly supported by finding of other studies used carotenoids for fish pigmentation (Li et al. 2007; Choubert et al. 2009; Doolan et al. 2009; Brown et al. 2016). The control and the fish fed with 50 and 100mg astaxanthin kg⁻¹ diet demonstrated almost the same CV% of the muscle carotenoid content. The most familiar reactions of carotenoid metabolism in fish are primarily oxidative (Kitahara 1983; Schiedt et al. 1988; Matsuno 2001). Fish can bioconvert main carotenoids through reductive metabolic pathways (Matsuno 2001; Rajasingh et al. 2006; Yasir & Qin 2010). The basal diet which is used in this study was made from different ingredients including fish oil, fish and meat

meals as well as rice and wheat meals. Although the control had not been fed with dietary astaxanthin supplements, the presence of carotenoids in the basal diet ingredients is possible. As the muscle carotenoid content is expressed as total carotenoids, ingestion and accumulation of these carotenoids and final products of bioconversion of any kinds of these pigments through reductive metabolic pathways could increase the deposited carotenoid amount. Several studies have reported minor improvement in pigmentation of control fish (No & Storebakken 1991; Christiansen & Torrissen 1996; Kalinowski et al. 2007; Li et al. 2007; Yi et al. 2014).

Neither the feed conversion ratio (FCR), specific growth rate (SGR), condition factor (CF) nor final mean body weight of the juvenile rainbow trout were significantly affected by the dietary supplementation of synthetic astaxanthin at the tested concentration after a 30-day trial. In all experimental groups, fish demonstrated natural growth throughout the experiment. These results are well in accordance with the findings of other studies carried out with rainbow trout in which administration of astaxanthin was ineffective in fish growth parameters and feed utilization (Storebakken & Choubert 1991; Thompson et al. 1995; Nickell & Bromage 1998; Řehulka 2000; Amar et al. 2001; Page & Davies 2006; Rahman et al. 2016). Also the present results are in line with the findings of the studies conducted on the other species like Atlantic salmon, *Salmo salar* (Baker et al. 2002; Olsen & Baker 2006), *Cyprinus carpio* (Paripatananont et al. 1999), red porgy *P. pagrus* (Chatzifotis et al. 2005; Kalinowski et al. 2005; Tejera et al. 2007), *S. aurata* (Gomes et al. 2002), large yellow croaker *Larimichthys croceus* (Yi et al. 2014), characins *Hyphessobrycon eques* (Steindachner, 1882) (Wang et al. 2006), Australian snapper, *P. auratus* (Doolan et al. 2009), flame-red dwarf gourami, *Trichogaster lalius* (Hamilton, 1822) (Baron et al. 2008), discus *Symphysodon aequifasciatus axelrodi* (Liu et al. 2016) and olive flounder, *Paralichthys olivaceus* (Pham et al. 2014). However, some other studies revealed the positive

effects of astaxanthin supplementation diet on growth parameters (Torrissen 1984; Boonyaratpalin & Unprasert 1989; Torrissen 1989; Christiansen et al. 1995; Christiansen & Torrissen 1996; Akhtar et al. 1999; Kalinowski et al. 2011; Sun et al. 2012; Li et al. 2014).

Carotenoids like astaxanthin are categorized as micronutrients in fish feeding. So, the physiological effects of these compounds are not directly inserted into somatic growth as they act and put effects, such as antioxidants, indirectly in various physiological aspects and may ultimately result in improved growth. Consequently, prolonged dietary supplementation with carotenoids might be required to observe any improvements on growth performance. Nickell & Bromage (1998) showed that in the rainbow trout growth improvements were not obtained in the first 330 days of the experiment.

Based on the results of the present study, it can be concluded that the supplementation of astaxanthin significantly improved the skin and muscle carotenoid content of immature rainbow trout without affecting the growth performance. According to the obtained results, dietary inclusion of 200mg astaxanthin kg⁻¹ diet for a period of 4 weeks is necessary for improvement of muscle and skin pigmentation.

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مقاله پژوهشی

تأثیر آستاگزانتین خوراکی بر رشد، رنگدانه پوست و عضله در قزل آلی رنگین کمان نابالغ، *Oncorhynchus mykiss* (Walbaum, 1792)

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چکیده: در این مطالعه تأثیر آستاگزانتین خوراکی بر عملکرد رشد و میزان رنگدانه پوست و عضله در قزل آلی رنگین کمان نابالغ مورد بررسی قرار گرفت. چهار تیمار غذایی با مکمل آستاگزانتین حاوی ۵۰، ۱۰۰، ۱۵۰ و ۲۰۰ میلی گرم آستاگزانتین به ازای هر کیلوگرم غذا تهیه شد. رژیم غذایی پایه بدون استفاده از مکمل آستاگزانتین به عنوان کنترل در نظر گرفته شد. ماهیان قزل آلی نوجوان با میانگین وزن یکسان (20.07 ± 1.45 گرم) به مدت ۳۰ روز با تیمارهای غذایی ساخته شده تغذیه شدند. تفاوت معنی داری در افزایش وزن، شدت رشد ویژه، فاکتور وضعیت بدن، درصد بازماندگی و ضریب تبدیل غذایی در بین تیمارهای مختلف دیده نشد ($P > 0.05$). میزان کاروتنوئید پوست به طور معنی داری تحت تأثیر مکمل های غذایی تغییر کرد ($P < 0.05$)، به نحوی که کمترین میزان کاروتنوئید در گروه کنترل و نیز ماهیان تغذیه شده با دوز ۵۰ میلی گرم آستاگزانتین به ازای هر کیلوگرم غذا سنجیده شد ($P < 0.05$). همچنین، ماهیان تغذیه شده با دوز ۲۰۰ میلی گرم آستاگزانتین به ازای هر کیلوگرم غذا بیشترین میزان کاروتنوئید پوست را دارا بودند ($P < 0.05$). هرچند میزان کاروتنوئید عضله در تمام گروه های آزمایشی روند افزایشی را نشان داد، اما این افزایش در ماهیانی که دوزهای بالاتری از آستاگزانتین را از طریق غذا دریافت کرده بودند، به طور معنی داری بیشتر بود ($P < 0.05$). بر اساس نتایج حاصل، می توان بیان نمود که در قزل آلی رنگین کمان نابالغ، در مقایسه با پوست، آستاگزانتین به طور عمده در بافت عضله ذخیره می شود. همچنین مکمل غذایی با آستاگزانتین جهت افزایش شدت رنگدانه پوست در قزل آلی رنگین کمان نابالغ لازم و ضروری می باشد.

کلمات کلیدی: آزادماهیان، پوست، رشد، عضله، کاروتنوئید.