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The effects of lucantin red, yellow and astaxanthin on growth, hematological, immunological parameters and coloration in the Tiger Oscar (*Astronotus ocellatus* Agassiz,1831)

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Abstract

The present study aimed to determine the effects of lucantin red and yellow and also astaxanthin on the growth, hematological and immunological parameters, and coloration of the tiger oscar (*Astronotus ocellatus*). To this end, 120 fingerlings of tiger oscar were equally and randomly divided into 12 aquariums. The fish were fed with experimental diets for 8 weeks. Treatments included diets containing no pigment (control), 100 mg kg^{-1} lucantin red, 100 mg kg^{-1} lucantin yellow, and 100 mg kg^{-1} astaxanthin, with three replicates apiece. At the end of the trial, growth factors, hematological and immunological parameters, and coloration were measured and the obtained data were statistically analyzed. The results showed that there is a significant difference between the control group and other treatments fed with pigments in terms of growth factors (p<0.05). However, no significant difference was found between treatments fed with pigments in this regard (p>0.05). The results indicated no significant difference between treatments in hematological parameters (p>0.05). The results demonstrated that there is a significant difference between the control group and other treatments in terms of lysozyme activity. In addition, a significant difference was observed between treatments 1 and 3 in this regard (p<0.05). However, there was no significant difference between treatments in other immunological parameters (total immunoglobulin, IgM). The statistical analyses suggested no significant difference between experimental treatments in terms of total carotenoids in the blood (p>0.05). Statistical analysis of color intensity variation revealed a significant difference between the control group and other treatments (p<0.05). The study findings showed that lucantin red and yellow and astaxanthin can improve growth factors and coloration and increase lysozyme activity in Tiger Oscar.

Keywords: Astaxanthin, *Astronotus ocellatus*, Lucantin, Coloration, Blood

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Introduction

The fish color is an important indicator in the aquaculture industry (Li *et al*., 2014). Along with the growth of aquaculture in the world, the industry of ornamental fishes propagation and rearing is expanding (Erdogan *et al*., 2012; Guroy *et al*., 2012). The color of ornamental fishes is one of the most important qualitative criteria in determining their market value. Accordingly, a group of carotenoid pigments may grow in the skin of the fish influenced by using of special diet (Sornsupharp *et al*., 2015). A wide range of artificial and natural carotenoids are used as nutritional supplements in aquaculture to improve skin color (Storebakken and No, 1992; Paripatananont *et al*., 1999; Gouveia *et al*., 2003; Hancz *et al*., 2003; Chien and Shiau, 2005; Kalinowski *et al*., 2005). As natural antioxidants, carotenoids play a major role in the health of living organisms by neutralizing the free radicals caused by the normal activity of cells and environmental stress (Chew, 1995). Various factors affect the coloration of the fish such as fish size and age, fecundity course, diet composition, the source and concentration of carotenoid, sexual maturity, and genetic factors. In addition, water temperature, salinity, and light intensity are environmental parameters that influence fish coloration (Torrissen *et al*., 1989; Storebakken and No, 1992). Astaxanthin is the most important carotenoid pigment found in aquatic organisms (Christiansen *et al*., 1996; Guerin *et al*., 2003) and controls some biological functions including the prevention of the oxidation of essential unsaturated fatty acids, protection against negative effects of ultraviolet light, vitamin A generation, immunity reactions, bio-coloring, and improvement of growth and reproductive behaviors (Torrissen *et al*., 1989; Lorenz and Cysewski., 2000). Mammals and most fishes are not able to synthesize the carotenoids they need and crustaceans also have a limited ability to convert other pigments to astaxanthin. Therefore, it is advisable that this pigment is directly added to the diet (Meyers, 1977; Guerin *et al*., 2003). The Tiger Oscar (*Astronotus ocellatus*) is a large, boldly colored South American cichlid characterized by its unique personality and striking intelligence. It is considered a popular aquarium fish in Europe and the U.S.A (Staeck and Linke, 1985; Loiselle, 1995; Keith *et al*., 2000). Lucantin red and yellow are other types of carotenoid pigments. The present study aims to determine the effect of three pigments on growth factors, hematological and immunological parameters, and coloration of the tiger oscar.

Materials and methods

In this study, 120 fingerlings of tiger oscar, with a mean weight of 7.5±0.5 grams, were provided from an ornamental fishes propagation and rearing center in Guilan Province. The subjects were complete the same age and were the offspring of a single pair of brooders. After adaptation to the experimental environment, the subjects were randomly and equally assigned to 12 aquariums sized 50×40×33 (10 fish in each aquarium). Every 3 aquariums represent a single treatment with three replicates. Experimental treatments were as follows:

-Control: Commercial feed without food additives

-Treatment 1: Commercial feed containing 100 mg per kg of lucantin red
-Treatment 2: Commercial feed containing 100 mg per kg of lucantin yellow
-Treatment 3: Commercial feed containing 100 mg per kg of astaxanthin

The base diet was Biomar feed which contained 54% protein, 18% raw fat, 10% cellulose, 10% ash, and 1.6% total phosphorus. The subjects were fed twice a day (morning and night) by 3% of their body weight for 8 weeks (Ghiasvand and Shapoori, 2006).

The pigments used in this research were purchased from BASF Company, Germany. Astaxanthin, 3,3'-dihydroxy-ß-carotene-4,4'-dione, was used as a purple powder with a particle size less than 0.6 mm and completely soluble in 40°C water. The chemical formula of Lucantine red and Lucantin yellow was 4,4'-dioxo-β-carotene and β-apo-8'-carotenoic acid ethyl ester, respectively. The desired amount of pigments was dissolved in 150 ml distilled water by an electromagnetic device (hot plate magnetic) and a magnet with a temperature of 50°C. Then, the resulting solution was sprayed on the food. The feed containing pigments was dried in a gentle drying machine, kept in a dark and cool place for a while, and then placed in a freezer until the end of the trial.

The mean water temperature, dissolved oxygen, pH, water hardness (DH), total ammonia before feeding, and ammonia after feeding during the trial were 27.15±1.32, 7.63±3.24, 8.0±3.33, 231.0±99.20, 0.1 mg L^{-1}, and 0.2 mg L^{-1}, respectively.

Measurement of growth factors and survival rate

The length of subjects was measured by using a caliper with a precision of one millimeter and their weight was measured by a digital scale with a precision of 0.001 gram. To determine the growth status of subjects and performance of different treatments, survival rate, body weight increase, feed conversion ratio, special growth rate, weight gain, condition factor and length were calculated using the following equations:

Survival rate (SVR)=(S-D)/S×100 (Mazurkiewicz *et al*., 2008)

S=the number of subjects

D=the number mortalities

Body weight increase (BWI)=100×(BW_1 – BW_0)/BW_0 (Hung *et al*., 1989)

BW_0= initial weight (g)

BW_1= final weight (g)

Feed conversion rate (FCR)=F/W_1–W_0) (Ronyai *et al*., 1990; Abdelghany and Ahmad, 2002)

F=the amount of food consumed by the fish

W_0= the mean primary biomass (g)

W_1= the mean final biomass (g)

Special growth rate (SGR)= =[(LnW_1 - LnW_0) / t] ×100 (Ronyai *et al*., 1990)

W_0=the mean primary biomass (g)

W_1=the mean final biomass (g)

t=time period (day)

Weight gain (WG)=final weight (g)–initial weight (g) (Ricker, 1979; Tacon, 1990)

Condition factor (K)=W/L^3×100(Ojolick *et al*., 1995)

W= fish weight (g)

L= fish length (cm)

Length=final length(cm)–initial length(cm)

Hematological and Immunological parameters

In order to measure hematological parameters at the end of the trial, 3-6 fish from each treatment were randomly selected and anesthetized using the extract of clove powder (0.5 ml in one liter of water). Then, after drying the fish, blood sampling from the caudal peduncle were done using heparinized insulin syringes, and blood samples were poured into microtubes containing 20 µl heparin. The microtubes were numbered and prepared for hematological tests in the next steps (Thrall, 2004). For serological studies, blood samples in Eppendorf tubes without heparin were centrifuged at 300 rounds per minute for 10 minutes (Labofuge, Heraeus Sepatch, Germany). Then, the serum was separated, poured into new Eppendorf tubes using a sampler, and stored at -80°C. Red blood cells count (RBC), hematocrit (HCT), hemoglobin (HB), white blood cells count (WBC), differential white blood cells count (lymphocytes, eosinophils, neutrophils, and monocytes), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were the hematological parameters measured in this study (Klontz, 1994). To measure lysozyme, the method proposed by Clerton *et al*. (2001) was used, and immunoglobulin (IgM) and total immunoglobulin were measured by the method of Siwicki and Anderson (1993).

Measurement of total carotenoid and coloration

The method of Weber (1988) was used to measure total carotenoid. In addition, the procedure proposed by Yam and Papadakis (2004) was used for determining color variation in subjects. This method is based on the processing of images taken by a digital camera at the same light intensity and conditions. To this end, 6 fish were randomly selected from each replicate and anesthetized in the extract of clove powder. Then, some images of their left side were taken by a Cannon digital camera (12.1 pixels) from a distance of 30 cm. The intensity of colors was measured at caudal peduncle or R.O. area, which is a common point in all strains of oscar. Imaging was done in 4 stages: before feeding with diets containing pigments, 10 days after feeding with diets containing pigments, 20 days after the second imaging, and 27 days after feeding with diets containing pigments.

Data analysis

The Kolmogorov-Smirnov test was used to test the normal distribution of data and the homogeneity of variances was examined using Levene's test. In addition, one-way analysis of variance (ANOVA) was used for statistical analysis of data. The mean values were compared using ANOVA and Duncan's multiple range test in SPSS, at the 95% interval of confidence. Excel 2010 was used to draw charts.

Results

According to Table 1, significant difference was observed between the control and other treatments fed with pigments in growth factors (survival rate, weight gain, FCR, SGR, final weight, condition factor, and length increase) (p<0.05). The results of hematological and immunological parameters assessment have been presented in Tables 2 and 3.

Table 1: The mean values of growth factors (mean ± SD) in different treatments. Different letters (a-d) indicate a significant difference (p<0.05).

Diets	SVR(%)	BWI(%)	FCR	SGR(%)	WG(g)	K	Length(cm)
Control	85±0.7^{a}	101.77±1.51^{a}	1.85±0.27^{b}	1.25±0.13^{a}	7.63±1.13^{a}	2.96±0.27^{c}	0.90±0.14^{a}
Treatment 1	100±0^{b}	280±1.32^{bc}	0.92±0^{abc}	2.38±0.21^{bc}	21.02±0.25^{bc}	2.53±0.14^{ab}	3.30±0.21^{bc}
Treatment 2	100±0^{b}	254±1.90^{b}	1.1±0.08^{c}	2.26±0.09^{b}	19.12±1.42^{b}	2.51±0.17^{ab}	3.08±0.04^{b}
Treatment 3	100±0^{b}	271±1.34^{bc}	0.90±0.04^{abc}	2.34±0.06^{bc}	20.36±1.01^{bc}	2.4±0^{ab}	3.41±0.12^{bcd}

Table 2: The mean values of hematological parameters (mean ± SD) in different treatments.

Diets	WBC ($N\times10^{3}$)	RBC ($N\times10^{6}$)	HB (g dl^{-1})	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g dl^{-1})	Neutrophilis (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Control	5050±1.7	1.88±0.53	7.55±0.07	7.55±0.07	173±0	40±0	23±0	26.5±0.7	70.5±1.36	6±1.41	0
Treatment 1	4550±2.37	1.81±0.40	7.4±0.28	7.4±0.28	168±2.82	41±0	24±0	26±1.41	69.5±2.12	3±2.82	0
Treatment 2	4550±2.23	1.62±0.66	6.65±1.2	6.65±1.2	172.5±2.77	40.5±0.7	23.5±0.7	22.5±3.53	72±2.82	5±0	0.5±0.7
Treatment 3	5200±2.45	1.86±0.99	7.25±0.35	7.25±0.35	163.5±2.53	39±0	23.5±0.7	24.5±2.95	70.5±1.77	4.5±1.48	0.5±0.7

In addition, there was no significant difference between treatments during the trial (56 days) in terms of hematological parameters (p>0.05) (Table 2).

Table 3: The mean values of immunological parameters (mean ± SD) in different treatments. Different letters (a-d) indicate a significant difference (p<0.05).

Diets	IgM (ml dl^{-1})	Total Immunoglobulin (mg ml^{-1})	Lysozyme (µg ml^{-1})
Control	12.75±2.47	19.75±0.35	19.5±3.53^{a}
Treatment 1	14.6±0.56	20.5±1.12	35±1.41^{abc}
Treatment 2	14.95±1.48	19±1.82	45.5±1.67^{abcd}
Treatment 3	14.1±1.55	17.75±1.76	75±1.72^{d}

The highest lysozyme activity was related to the treatment fed with astaxanthin, and there was a significant difference between the control other treatments fed with astaxanthin (p<0.05) (Table 3).

Table 4 shows the results on the measurement total carotenoid and intensity variation of the main and primary skin colors in 4 steps.

Table 4: The mean values of total carotenoid and intensity variation of the main and primary skin colors (mean±SD) in different treatments. Different letters (a-d) indicate a significant difference (p<0.05).

Diets	Total Carotenoid (µg ml^{-1})	Stages	R	G	B	C	M	Y	K
Control	2.9±0.14	Stage1	78.77±1.02^{a}	109.78±2.5^{a}	101±3.9^{a}	37.33±3.1^{a}	46.5±2.29^{a}	48.66±1.2^{a}	28.66±2.1^{a}
		Stage2	85.66±5.56^{a}	83.88±3.02^{a}	60.99±1.4^{d}	48.55±1.57^{d}	43.66±4.66^{a}	63.88±2.1^{a}	43.33±2.21^{a}
		Stage3	173±3.09^{a}	127±3.64^{cd}	32.66±1.71^{b}	20.66±3.46^{cd}	56.44±1.40^{a}	93.55±3.14^{a}	28.55±1.3^{c}
		Stage4	120±1.73^{a}	80.33±1.33^{bc}	35.22±3.51^{b}	38.66±2.51^{d}	50.77±1.89^{a}	87.33±2.6^{a}	38.22±1.37^{b}
Treatment 1	6.05±0.49	Stage1	78.49±0.27^{a}	109.71±0.24^{a}	101±0.91^{a}	37.54±0.72^{a}	46.5±0.7^{a}	48.57±0.45^{a}	28.43±0.18^{a}
		Stage2	143±5.50^{bc}	86.55±2.36^{abc}	42.88±2.2^{abcd}	28.11±1.68^{c}	58.66±1.61^{bc}	86.99±1.69^{bcd}	24.77±2.5^{d}
		Stage3	193.22±1.54^{b}	79.88±2.11^{b}	15.88±1.07^{a}	16.88±2.83^{c}	78.88±1.76^{c}	100±0^{b}	7.44±2.3^{b}
		Stage4	176±1.14^{b}	80.10±2.87^{c}	20.66±1.65^{a}	19.77±2.02^{abc}	68.22±1.01^{c}	95.88±1.50^{b}	12.55±1.89^{a}
Treatment 2	6.75±1.62	Stage1	78.52±0.33^{a}	109.08±0.24^{a}	101±0.84^{a}	37.60±0.92^{a}	46.48±0.04^{a}	48.28±0.55^{a}	28.79±0.32^{a}
		Stage2	154.66±6.19^{bcd}	111.55±2.19^{d}	27.21±2.71^{bcd}	25.99±1.65^{bcd}	48.22±2.66^{ab}	90.22±2.67^{cd}	26.33±2.60^{d}
		Stage3	211.88±1.34^{c}	120±1.44^{c}	14.77±1.24^{a}	10.77±1.26^{ab}	62.22±2.78^{b}	99.66±0^{b}	6.55±1.5^{a}
		Stage4	177±2.89^{b}	112±2.69^{d}	19.1±1.66^{a}	20.66±1.85^{bc}	60.88±1.57^{a}	97.44±1.27^{b}	12.99±2.51^{a}
Treatment 3	5.6±1.54	Stage1	78.22±0.12^{a}	109.31±0.54^{a}	101.77±0.17^{a}	36.14±0.44^{a}	46.17±0.82^{a}	48.17±0.39^{a}	28.21±0.49^{a}
		Stage2	174.55±1.85^{d}	101.11±2.54^{bcd}	49.66±2.65^{d}	21.22±1.19^{ab}	65.66±1.20^{cd}	85.99±1.33^{bc}	13.44±1.96^{bc}
		Stage3	190±2.98^{b}	62.77±1.38^{a}	15.44±1.84^{a}	17.77±1.46^{c}	83.44±1.19^{d}	99.88±1.19^{b}	7.99±1.15^{ab}
		Stage4	176±2.79^{b}	68.66±1.6^{ab}	29.99±2.3^{a}	20.55±1.07^{bc}	82.21±1.5^{d}	99.33±1.33^{b}	11.44±1.57^{a}

There was no significant difference between the control other treatments fed with pigments in total carotenoid of blood (p>0.05) (Table 4).

Variation in the intensity of primary colors (RGB) and secondary colors (CMYK) in the first stage of imaging (before beginning of feeding with pigmented food)

In this stage of imaging, no pigmented feed was given to the fish. As a result, no significant difference was observed between treatments in the intensity of primary and secondary colors (p>0.05) (Table 4).

Variation in the intensity of primary colors (RGB) and secondary colors (CMYK) in the second and third stages of imaging (10 and 20 days after the beginning of feeding with pigmented food)

Since the fish were fed with pigmented food in the second and third stages of imaging, a significant difference was found between the control and treatments fed with pigmented food in the intensity of primary and secondary colors (p<0.05) (Table 4). In terms of color intensity, because of feeding the fish with pigmented food, the intensity of red (R) showed a significant difference from other primary colors (G and B) and primary colors (K, M, C). In other words, with the increase of red color in the skin, the intensity of blue, green, turquoise, and black somewhat reduces. This suggests that the use of pigmented food increased the intensity of red in caudal area of fish. In all stages of imaging, R and Y coefficients led to the highest increase in color intensity in this area.

Variation in the intensity of primary colors (RGB) and secondary colors (CMYK) in the fourth stage of imaging (27 days after the beginning of feeding with pigmented food)

There was a significant difference between the control and treatments fed with pigmented food (p<0.05) (Table 4). Given that feeding was stopped in this stage of imaging, the red color in the caudal area caused no significant difference between treatments, except for the treatment fed with lucantin yellow. This indicates that pigments are stored the tissue, muscle, and liver and their effect does not decline after the end of feeding with pigmented food.

Discussion

The survival rate in all treatments, except the control, was 100%. There was a significant difference between the control group and other treatments in this regard (p<0.05). The highest weight gain was related to treatment 1 and the lowest value was observed in the control group. There was a significant difference between the control and other treatments in terms of weight gain, while such a difference was not found between treatments 1, 2, and 3 (p>0.05). The lowest and the highest FCR belonged to the treatment fed with astaxanthin-containing diet and the control, respectively. In this regard, a significant difference was found between the control and the treatment fed with the diet containing lucantin yellow. The highest and the lowest SGR were related to treatment 1 (2.38±0.21) and the control (1.25±0.13). There was a significant difference between the control and other treatments (p<0.05). The highest final weight was found in the treatment fed with

the diet containing lucantin red and the lowest value was related to the control, which suggests a significant difference between the control and other treatments. In terms of condition factor, there was a significant difference between the control and other treatments. In addition, a significant difference was observed between the control and other treatments in terms of increased length. Verakunpiriya *et al*. (1997) stated that pigment sources can improve growth factors. Prescription of 5% aloe vera causes a significant increase in weight, SGR, and condition factor and reduction of FCR in oscar (Saeedi *et al*., 2014). Beiranvand *et al*. (2015) showed that addition of spirulina powder to the diet of the Zebra Danio (*Danio rerio* Hamilton,1822) increased body weight and SGR. Amar *et al*. (2001) reported that carotenoids are able to improve the growth, the immune system, and survival rate of fish. Moreover, they can cause protection against harmful effects of fat oxidation (Liebler, 1993; Waagbo *et al*., 2003). There are different views about the effect of carotenoid pigments on growth factors of different fish species. While some results indicate that these pigments cannot improve growth factors in rainbow trout, Atlantic salmon, cod, Arctic char, and red porgy (Storebakken and Goswami, 1996; Barbosa *et al*., 1999; Mansour *et al*., 2006; Tejera *et al*., 2007; Sawanboonchun *et al*., 2008; Sheikhzadeh *et al*., 2012), the results of some other researchers suggest the positive effect of these pigments on growth improvement. This difference may be attributed to fish species, development stages, and type of carotenoid. Li *et al*. (2014) reported that diets containing astaxanthin and *Haematococcus pluvialis* efficiently improved growth factors in large yellow croaker. Specifically, weight gain was significantly higher in fish fed with *H. pluvialis* than those fed with the diet containing astaxanthin. The accumulation of fat influenced by the total body fat and the condition factor can justify this finding. Christiansen and Torrissen (1995) stated that small amounts of astaxanthin (1.5 mg kg^{-1}) can lead to the highest growth and survival in fries of Atlantic salmon (*Salmo salar* Linnaeus,1758). Christiansen *et al*. (1995) showed that feeding of Atlantic salmon and Salmon salar pars with semi-purified diets contain 60 mg kg^{-1} astaxanthin for 315 days significantly improved their growth compared to the control group. In the present study, the subjects fed with diets containing pigments showed the highest BWI, SGR, WG, and increased length and the lowest FCR compared to the control. There was a significant difference between all treatments in terms of growth factors. It seems that the addition of these pigments to the diet of tiger oscars has been effective in the improvement of their growth.

The results of hematological parameters measurement at the end of the trial presented no significant difference between treatments in any of the parameters, although some of them underwent a reduction or increase during the trial. Regarding hematological parameters and blood cell indices, the results of this study were both consistent and inconsistent with the findings of previous studies. Rezaei *et al*. (2013) studied the effect of adding the extract of *Zhumeria majdae* (150,300,600 mg kg^{-1})

to the diet on growth factors and hematological and immunological parameters in catfish (*Pangasianodon hypophthalmus*) and concluded that the extract of this plant has no significant impact on hematological parameters. Ansarifard *et al*. (2018) studied the Effects of *Arthrospira platensis* on growth, skin color and digestive enzymes of Koi, *Cyprinus carpio* Their results indicated inclusion of 5-10% *A. platensis* in diets has a significant positive effect on growth rate, pigmentation, and improvement of digestive and liver enzymes activities in koi fish. Tukmechi *et al*. (2011) stated that some factors such as environmental factors, especially due to the coldness of the fish, seasons, salinity, photoperiod, temperature, density, physiological parameters, species, reproductive cycle, puberty status, age, gender, nutritional conditions, sampling time and method, and the accuracy and sensitivity of measurement methods can affect growth factors and survival and make a difference in the interpretation of researchers.

Some studies show that feeding of fish and poultry with Spirulina can increase body resistance to diseases, improve survival rate and the growth, and enhance the function of the immune system (Hayashi *et al*., 1998; Belay, 2002). Li *et al*. (2014) reported that increasing the levels of astaxanthin and *H. pluvialis* in the diet of large yellow croaker (*Pseudosciaena crocea*) increases the activity of lysozyme. The level of lysozyme is one of the important indicators of innate immunity in fish (Saurabh and Sahoo, 2008). Previous studies on mammals show that lysozyme can be activated by diets containing astaxanthin (Chew *et al*., 2011; Park *et al*., 2011).

The main objective of stimulants is to improve non-specific immunity. Due to the higher efficiency of the nonspecific immune system than the specific immunity (compared to warm-blooded animals), the effect of stimulants is more prominent in the immune system of fishes. Lysozyme is released into the blood by neutrophils and macrophages (Sakai, 1999). Chien *et al*. (2003) showed that astaxanthin can stimulate the immune system of juveniles of the giant tiger prawn (*Penaeus monodon*). Generally, increased lysozyme activity in the serum and mucus of fish can be indicative of the immune system stimulation and improvement of the immune response. By enhancing the complement system and lysozyme, carotenoid pigments increase the total number of leukocytes and phagocytes and thereby cause the stimulation of the immune system, increased immunity, and resistance to pathogens. Wang *et al*. (2015) reported that dietary astaxanthin in Pacific white shrimp (*Litopenaeus vannamei*) significantly affects the hemolymph immunological index, including total haemocyte counts, phagocytic activity of haemocyte, serum anti-superoxide radical activity, serum phenoloxidase activity, serum anti-bacterial activity and serum bacteriolytic activity. In the present study, subjects of Treatment 3 presented the highest lysozyme activity compared to the control and treatments 1 and 2. Li *et al*. (2014) stated that the effect of astaxanthin and *H. pluvialis* on total immunoglobin of long yellow croaker is negligible. Previous studies on warm-blooded animals have

indicated that beta-carotene and canthaxanthin can improve the proliferation of T and B cells (Bendich and Shapiro, 1986). However, there is no evidence of the effect of carotenoids on the improvement of immunoglobin products.

Despite the increase of total carotenoid in treatments 1, 2, and 3 compared to the control, it was not statistically significant. Variation of the intensity of the primary and secondary colors on the skin was noticeable in all treatments during the four stages of imaging, and statistical analyses show a significant difference between the control and other treatments in this regard. Mashalchi *et al*. (2010) showed that diets containing astaxanthin and danualila changed the skin color into red in white oscars. Hassaninia *et al*. (2016) stated that the addition of lucantin pink to the diet of oscar can change their skin color and significantly increase the intensity of red in this fish. Choubert and Heinrich (1993) and Sommer *et al*. (1991) reported that high levels of sterilized astaxanthin and healthy spores with low absorbency in non-homogenized healthy algae produce lower total carotenoid in the skin and muscles of rainbow trout compared to astaxanthin and homogenized algae. Bjerkeng (2000) stated that coloration of fish, in addition to biological factors, is influenced by pigments content of the diet, the concentration of pigments, duration of feeding, and diet composition. Pham *et al*. (2014) studied the effects of carotenoid sources of diet on the olive flounder (*Paralichthys olivaceus*) and concluded that the red color of this fish increases by feeding on diets containing carotenoids, but the brightness, clarity, and yellow color of the skin are not affected by carotenoid sources. Safari and Mehraban Sang Atash (2015) showed that the extract of achiote (*Bixa orellana*) increases the level of carotenoids in the blood of rainbow trout. Moreover, they have been stated that there is a positive correlation between the carotenoid content of blood and final weight and SGR and a negative relationship between the carotenoid content of blood and FCR. This is consistent with the findings of the present study. Shapoori *et al*. (2012) believe that the difference in the color intensity caused by natural and artificial pigments are due to the quality, concentration, and the absorption period of these materials. Carotenoids are a major ingredient of diets but their distribution and intensity are probably influenced by genetic factors. Generally, chromatophores in fishes include melanophores, xanthophores, erythrophores, and iridophores that are responsible for the revealing of colors on the skin of fish. However, the pigment patterns in fish are affected by the different position of chromatophores. In addition, the pattern of pigment cells may be caused by patterning mechanisms away from local environmental guidance or the interaction between neighboring chromatophores (Kelsh, 2004). Sornsupharp *et al*. (2015) showed that diets containing carotenoids can increase the total carotenoid content of color in the skin and muscles of flowerhorn cichlids. This is due to the concentration of carotenoids in the diet, which plays a major role in the skin coloring in this species. It seems that in the present study, treatment 1 has a better performance than other treatments.

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References

Abdelghany, A.E. and Ahmad, M.H., 2002. Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. *Aquaculture Research,* 33, 415-423.

Amar, E.C., Kiron, V., Satoh, S. and Watanabe, T., 2001. Influence of various dietary synthetic carotenoids on bio-defence mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research, 32*, 162–173.

Ansarifard, F., Rajabi Islami, H., Shamsaie Mehrjan, M., & Soltani, M., (2018). Effects of Arthrospir platensis on growth, skin color and digestive enzymes of Koi, Cyprinus carpio. *Iranian Journal of Fisheries Sciences, 17*(2), 381-393.

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

Beiranvand, M., Ghaeni, M. and Velayatzadeh, M., 2015. Impact of *Spirulina* sp. on growth and food intake in *Danio rerio* Hamilton, 1822. *Nova Biologica Reperta*, 2(**3**), 207-215.

Belay, A., 2002. The potential application of *spirulina* (*Arthrospira*) as a nutritional and therapeutic supplement in health management. *American Nutraceutical Association*, 5(**2**), 27-48.

Bendich, A. and Shapiro, S.S., 1986. Effect of β-carotene and canthaxanthin on the immune responses of the ra. *J. Nutrition*, 116, 2254-2262.

Bjerkeng, B., 2000. Carotenoid pigmentation of salmonid fishes recent progress (L. E. In: Cruz-Sua´rez, Rique-Marie, D., Tapia-Salazar, M., Overa-Novoa, M.A., y Civera-Cerecedo, R. (Eds.),Avances en Nutricio´n Acuı´cola V. Memorias del V Simposium Internacional de Nutricio´n Acuı´cola 19–22 Noviembre, 2000. Me´rida, Yucata´ Internacional de Nutricio´n Acuı´cola 19–22 Noviembre, 2000. and Y. n. Me´rida Eds.

Chew, B.P., 1995. Antioxidant vitamins affect food animal immunity and health. *Journal of Nutrition*, 125, 1804– 1808.

Chew, B.P., Mathison, B.D., Hayek, M.G., Massimino, S., Reinhart, G.A. and Park, J.S., 2011. Dietary astaxanthin enhancesimmune response in dogs. *Veterinary Immunology and Immunopathology*, 140, 199-206.

Chien, Y.H., Pan, C.H. and Hunter, B., 2003. The resistance to physical stresses by *Penaeus monodon* juveniles fed diets supplemented with astaxanthin. *Aquaculture*, 216(**1-4**), 177-191.

Chien, Y.H. and Shiau, W.C., 2005. The effects of dietary supplementation of algae and synthetic astaxanthin on body astaxanthin, survival, growth, and low dissolved oxygen stress resistance of kuruma prawn, *marsupenaeus japonicus* Bate. *Journal of Experimental Marine Biology and Ecology*, 318, 201–211.

Choubert, G. and Heinrich, O., 1993. Carotenoid pigments of the green alga *Haematococcus pluĺialis*: assay on rainbow trout, *Oncorhynchus mykiss*, pigmentation in comparison with synthetic astaxanthin and

canthaxanthin. *Aquaculture*, 112, 217-226.

Christiansen, R., Lie, O., Torrissen, O.J. and Waagbo, R., 1995. Antioxidant status and immunity in Atlantic salmon (*Salmo salar* L.), fed semi-purified diets with and without astaxanthin supplementation. *Journal of Fish Diseases*, 18, 317-328.

Christiansen, R. and Torrissen, O.J., 1995. Growth and survival of Atlantic salmon (*Salmo salar* L.), fed different dietary levels of astaxanthin: first-feeding fry. *Aquaculture Nutrition*, 1, 189-198.

Christiansen, R. and Torrissen, O.J., 1996. Growth and survival of Atlantic salmon, *Salmo salar* L. fed different dietary levels of astaxanthin. Juveniles. *Aquaculture Nutrition*, 2, 55-62.

Clerton, P.T.D., Verlhac, V., Gabraudan, J. and Deschaux, P., 2001. Dietary vitamin E and rainbow trout (*Oncorhynchus mykiss*) phagocyte functions: effect on gut and on head kidney leucocytes. *Fish and Shellfish Immunology*, 11, 1-13.

Erdogan, F., Erdogan, M. and Gumus, E., 2012. Effect of dietary protein and lipid levels on growth performances of tow African Ciclids (*Pseudotropheus socolofi*) and (*Haplochromis ahli*)., *Turrkish Journal of Fisheries and Aquatic Sciences*, 12, 453-458.

Ghiasvand, Z. and Shapoori, M., 2006. The effect of synthetic and natural pigments on the colour of the Albino Oscar (*Astronotus ocellatus* sp., Agassiz, 1831). *Marine Biology*, 1(**3**), 78-85.

Gouveia, L., Rema, P., Pereira, O. and Empis, J., 2003. Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. *Aquaculture Nutrition*, 9(**2**), 123-129.

Guerin, M., Huntley, M.E. and Olaizola, M., 2003. *Haematococcus* astaxanthin: applications for human health and nutrition. *Trends in Biotechnology*, 21, 210–216.

Guroy, B., Sahin, I., Mantoglu, S. and Kayali, S., 2012. *Spirulina* as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid(*Pseudotropheus acei*. *Aquaculture International*, 20, 869–878.

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

Hassaninia, A., Vahabzadeh Roudsari, H. and Sadeghpour, A., 2016. Effect of Pink Leucantin on White Oscar (*Astronotus ocellatus*) Skin *Aquaculture Development*, 10(**1**), 23-31.

Hayashi, O., Hirahashi, T., Katoh, T., Miyajima, H., Hirano, T. and Okuwaki, Y., 1998. Class specific influence of dietary *Spirulina platensis* on antibody production in mice. *Journal of Nutritional Science and Vitaminology*, 44, 841-851.

Hung, S.S.O., Lutes, P.B. and Storebakken, T., 1989. Growth and feed efficiency of whitesturgeon (*Acipenser transmontanus*) sub yearling at different feeding rates. *Aquaculture*, 80, 147-153.

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

Keith, P.O.Y., Bail, L. and Planquette, P., 2000. Atlas des poissons d'eau douce de Guyane (tome 2, fascicule I). Publications scientifiques du Muséum national d'Histoire naturelle, Paris, France. 286 P.

Kelsh, R.N., 2004. Genetics and evolution of pigment patterns in fish. *Pigment Cell Research*, 17, 326–336.

Klontz, G.W., 1994. Fish hematology. In: Techniques in Fish Immunology. In J. S. Stolen, Fletcher, T.C.,Rowley, A.F., Kelikoff, T.C., Kaatari, S.L., Smith,S.A., (Ed.), (Vol. 3). SOS Publications Fair Haven,New Jersey, USA. pp.121-132.

Li, M., Wu, W., Zhou, P., Xie, F., Zhou, Q. and Mai, K., 2014. Comparison effect of dietary astaxanthin and *Haematococcus pluvialis* on growth performance, antioxidant status and immune response of large yellow croaker *Pseudosciaena crocea*. *Aquaculture*, 434, 227–232.

Liebler, D.C., 1993. Antioxidant reactions of carotenoids. *Annals of the New York Academy of Sciences*, 691, 20–31.

Loiselle, P.V., 1995. The cichlids of Jurassic Park. Cichlid News Magazine. pp.18-23.

Lorenz, R.T. and Cysewski, T., 2000. Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. *Tibtechnology*, 18, 160–166.

Mansour, N., McNiven, M.A. and Richardson, G.F., 2006. The effect of dietary supplementation with blueberry, a-tocopherol or astaxanthin on oxidative stability of Arctic char (*Salvelinus alpinus*) semen. *Theriogenology*, 66, 373-382.

Mashalchi, M., Alishahi, M., Javaheri Baboli, M. and Hejazi, M.A., 2010. Comparison between the effects of astaxanthin and *Dunaliella salina* on coloration and immune response of *Astronorus ocellatus*. *Marine Biology*, 2(**6**), 75-83.

Mazurkiewicz, J., Przyby, A. and Golski, J., 2008. Usability of some plant protein ingredients in the diets of Siberiansturgeon *Acipenser baerii* Brandt. *Archives of Polish Fisheries*, 17, 45-52.

Meyers, S.P., 1977. Using crustacean meals and carotenoid fortified diets. *Feedstuffs*, 38, 26-27.

Ojolick, E.J., Cusack, R., Benfey, T.J. and Kerr, S.R., 1995. Survival and growth of all-female diploid and triploid rainbow trout (*Oncorhynchus mykiss*) reared at chronic high temperatures. *Aquaculture*, 131, 177-187.

Paripatananont, T., Tangtrongpairoj, J., Sailasuta, A. and Chansue, N., 1999. Effect of astaxanthin on the colouringation of goldfish (*Carassius auratus*). *Journal of the World Aquaculture Society, 63*, 454–460.

Park, J.S., Mathison, B.D., Hayek, M.G., Massimino, S., Reinhart, G.A. and Chew, B.P., 2011. Astaxanthin stimulates cell-mediated and humoral immune responses in cats. *Veterinary*

Immunology and Immunopathology, 144, 455–461.

Pham, M.A., Byun, H.G., Kim, K.D. and Lee, S.M., 2014. Effects of dietary carotenoid source and level on growth, skin pigmentation, antioxidant activity and chemical composition of juvenile olive flounder *Paralichthys olivaceus*. *Aquaculture*, 31, 65–72.

Rezaei, M.H., Sourinejad, I., Soltanian, S. and Yousefzadi, M., 2013. The effects of dietary *Zhumeria majdae* extract on growth indices, hematology and immunology of catfish *Pangasianodon hypophthalmus*. *Aquatic Ecology*, 3(**1**), 8-19.

Ricker, W.E., 1979. Growth rates and models. *Fish physiology*, 8, 678-743.

Ronyai, A., peteri, A. and Radics, F., 1990. Cross breeding of starlet and lena river sturgeon. *Aquaculture, Hungrica szarwas*, 6, 13-18.

S**aeedi M.A., Salimi B., Mahmoudi N. and Jalili, S., 2014.** Effect of Aloe Vera extracts on the growth, survival factors of Oscar fish (*Astronatus Ocellatus*). *Breeding and Aquaculture Sciences Quarterly*, 1(**1**), 55-66.

Safari, O. and Mehraban Sang Atash, M., 2015. The effects of dietary supplement of annatto (*Bixa orellana*) seedmeal on blood carotenoid content and fillet color stability in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 437, 275–281.

Sakai, M., 1999. Current research status of fish immunistimulants. *Aquaculture*, 172, 63-92.

Saurabh, S. and Sahoo, P.K., 2008. Lysozyme: an important defence molecule of fish innate immune system. *Aquaculture Research*, 39, 223–239.

Sawanboonchun, J., Roy, W.J., Robertson, D.A. and Bell, J.G., 2008. The impact of dietary supplementation with astaxanthin on egg quality in Atlantic cod broodstock (*Gadus morhua*, L.). *Aquaculture*, 283, 97-101.

Shapoori, M., Ghiasvand, Z. and Jamili, S., (2012). The study of synthetic and natural pigments on the colour of the Albino Oscar. *International Journal of Marine Science and Engineering*, *2*(3), 203-206 .

Sheikhzadeh, N., Tayefi-Nasrabadi, H., Oushani, A.K. and Enferadi, M.H., 2012. Effects of *Haematococcus pluvialis* supplementation on antioxidant system and metabolism in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, *38*, 413-419.

Siwicki, A.K. and Anderson, D.P., 1993. Non-Specific defence mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages,lysozyme activity in serum and organs and total immunoglobulin level in erum. Disease diagnosis and prevention methods. Olsztyn, Poland: FAO-project GCP/INT/JPA, IFI. pp. 105-112.

Sommer, T.R., Potts, W.T. and Morrissy, N.M., 1991. Utilization of microalgal astaxanthin by rainbow trout. *Aquaculture*, 94, 79–88.

Sornsupharp, B., Lomthaisong, K. and Dahms, H.U., 2015. Effects of dried fairy shrimp *Streptocephalus sirindhornae* meal on pigmentation and carotenoid deposition in flowerhorn cichlid; *Amphilophus citrinellus* (G€unther, 1864) 3 Cichlasoma trimaculatum (G€unther, 1867).

Aquaculture Research, 46(**46**), 173-184.

Staeck, W. and Linke, H., 1985 American Cichlids II: Large Cichlids, A Handbook for Their Identification, Care, and Breeding. Tetra-Verlag, Melle, Germany.

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

Storebakken, T. and No, H.K., 1992. Pigmentation of rainbow trout. *Aquaculture,* 100, 209–229.

Tacon, A.G.J., 1990. Standard methods for the nutrition and feeding of farmed fish and shrimp. Argent Laboratories Press. 454 P.

Tejera, N., Cejas, J.R., Rodríguez, C., Bjerkeng, B., Jerez, S., Bolaños, A. and Lorenzo, A., 2007. Pigmentation, carotenoids, lipid peroxides and lipid composition of skin of red porgy (*Pagrus pagrus*) fed diets supplemented with different astaxanthin sources. *Aquaculture,* 270, 218–230.

Thrall, M.A., 2004. Veterinary Hematology and Clinical Chemistry. Lippincott Williams and Wilkins USA 241, 277-288.

Torrissen, O.J., Hardy, R.W. and Shearer, K.D., 1989. Pigmentation of salmonids- carotenoid deposition and metabolism. *Reviews in Aquatic Science,* 1, 209–225.

Tukmechi, A., Rahmati, H.R., Manaffar, R. and Sheikhzadeh, N., 2011. Dietary administration of beta-mercapto-ethanol treated *Saccharomyces cervisiae* enhanced the growth, innate immune response and disease. *Fish and Shellfish Immunology,* 30, 923-928.

Verakunpiriya, V., Watanabe, K., Mushiake, K., Kawano, K., Kobayashi, T., Hasegawa, I.,Kiron, V., Satoh, S. and Watanabe, T., 1997. Effect of krill meal supplementation in softdry pellets on spawning and quality of egg of yellowtail. *Fisheries Science,* 63, 433–439.

Waagbo, R., Hamre, K., Bjerkas, E., Berge, R., Wathne, E., Lio, O. and Torstensen, B., 2003. Cataract formation in Atlantic salmon, *Salmo salar* L., smolt relative to dietary pro and antioxidants and lipid level. *Journal Of Fish Diseases,* 26, 213–229.

Wang, H., Dai, A., Liu, F. and Guan, Y., 2015. Effects of dietary astaxanthin on the immune response, resistance to white spot syndrome virus and transcription of antioxidant enzyme genes in Pacific white shrimp *Litopenaeus vannamei*. *Iranian Journal of Fisheries Sciences,* 14(**3**), 699-718.

Weber, S., 1988. Determination of stabilized, added astaxanthin in fish feeds and premixes with HPLC. In H. E. Keller (Ed.), Analytical Methods for Vitamins and Carotenoids in Feeds Roche Publication No. 2264 Basel, Switzerland. pp.59-61.

Yam, K.A. and Papadakis, S.E., 2004. A Simple digital imaging method for measuring andanalyzing color of food surfaces. *Journal of Food Engineering,* 61, 137-142.