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Efficacy of Astaxanthin Fed to Rainbow Trout (*Oncorhynchus mykiss*): Effect on Growth, Pigmentation and Blood Indices

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Abstract

In this study, the effect of astaxanthin on growth, pigmentation and blood factors were studied in rainbow trout weighted average (93±0.64 g) for 8 weeks. Two treatments and three replicates were employed as follows: Treatment A: fed with a commercial diet (control), treatment B: 41 mg/kg astaxanthin. All treatments were fed in hours 8, 12, 16, respectively. It should be considered that the feeding rate of biomass weight was examined after each took biometrics. Finally, blood samples were taken from the fish. The results revealed a significant difference between treatments in terms of weight and length ($p<0.05$) and no significant difference ($p>0.05$) in food intake and growth performance parameters, including FCR, SGR, GR, BWI%, and survival rate between treatments. In terms of pigmentation between experimental treatments, astaxanthin was created more change color than the control group. Results of blood factors showed that between WBC, RBC, Hb, HCT, Lymphocytes, Neutrophils, Monocytes and IgM were significant differences between treatments ($p<0.05$). So we can conclude that 41mg/kg astaxanthin dietary, due to economy, would be proper diet in the feeding of rainbow trout propagation.

Keywords: Rainbow Trout ; Astaxanthin; Pigment; Coloration; Growth; Blood Factor

Introduction

The production of rainbow trout depends on the responses of the consumer market. Consumers require fish with better flesh quality and appearance. Carotenoids, which belong to the natural constituents of salmonid fish feed, may help meet the latter requirement [1]. It is important to use carotenoids in fish feeds. These give yellow, red and pink to the skin, flesh and eggs of fish [2]. Pigments play an important role in the diet of animals and animal feed production industry. The carotenoids in the normal and abnormal environment fish are available and have positive effects on fish. Muscle coloration in the natural environment and fish that are fed with natural food, due to its accumulation in muscle is orange [3]. Carotenoids are the main pigments of many aquatic animals. In salmonids, astaxanthin is responsible for the typical red colour of the flesh [4-6].

As is the case with other, salmonids cannot endogenously synthesize astaxanthin; therefore, it must be supplemented in the fish's ration. Research also indicates additional benefits from dietary carotenoids beyond the resulting coloration [7]. Salmon meat staining depends on factors such as ration diet, growth rate, management conditions,

amount and duration of use of pigments, etc. Also, it could be useful to create the color of carotenoids in fish flesh, increase growth, boost the immune system by increasing antibody production, reduce stress, increase survival and fertilization, etc [8-11]. Astaxanthin, which is the most effective on pigmentation, exists densely in water organisms such as Gammarus, copepods and starfish [12-14]. One of the most important roles of the pigments is biological functions related to growth, reproduction and tissue health in salmonids and shrimp, possibly due to the compound's strong antioxidant properties [7]. It is not harmful to human and could be anticancer and antioxidant [15]. It provides salmon flesh with its characteristic rich pink-red color and accounts for more than 90% of the total carotenoid content found in the flesh of wild salmonids (salmon and trout). In nature, salmonids absorb astaxanthin from the consume of crustaceans. The absorbed carotenoid is then transported in the blood to the muscles and skin where it is deposited [7].

Several studies on astaxanthin were published by Christiansen who examined the issue from the viewpoint of the effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon [16], growth and survival of Atlantic salmon juveniles [17], and first-feeding fry [18], antioxidant status and immunity in Atlantic salmon [19] and effects of astaxanthin and vitamin A on growth and survival during the first feeding of Atlantic salmon [20]. Besides pigmentation, carotenoids are involved in certain physiological functions, as pointed out by Nakano, et al. [21] who studied the biochemical characteristics of the liver and blood in rainbow trout fed a diet supplemented by red yeast (*Phaffia rhodozyma*) containing astaxanthin as its principal carotenoid pigment or synthetic astaxanthin. It follows from the literature survey that there is little knowledge of the effect of astaxanthin on blood factors and no investigations on growth indicators of the rainbow trout. The purpose of this study was an investigation on the effect of this pigment on the growth and coloration and blood indices of the rainbow trout.

Materials and Methods

Feeding Experiment

The experiment was conducted in pools of Mr.Sarshar farm that was located in the 3000 Road of Tonekabone region of Mazandaran province. Pools dimension in this experiment were (4m×3m×1m) and period was 60 days in the autumn season. The fish were fed 3 times daily. On days 0, 20, 40 and 60, all fish remaining in each cage were weighed. Throughout the experiment, the temperature, dissolved oxygen level and pH of the rearing water were 8.64±0.965 °C, 9.12±0.379 mg1-1, 7.1±0.293, respectively. Water supply was that originated from 3000 rivers.

The two different treatments used in the experiment were as follows:
Treatment A: fed with commercial normal diet (control group)
Treatment B: Diet supplemented with Astaxanthin 41 mg/kg

The commercial basal diet was supplied by Faradane Company (Table 1) and synthetic astaxanthin was produced by Behparvar Company. The experimental diet was made by adding the requisite amounts of the different pigment sources to a commercial diet. The food processor was done every tertian. For preparation ration, initially small amounts of oil with pigments are well mixed and spray to pellets. Then the daily food of each pool was weight with a digital scale and packed.

Chemical Composition	Percentage (%)
Crude protein	48
Crude fat	12
Crude ash	12
Dry matter	10

Table 1 : Nutritional composition of control diet (basal diet).

300 pieces of Rainbow trout with an average weight of (93±0.64 g) in a completely randomized design were compared for 8 weeks in the same breeding conditions. Fish were fed three times a day (8, 12, 16) and feed required per day according to the weight of biomass in different periods (usually after each bioassay) was calculated. Once every 20 days, randomly, 10 number of fish selected for biometry from each replicate, and then weighted with a digital scale with precision (0.01 g) and the length was measured by millimetre ruler. To reduce stress in fish during the biometry, Feeding was discontinued 12 hours before and after the biometry. Blood tests were done at the end of the research period that included RBC, WBC, HCT, Hb, IgM, Lym and Mono. The weight and length of fish in each pool, Statistical calculations values of FCR, percentage of BWI, SGR, GR, CF and survival rate were calculated.

Colour Measurements

During the experimental period, trout were sampled four times. The first sampling was done at the beginning of the experiment, and the subsequent samplings were carried out at 20, 40 and 60 days. In all of the samplings, six trout were sacrificed. Each trial included three replicates. The total weight and total length of each trout were measured, and three fillets were obtained per trout to match with

SalmoFanTM.

Preparation of the Blood Samples

Blood samples were taken from 10 fish that caught randomize. The sampling was performed 20 hours after the last feeding during the forenoon, the samples were taken by puncturing the caudal veins immediately after catching and a stunning amount of 2cc. Blood taken from the fish into micro test tubes containing a drop of heparin (anticoagulant) was poured and shake gently until completely mixed blood and heparin. To prepare serum hemolysis mentioned tubes were centrifuged at 3500 RPM for 15 min. Then, serum was removed by the sampler tool of hemolysis tube and then was transferred into micro test tubes with a volume of 2cc [22].

Statistical Analysis

The data analyzed by SPSS 13 and Excel 2003 soft wares. For statistical analysis, The homogeneity of the control data, Averages of blood factors were examined (Shapiro Wilk test) Through Kruskal-Wallis and Man-Witney nonparametric test and the average weight and length measurements were calculated by ANOVA, The confidence level was %95 and Duncan Test was employed.

Results

Water physicochemical parameters were measured including dissolved oxygen, temperature, pH its effect on feeding, breeding all the time these factors were carefully controlled (Table 2). Results of water quality parameters were revealed no significant difference during the breeding period (p> 0.05).

Factor	Average	Min	Max
Oxygen	9.12±0.379	8.14	9.91
Temperature	8.64±0.965	7.11	9.9
pH	7.1±0.293	6.6	7.8

Table 2 : Water physicochemical parameters.

Growth data of the fish on days 0, 20, 40 and 60 are summarized in Table 3. The first average weight was 93±0.64 g and mean total length was 18.1 ± 0.25 cm. Fish biometry performed based on weight during the breeding period and revealed a significant difference between treatments ($p<0.05$) But there was no significant difference in terms of length ($p>0.05$) (Table 3 and 4). The lowest weight found in the control group and the greatest weight was received in astaxanthin group but no significant difference was found in terms of performance parameters including growth and food consumption FCR ‹SGR‹ GR‹ %BWI, CF and survival rate between different treatments was observed ($P>0.05$) (Table 5). The most SGR, BWI, GR, CF and survival rate was found related to astaxanthin and most FCR related to control group (Table 5).

Diets	0 day	20 day	40 day	60 day
Control	93±0.64 a	113.95±1.02 a	132.46±2.18 a	157.62±2.48 a
Astaxanthin	93±0.64 a	116.65±1.68ab	138.67±1.42 b	168.29±2.99 b

Table 3: Mean weight of the fish fed various diets.
Each value is a mean ± SE. (n = 3 replicates). Each replicate consists of measurements from 10 fish. The means with different letters in each column denote a significant difference ($P < 0.05$).

Diets	0 day	20 day	40 day	60 day
Control	18.1±0.25 a	18.83±0.17 a	22.54±0.45 a	24.06±0.13 a
Astaxanthin	18.1±0.25 a	19.33±0.44 ab	22.66±0.8 ab	24.5±0.2 ab

Table 4: Mean length of the fish fed various diets.
Each value is a mean ± SE. (n = 3 replicates). Each replicate consists of measurements from 10 fish. The means with different letters in each column denote a significant difference ($P < 0.05$)

Diets	FCR	SGR	BWI%	GR	CF	survival
Control	4.55±0.28	35.28±0.22	19.02±1.29	1.26±0.06	1.13±0.03	91.33±2.31
Astaxanthin	4.54±0.62	36.48±0.62	21.39±3.02	1.48±0.19	1.15±0.04	95.33±2.31

Table 5: Mean growth parameters of the fish fed various diets.
Each value is a mean ± SE. (n = 3 replicates). Each replicate consists of measurements from 10 fish. The means with different letters in each column no significant difference ($P > 0.05$).

Treatments Parameters	Control	Astaxanthin
RBC	1006111±169314	1347000±253519
WBC	4133.3±715.9	9233.3±1019.8
Hb gr/dl	6.51±1.27	8.68±1.65
HCT%	37.11±7.32	43.89±7.39
Lym	62.44±12.3	96.11±2.85
Mono	2.22±2.59	2.67±2.29
Neu	2.67±2.45	3.56±5.64
IgM	9.63±1.35	29.67±24.54

Table 6: Mean blood factors of the fish fed various diets. Each value is a mean ± SE (n = 3 replicates). Each replicate consists of measurements from 10 fish.
*(Based on Nonparametric Kruskal-Wallis and manwitny tests, the separate character is not used (a,b))

Results revealed that coloration of the fish was fed with astaxanthin showed significant change color than the control group was created. According to the SalmoFanTM, degree of color was 28.

In this experiment, blood factors including RBC, WBC, Hematocrit, Hemoglobin, Lymphocytes and IgM revealed significant differences between treatments ($p<0.05$) but Monocytes and Neutrophils, were showed no significant difference between treatments ($p>0.05$) (Table 6). Most RBC, WBC, Hb, HCT, Neu, Mono, Lym and IgM were associated with astaxanthin.

Discussion

So the carotenoids should be used as a food supplement. The carotenoid pigments in salmon biology are a sign of normal matter. In the present study, growth of rainbow trout was affected by the addition of synthetic carotenoids into their diet, because at the end of the feeding trial high increase their initial weight was achieved.

Rations used in these experiments revealed Significant differences concerning weight and blood factors of the twenty-second day ($p<0.05$) and treatment astaxanthin provided better conditions than the control group for the fish. Much research in this area used in aquaculture. However, conflicting results have been reported. The results of this experiment with studies in Atlantic salmon fed astaxanthin [16,18]، *Rodeus uyekii* fed astaxanthin and *Penaeus monodon* fed with Dunaliella extract [23] Is consistent. The results with research in rainbow trout fed with synthetic astaxanthin [21,24] and Penaeus monodon and *Penaeus japonicus* using astaxanthin, beta-carotene and canthaxanthin [25-28] been done is contradictory. This paradox may react differently to the cultured species or different life stages it is associated. Among the possible causes of weight gain can be positive effect astaxanthin on metabolism, Accelerate and increase the efficiency of digestion and absorption of nutrients [229], Sufficient duration of the pigment, Suitability of fish weights considered in this study and most importantly, The optimal diet for maximum growth Irritable and fish growth in the use of astaxanthin noted. Kurnia, et al. [30] in his studies Examined Three experimental diets contained 30 mg Asx/kg diet of synthetic Asx, marine bacteria or combined synthetic Asx and marine bacteria. One diet was served as a control diet. The fish fed diet supplemented both marine bacteria and synthetic Asx provided the highest total carotenoids and Asx content in the skin and muscle. In studies on the survival of Penaeus monodon fed with astaxanthin [31] is similar. astaxanthin coloration due to Salmo Fan was 28º that with findings similar to those of Mehrabi, et al. [32] ; Yanar, et al. [33]; Mollaei and Zarinfar [34], Ghyasvand, et al. 2009 [32-34] and with Bjerkeng, et al. [35]; Erdem [36]; Ando, et al. [37] is contradicted. This difference may be due to rearing conditions and age of the fish is feeding time.

Pigments also raise the immune system and increase the resistance which with findings of (Amaninejad et al. [38]; Bjerkeng, et al. [35]; Torrisen, et al. [39] was similar. Amaninejad announced that carotene in algae due to increased resistance of the immune system. Treatments in this study, which were fed with astaxanthin had increased immune system compared to control diet. Most RBC, WBC, Hb, HCT, Lym, Neu and IgM treated to astaxanthin. Faghani [40] reported the level of Hct 21/1±0.65, HB 5/23±0.64 and Lym 82±5/1. Rehulka and coworkers in 2009, examined the effect of astaxanthin on blood factors on rainbow trout and level of RBC (1.06 vs. 1.15 T/l)، HB) 71.8 vs. 76.5 gr/l), Hct (0.386 vs. 0.422) announced. Mccarthy and colleagues (1973) number of RBC (1.7 -1.2)×106 per cubic millimeter count, hemoglobin 9-7 g dl and hematocrit 32-45% reported. Haley & Weiser [41] reported the average number of RBC (1.5 ± 0.16)×106 per cubic millimeter count in rainbow trout. Astaxanthin was found to be more effective than the control group. Although salmonids absorb astaxanthin 10-20 times as other pigments [42]. This development is important for commercial aquaculture and thus be market-friendly.

Conclusion

In conclusion, the treatment that fed with astaxanthin in terms of coloration, growth and immune resistance were in better condition than the control group. According to studies and information gaps can be the use of animal sources contain carotenoids due to expensive synthetic carotenoid pigments and purification by knowledge and technology of biological pigments, use appropriate levels of carotenoids in the diet of male and female productive aquatic, Generation

to provide better quality for future studies were suggested.

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