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Study on Effect of Red Bell Pepper on Growth, Pigmentation and Blood Factors of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: The effect of red bell pepper andastaxanthin on growth,pigmentation and blood factors of rainbow trout weighted average (93±0.64g) were studiedfor 8 weeks. Five treatments(three replicates/treatment) were employed as follows: treatmentA:fed with commercial diet (control), treatment B: 41 mg/kg AS (astaxanthin as positive control), treatment C: 33 mg/kg RP (Red pepper), treatment D: 44 mg/kg RP, treatments E: 55 mg/kg RP. All treatments were fed in hours 8,12,16, respectively. It should be considered that the feeding rate of biomass weight after each took biometrics. Finally, blood samples were taken from the caudal vein of fish. The results showed 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 Food conversion ration, Specific growth rate, Growth rate, Body weight increase and survival rate but the parameter of Condition factor showed a significant difference between treatments ($p<0.05$). In terms of pigmentation between experimental treatments, red bell pepper 55 mg kg was created more change color than its group treatments and control groupresults of blood factors showed that between WBCs, RBCs, Hb, HCT, Lymphocytes and IgMhad significant differences between treatments ($p<0.05$), but the factors of Monocytes and Neutrophils, there was no significant difference between treatments ($p>0.05$). The results showed that the use of vegetable ingredients in diets for rainbow trout is an effective and affordable and the diet of 55mg/kg red bell pepper is available diet in raising rainbow trout.

Key words: Red Bell Pepper · Astaxanthin · Rainbow Trout · Pigmentation · Growth

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*), is one of the most important commercial species salmonidae, which are grown widely in many countries. Now, this fish have valuable share in the human food supply [1]. Fish nutrition has an important impact on several parameters directly influencing the quality of fish [2]. The carotenoids in the normal and abnormal environmentfish are available and have positive effects on fish, including increased growth, boost the immune system by increasing antibodies are produced [3-7]. The pigments in the body of fish can't be made and should be added to the diet [3]. Using compounds as plant food is used in pigments production. Today, research on the use of this material is ongoing [8, 9]. Pigments play an important role in the diet of animals and animal feed industries. Carotenoids are group of natural pigments and micronutrient elements that is necessary to be added to fish diets. Astaxanthin is the major carotenoid pigment that found in aquatic animals [4, 10] and important biological functions, including preventing the oxidation of unsaturated

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essential fatty acidsPUFA, protection against the negative effects of ultraviolet light to produce vitamin A, an immune response, controls of Growth and reproductive behavior and improve [11, 12] Red Bell peppers of mainvegetables, family of the *Solanaceae*, genus *Capsicum* and the species are *Capsicum annum*. Red bell peppers are a rich source of vitamins A and C that compounds are strong antioxidants such as vitamin C and carotene. Capsanthin in pepper, especially, common pepper has a chemical composition that stimulates the immune system and helps in attacking infectious agents [13]. Recently, the most promising alternatives to synthetic astaxanthin for salmonids pigmentation are the red yeast *Phaffiarhodozyma* [14, 15], *Haematococcus* algae [11, 16], *Longostilla*, *Pleuroncodesplanipes* [17] and *Chlorella vulgaris* [8]. In addition, red pepper (*Capsicum annum*), which are abundant and rich in carotenoid pigment, could be considered alternative sources. It was shown that red pepper could be used for pigmentation of salmonids [18-21]. However, it is still not clear what percentage of red pepper can be used in rainbow trout diets or what effects it has on growth. No investigations about effects of Red bell pepper on blood factors have been published. In the present study, the effects of diets including various proportions of Red bell pepper on the growth and blood factors of rainbow trout were examined in comparison to synthetic astaxanthin as well as their potential as additives in rainbow trout diets.

MATERIALS AND METHODS

Experimental Procedure and Feeding Trial: The experiment was conducted in pools was located in the 3000 Road of Tonekabone region of Mazandaran province. Pools dimension in this experiment were (4m×3m×1m) and time period was 60 days. 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±0.379mg1^{-1}, 7.1±0.293, respectively. Water supply was river.

The five different treatments used in the experiment were as follows:

Treatment A: fed with commercial normal diet (control)
Treatment B: Diet supplemented with AS 41 mg/kg (positive control)
Treatment C: Diet supplemented with AS 33 mg/kg
Treatment D: Diet supplemented with AS 44 mg/kg
Treatment E: Diet supplemented with AS 55 mg/kg

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

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

The commercial basal diet was supplied by Faradane Company (Table 1), synthetic astaxanthin, by Behparvar Company, whereas the red bell pepper wasprovided by local producers. This plant was dried in the shade, powder. The experimental diets were made by adding the requisite amounts of the different pigment sources to a commercial diet. Food processor was done every tertian. For Preparation ration, initially small amount of oil with pigments are well mixed and spray to pellets. Then thedaily food of each pool with a digital scale was weight and packed.

750 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 feeding three times a day [8, 12, 16] and feed required per day according to the weight of biomass in different time periods (usually after each bioassay) was calculated. Once every 20 days, randomly, 10 fish were selected for biometry from each replicate, weighed with a digital scale with precision (0.01 g) and length was measured by millimeter ruler. In order 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 period that included RBCs, WBCs, HCT, Hb, IgM, Lym and Mono. The weight and length of fish in each pool, statistical calculations values of FCR, %BWI, SGR, GR, CF, survivalwere 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 was measured and three fillets were obtained per trout in order to matching with Salmo Fan™.

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 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 [22]. Measurements of red blood cells (RBC) and white blood cells (WBC) by soluble Lewis, neubauer. Hemoglobin (Hb) was measured with by instrumental methods use SYSMEXLYS, Hematocrit (HCT) was determined by spinning blood samples in heparinized capillary tubes in micro-hematocrit and micro-centrifuge Hettichby far (14,000 rpm). IgM bymethod of Nephelometry and for calibration and testing control used Binding Site of England was made.

Statistical Analysis: The data analyzed by SPSS 13 and Excel 2003 soft ware. For statistical analysis, the homogeneity of the control data, averages obtained from measurements of blood factors (Shapiro Wilk test) through Kruskal-Wallis and Man-Witney nonparametric test and the average weight and length measurements by ANOVA, The confidence level was %95 and Duncan Test were employed.

RESULTS

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

Growth data of the fish on days 0, 20, 40 and 60 are summarized in Table 3. The results showed that response fish to different levels of RP in the diet is different. In study, the first average weight was 93±0.64 g and mean total length 18.1±0.25 cm and in the end of test, there was significant difference between the experimental treatments than the control treatment (p>0.05). On these sampling days, the highest weight gain was displayed by 55mg/kg red pepper, followed by the groups of 33mg/kg, 44mg/kg, astaxanthin and control group (P<0.05) (Table 3, 4). The most SGR, BWI, GRand survival related to55mg/kg red pepper (Table 5).

Results revealed that coloration of the fish was fed with red pepper significant change in their meat than the control were created. According to the results of the greatest color change at the end of term was related to treatment E (55mg/kg RP) that the grade of color with SalmoFan™ was 32° and treatments B, C,D respectively, were 27°, 29° and 30°.

In this experiment, blood factors including RBCs, Hemoglobin, Hematocrit, WBCs, Lymphocytes and IgMhad significant differences between treatments (p<0.05) but Monocytes and Neutrophils, there was no significant difference between treatments (p>0.05) (Table 6). Most RBCs, WBCs, Hb, HCT, Neu and IgM associated to treatment of 55mg/kg red pepper. Most Mono and Lym, respectively related to44mg/kg red pepper and astaxanthin. The Lowest RBCs, Hb, HCT, Lym-related control, WBCs and IgM-related treatment D and the lowest Mono related to treatment E.

DISCUSSION

Fish, like other animals can't produce carotenoids. In normal conditions, these materials include food by algae; crustaceans removed etc. provide rich carotenoids. So the carotenoids in conditions should be used as a food supplement [23]. Obviously, the carotenoid pigments in salmon biology are a sign of normal matter. Very high survival rates, good growth performance of fishes and the

Table 2: Water physicochemical parameters

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 3: Mean weight (g) of the fish fed various diets

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±1.68^{ab}	138.67±1.42^{b}	168.29±2.99^{b}
33 mg/kg Red Bell Pepper	93±0.64^{a}	120.49±0.8^{ab}	149.11±0.31^{d}	182.37±3.16^{d}
44 mg/kg Red Bell Pepper	93±0.64^{a}	121.33±0.4^{c}	150.77±1.22^{de}	181.01±8.14^{d}
55 mg/kg Red Bell Pepper	93±0.64^{a}	122.14±0.5^{d}	154.68±0.42^{e}	188.84±1.6^{e}

Data are presented as Mean ± SE. Each replicate consists of measurements from 10 fish

The means with different letters in each column denote a significant difference (P<0.05)

Table 4: Mean length (cm)of the fish fed various diets

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]
33 mg/kg Red Bell Pepper	18.1±0.25[a]	20.21±0.63[bcd]	23.43±0.09[c]	25.94±0.05[e]
44 mg/kg Red Bell Pepper	18.1±0.25[a]	20.67±0.51[cd]	23.5±0.24[c]	26.31±0.25[ef]
55 mg/kg Red Bell Pepper	18.1±0.25[a]	21.1±0.3[d]	23.73±0.15[c]	26.67±0.23[f]

Data are presented as Mean ± SE. Each replicate consists of measurements from 10 fish
The means with different letters in each column denote a significant difference (P<0.05)

Table 5: Mean growth parameters of the fish fed various diets

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[c]	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
33 mg/kg Red Bell Pepper	4.5±0.26	37.07±0.41	22.31±1.73	1.66±0.13	1.04±0.015[ab]	96±2
44 mg/kg Red Bell Pepper	5.29±1.68	36.47±1.48	20.06±5.41	1.51±0.41	0.993±0.04[a]	96±2
55 mg/kg Red Bell Pepper	4.64±0.46	37.21±0.33	22.09±1.66	1.71±0.11	0.996±0.019[a]	97.33±3.06

Data are presented as Mean ± SE. Each replicate consists of measurements from 10 fish
The means with different letters in each column denote a significant difference (P<0.05)

Table 6: Mean blood factors of the fish fed various diets

Treatments Parameters	Control	Astaxanthin	33mg/kg RP	44mg/kg RP	55mg/kg RP
RBC (× 10^6/µL)	1006111±169314	1347000±253519	1291889±144852	1261667±229633	1455778±399078
WBC (/µL)	4133.3±715.9	9233.3±1019.8	4311.1±899.2	4066.7±926	10511.1±5463.8
Hb (gr/dl)	6.51±1.27	8.68±1.65	8.96±0.91	8.6±1.44	9.74±2.48
HCT(%)	37.11±7.32	43.89±7.39	45.78±5.7	43.78±6.74	50.78±12.5
Lym (%)	62.44±12.3	96.11±2.85	86.11±19.86	86.33±23.68	94.33±3.81
Mono (%)	2.22±2.59	2.67±2.29	1.33±1.58	3.33±2.55	1.22±1.2
Neu (%)	2.67±2.45	3.56±5.64	1.89±1.54	3.56±3.51	4±3.32
IgM (mg/l)	9.63±1.35	29.67±24.54	6.97±1.8	5.53±1.62	53.33±27.3

Data are presented as Mean ± SE. Each replicate consists of measurements from 10 fish.
*{On the basis of NaparametricKruskal-Wallis and Manwitny tests, the separate character are not used (a,b)}

Fig. 1: SalmoFan™

influence of growth parameters and food intake of diets showed that red bell pepper of plant pigment have any negative effects on health in rainbow trout. Several studies show [24, 25] that the use of high levels of plant material in diets of fish, especially carnivorous fish, retards their growth. The main reason for this has been considered due to containing high level cellulose ofthe plants. In addition, they might lead to negative effects on the taste of food, physical quality of the pellets and nutrition balance of diets [26]. However, the degree of this effect naturally depends on the feeding habit of the fish and the preparation of the diet. Rainbow trout is a carnivorous fish and, therefore, adding plants to their diets will be naturally restricted. Based on the results, the highest growth was related to treatment D and respectively lowest treatments B and C that similar conditions were attributed together. With increasing concentration of red pepper in the diet, weight gain of fish was ascending. In this regard, conflicting results have been reported. Results of this experiment with conducted studies on fingerling trout with plant carotenoids [27]. Carotenoids in salmonfishes [28] weresimilar, but studies on Monodon shrimp and rainbow trout fed with red pepper [21, 29, 30], Rainbow trout fed with red pepper [18] has been inconsistent. This paradox may associate react differently to the cultured species or different life stages. The addition of red pepper at each level increased the

pigmentation level. Although a significant difference in pigmentation appeared between the groups including the high dose of red bell pepper. As obsrved, treatment D had the highest color quality. Plant carotenoids can be stated with greater accumulation in the tissues, due to its vitamins and carotene, increased color. The findings with studies of [28, 31-34] Similarities and [21, 35, 36] has been inconsistent. These differences result from the quality, amount and period of capture, rearing conditions and age of the fish that to achieve desired results can be increased the amount of diet or feeding time. Pigments also raise the immune system and increase the resistance which with findings of [28, 31, 37] was similar. Amaninejad announced that carotene in algae due to increased resistance of immune system. Treatments in this study, which were fed with red pepper, had high immune system compared to control diet. Most RBCs, WBCs, Hb, HCT, Lym, Neu and IgM treated to 55 mg/kg red Bell pepper. Faghani *et al*. [38] reported the level of Hct 21.1±0.65, HB 5.23±0.64 and Lym 82±5.1. Rehulka [39], examined effect of astaxanthin on blood factors on rainbow trout and level of RBCs (1.06 vs. 1.15 T/l), HB) 71.8 vs. 76.5 g/l), Hct (0.386 vs. 0.422) announced. Mac Carthy *et al*. [40].

number of RBC (1.7-1.2)×106 per cubic millimeter count, hemoglobin 9-7 g dl and hematocrit 32-45% reported. Haley and Weiser [41] reported the average number of RBCs (1.5±0.16)×106 per cubic millimeter count in rainbow trout. The plant pigment was found to be more effective than astaxanthin although they contained equal amounts of carotenoids. The reason for this may be differences in carotenoid sources. So the natural pigments are comparable astaxanthin and highest absorb in fillet. This development is important for commercial aquaculture and thus be market-friendly.

In conclusion, treatments that fed with red pepper in terms of coloration, growth and immune resistance were in better condition than the control. Also plant pigments were due to its low cost and affordable can be named as an alternative to synthetic carotenoids. According to the study, effective dose for the red pepper can be 55mg/kg. Due to the different conditions of rearing, doses may be changed. It should be mentioned that can't be used for high doses of plant pigments. The main reason for this has been considered due to containing high level cellulose of the plant and can disrupt or stop the growth.

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