Contribution of Vitaton (B-Carotene) to the Rearing Factors Survival Rate and Visual Flesh Color of Rainbow Trout Fish in Comparison With Astaxanthin

M.Ghotbi, M.Ghotbi, and Gh. Azari Takami

***Abstract*—**In this study Vitaton (an organic supplement which contains fermentative β-carotene) and synthetic astaxanthin (CAROPHYLL® Pink) were evaluated as pro-growth factors in Rainbow trout diet. An 8 week feeding trial was conducted to determine the effects of Vitaton versus astaxanthin on rearing factors, survival rate and visual flesh color of Rainbow trout (*Oncorhnchynchus mykiss*) with initial weight of 196±5. Four practical diets were formulated to contain 50 and 80 (ppm) of β-carotene and astaxanthin and also a control diet was prepared without any pigment. Each diet was fed to triplicate groups of fish rearing in fresh water. Fish were fed twice daily. The water temperature fluctuated from 12 to 15 (C°) and also dissolved oxygen content was between 7 to 7.5 (mg/lit) during the experimental period. At the end of the experiment, growth and food utilization parameters and survival rate were unaffected by dietary treatments ($p>0.05$). Also, there was no significant difference between carcass yield within treatments ($p>0.05$). No significant difference recognized between visual flesh color (*Salmo*Fan score) of fish fed Vitaton-containing diets. On the contrary, feeding on diets containing 50 and 80 (ppm) of astaxanthin, increased SalmoFan score (flesh astaxanthin concentration) from <20 (<1 mg/kg) to 23.33 (2.03 mg/kg) and 27.67 (5.74 mg/kg), respectively. Ultimately, a significant difference was seen between flesh carotenoid concentrations of fish feeding on astaxanthin containing treatments and control treatment ($P<0.05$). It should be mentioned that just raw fillet color of fish belonged to 80 (ppm) of astaxanthin treatment was seen to be close to color targets (SalmoFan scores) adopted for harvest-size fish.

***Keywords*—**Astaxanthin, Flesh color, Rainbow trout, Vitaton, β-carotene,

I. Introduction

THE characteristic pink coloration of the flesh in Salmonids which is among the most important criteria [14] is caused by deposition of carotenoid pigments. Carotenoid pigments are biosynthesized by higher plants, algae, certain yeast and bacteria; whereas, all animals including fish, are believed to depend on the dietary supply for these pigments [3], [35]. Apart from being responsible for muscle coloration, carotenoids are involved in certain physiological functions of salmonid fishes, such as pro-vitamin a and pro-growth functions. For instance, astaxanthin may serve as a vitamin A precursor in Salmonid fishes [51], [20], [4], [5], and it has also been reported to have positive effects on growth and survival of salmonid fry during the start feeding period [27], [30], [38]. Furthermore, another study has indicated that a minimum dietary astaxanthin concentration is required for normal growth and survival of Atlantic salmon fry [26] which this effect has been linked to pro-vitamin A function of astaxanthin. Other studies have demonstrated positive effects of dietary astaxanthin on the growth of some aquatic animals. Red tilapia (*Oreochromis niloticus*) and Indian carp were reported to have improved growth when fed carotenoids [33], [22]. Such an improvement has recently been reported about some strogeon hybrids fed vitaton (organic source of ß-carotene) *(Sidorov and Koleman, 2005)*. Also, it has been shown that carotenoids improve growth and survival in some species of crustaceans such as *Homarus americanus* [26], *Penaeus japonica* [24], *Négre-Sadargues et al.,1993)* and *Penaeus monodon (Thongrod et al.,1995)*. In natural environments Salmonids achieve carotenoids through food chain, by eating their prey organisms, while in captivity these pigments must be supplemented to the diet. Currently these supplements are either manufactured by chemical synthesis or obtained from organisms that have the biosynthesizing ability. Astaxanthin, is the major carotenoid of Salmonid fishes [35]. Thus, astaxanthin is more efficiently utilized than other carotenoids for muscle pigmentation of rainbow trout *Oncorhynchus mykiss* [52]. The commercially most important sources of astaxanthin are chemically synthesized products that like any other synthetic products may have harmful effects on fish and human beings body. Also, astaxanthin is a costly ingredient in Salmonid diets [53], [54]. So taking account of the need for hygienic safety of foods and the importance of diet economical price, recently many research have focused on evaluating the effects of carotenoids on rearing attributes of Salmonids, during their life stages. Also, possibilities for replacement of currently used synthetic astaxanthin by another natural sources of carotenoids or organic pigmented products still require more investigation. So in the present experiment utilization of commercially and formulated astaxanthin (CAROPHYLL® Pink, 8% astaxanthin, Hoffman-La Roche, Basel, Switzerland) was tested in comparison with fermentative ß-carotene of Vitaton (≥6% ß-carotene, Vitan,

M. Ghotbi, Faculty of Agriculture and Natural Resources, Islamic Azad University, Science and Research branch, Tehran, Iran (e-mail: ghotbi.marjan@gmail.com).

M. Ghotbi, Faculty of Agriculture, Shahed University, Tehran, Iran (e-mail: mitra.ghotbi@gmail.com).

GH. Azari Takami, Faculty of veterinary medicine, University of Tehran, Tehran, Iran.

Ukraine) in diets for rainbow trout fish. The objective of the present study was to identify the effects of mentioned carotenoids on rearing factors, food conversion Ratio, survival rate and Carcass yield of growing rainbow trout fish.

II. Material and Methods

A. Fish and Feeding Management

The feeding experiment took place at Jajrood fish farm (Saied abad village, Tehran, Iran). Seven hundreds and fifty rainbow trout with mean initial weight of 196 (g) and mean length of 25 (cm) were distributed in 5 triplicates. Each two replicates stocked in rectangular concrete tanks (7.5 m× 0.87 m× 0.65 m) of hatchery that were divided in two parts by using perforated metal plates in the middle (50 fish per half a tank).

The tanks, set in parallel rows, supplied with fresh water of a deep well and drainage (ambient temperature between 12 to 15 (C°), pH 7.5, dissolved oxygen 7.5 (mg/lit)) at a rate of 2 to 2.5 (lit/sec). Prior to use, water was filtered through a sand filter to avoid contamination with zooplankton that could have provided a source of pigments (mainly astaxanthin).

Although, the tanks were indoor, they received a natural photoperiod (28 October to 22 December), except cloudy days which were supported with artificial illumination for 9 hours (7:00 h to 16:00 h). Before adopting experimental diets, fish were fed a commercial non-pigment supplemented feed (Chineh Feed Company, Tehran, Iran) thrice a day (Table. I).

During the feeding trial 4 experimental and a control diet were fed to the fish for 8 weeks. Fish were hand fed twice a day (00:7 h, 16:00 h) an equal quantity of feed 7 days per week at a rate of 2.5% to 3% of body weight per day (BWD) during the first and second months respectively. At each 2-week period an adjustment of the daily ration was made according to the fish growth. Dead fish were recorded and weighed for calculating feed conversion ratio (FCR).

B. Experimental Diets

Four experimental diets with identical basal composition (Table I) were supplemented with 2 given pigments, astaxanthin and ß-carotene at 2 levels, 50 and 80 mg per kg of feed, and one unsupplemented diet with a similar composition served as control. The two carotenoid sources used were Vitaton (7.1% ß-carotene, Vitan, Dnipropetrovs'k, Ukraine) (Table2) and commercial beadlets of 8% (W/W) astaxanthin content (CAROPHYLL® Pink, DSM, Basel, Switzerland). Both pigments were superficially added to the prepared pellets (4.5mm) in order to achieve 50 and 80 (ppm) doses. Two different levels of each pigments due to their bioavailability (35 and 56 (g) of Vitaton & 32 and 50 (g) of synthetic astaxanthin per 50 (kg of feed) were mixed with vegetable oil (amount of oil was approximately equal to 2% of feed mass~ 1(kg)) and stirred for about 20 minutes. After achieving relatively homogenized suspensions, they were distributed to the pellets while stirring them with an agitator. Pellets were allowed to absorb the suspensions for 4 hours then were stored at 0 (C°) prior to use.

C. Growth Trial

Random selection and returning were used to monitor the growth rate of the fish, in intervals of 2 weeks within an 8-week experimental period. 10% of fish in each test groups and control group (15 fish out of 150 for each treatment) were caught and anaesthetized with carnation powder to enable easy handling. The fish were weighed and measured at an accuracy of 5 (g) and 1 (mm). Prior to measuring the biometrical factors, the fish were starved for 24(h) in order to decrease stress. At the end of the trial, the fish were collected from each concrete tank and group weighed. From each replicate, 3 fish were sampled for muscle color assessment.

The body conformation traits studied included total body length, fork length, body height and body width. As to the condition parameters, the total weight of the fish and weight of the fish without viscera were recorded at an accuracy of 5 (g). These data were used to calculate the carcass yield (gutted body weight as% of body weight) and the body condition factor (body weight in g ×100/body length ^{3} in cm).

TABLE I
FOOD FORMULATION OF CHINEH FEED COMPANY

Ingredients		Feed type GF T2
Gross protein	Min	36%
Gross fat	Min	12%
Ash	Max	12%
NFE[a]		24%
Available p	Min	0.8%
Moisture	Max	9%

[a]Nitrogen Free Essence

D. Sampling and Recording

Fish per replicate were sampled and sacrificed using an overdose of anesthetic (carnation powder) at intervals of 4 weeks (0,4,8 weeks). Muscle color was visually assessed immediately post slaughter by using Roche *SalmoFan*™ (Hoffman-La Roche, Basel, Switzerland). The scale on the fan ranges from 20 to 34. Increasing scores represent increasing pigmentation. The mentioned visual assessment was done by the same three judges throughout the experiment.

E. Calculation and Statistical Methods

The following variables were calculated per group over 56 days: Average Weight Gain (AWG)= Final body weight - Initial body weight, Specific Growth Rate (SGR)= {(Ln Initial body weight-Ln Final body weight/days)}, Feed Conversion Ratio (FCR)= feed intake/weight gain, Protein Efficiency Ratio (PER)= weight gain/protein intake, Survival Rate (SR)= (final number of fish/Initial number of fish)×100, and the following parameters were measured on individual rainbow trout at the end of the trial, Body Condition Factor = (body weight/total length^3)×100, Carcass yield= (gutted body

weight/body weight)×100, where weights were in (g) and lengths were in (cm).

Rainbow trout muscle astaxanthin concentration was measured each 4 weeks, according to the following equation (Haffman La- Roche, Basel, Switzerland).

SalmoFan^{TM} Score= 4.179 Ln (Astaxanthin content mg/kg) +20.386

All data were subjected to analysis of variance (one-way ANOVA) and correlation analysis when appropriate using SPSS for windows. Differences between the means were tested by Tukey's multiple range test. A significance level of 5% was assumed.

TABLE II
VITATON INGREDIENTS

Vitaton Ingredients	Percentage
ß-carotene	7.1%
Vit B_1	0.196 mg%
Vit B_2	0.1671 mg%
Vit B_6	0.98 mg%
Vit B_{12}	0.0014 mg%
Vit E	27.6 mg%
Vit PP	1.33 mg%
Pantothenic Acid	3.5 mg%
Biotin	0.09 mg%
Lipid	44.7 mg%
Essential Amino Acids	9.055 mg%
Inessential Amino Acids	1.261 mg%

III. RESULTS

The initial total body weight of various fish groups were similar (approximately 196 (g)) (Table 3). After feeding the experimental diets for 56 days, the growth performance (weight gain and SGR) of the rainbow trout fish were not significantly affected by dietary treatments. The overall percentage of body weight increase was about 58.56% for treatments during the 56-day feeding trial, and SGRs varied from 0.77% d^{-1} (50 (ppm) of ß-Carotene) to 0.89% d^{-1} (80 (ppm) of ß-Carotene), though no significant difference were seen within them ($p>0.05$) (Table 3).

The overall feed conversion Ratio were relatively similar for all dietary treatments (2.4 kg dry feed eaten per kg body weight gain). A similar performance of diet was observed in term of protein efficiency values ($p>0.05$). So Neither vitaton nor astaxanthin significantly affected weight gain or FCR.

The survival rates showed no significant difference ($p>0.05$), although the highest values belonged to (80) ppm of Astaxanthin and ß-Carotene respectively (96% and 95.33%) (Table IV).

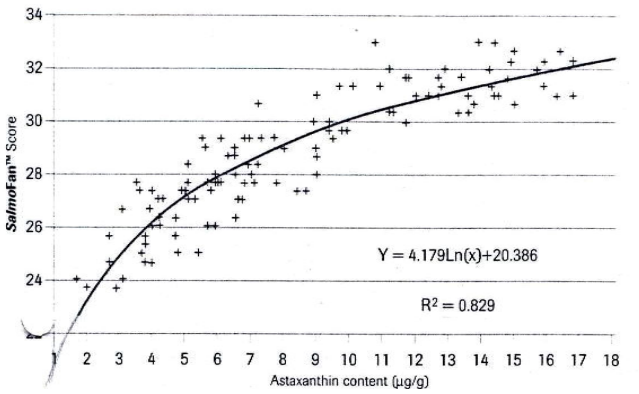

Fig. 3 *SalmoFan*TM Score versus chemical astaxanthin content in rainbow trout

Also, there was no significant difference between body condition factor (BCF) of fish fed 5 dietary treatments. At the end of the trial the biometric and slaughter parameters of rainbow trout fed on different diets, were reported in (Table IV). Among them the condition factor could be considered as rough descriptor of the morphologic quality of fish.

Ultimately, it was found that feeding diets including various types and levels of pigments did not affect the gross morphology (appearance) of rainbow trout relative to the control.

The visual color scores increased with increasing level of astaxanthin in the diet, and the *SalmoFan*^{TM} values were significantly different for each of the dietary astaxanthin levels. From<20 in control treatment to 23.33 and 27.67 for 50 and 80 (ppm) of astaxanthin treatments, respectively.

Although the variation in coloration were seen within the fish fed astaxanthin – supplemented diets and between them and control, no considerable difference was seen between the visual flesh color of fish fed ß-Carotene treatments and control treatment.

TABLE III
GROWTH PERFORMANCE AND EFFICIENCY OF RAINBOW TROUT FED THE EXPERIMENTAL DIETS OVER 56 DAYS.

Diet code	C	A50	A80	B50	B80	S.E.M
Initial Body Weight (IBW) (g)	195.66	199.33	194.66	195.66	197.66	5.02
Final Body Weight (FBW) (g)	304.66	313.66	305.66	303.00	327.00	5.02
Average Weight Gain (AWG) (g)	109.00	114.33	111.00	107.33	129.33	4.21
Specific growth rate (SGR)	0.78	0.81	0.80	0.77	0.79	0.24
Feed conversion ratio (FCR)	2.59	2.40	2.46	2.51	2.08	0.09
Protein efficiency ratio (PER)	1.12	1.16	1.14	1.10	1.33	0.42

TABLE IV
SOMATIC INDICES SLAUGHTER YIELD AND SURVIVAL RATE OF RAINBOW TROUT AT THE END OF 56 DAYS OF GROWTH

Diet code	C	A50	A80	B50	B80	S.E.M
Body Condition factor	1.291	1.318	1.338	1.320	1.324	0.14
Carcass yield (%WBW)	50.66	49.66	46.33	49.66	52.33	1.388
Survival rate (%)	91.33	92	96	93.33	95.33	0.920

IV. DISCUSSION

The pigment sources used in this experiment were chosen because of their different chemical properties and prices. CAROPHYLL® PINK is a product made by F.Hoffman-La Roche Ltd. According to the previous records, it contains 8% astaxanthin, which is the major carotenoid of wild salmonid fishes [35], [36] so it is efficiently used by trout fish.

Vitaton, another carotenoid source, is manufactured by vitan Ltd. This Non-GMO product is derived from yeast and contains≥ 6% ß-Carotene. (The package used in the current experiment contained 7.1% of ß-Carotene). Although ß-Carotene is not commonly utilized in salmonid diet, we used Vitaton in this experiment for the following two reasons. One was the economical price. It is almost 6 times cheaper than CAROPHYLL PINK. The other was the aim of this experiment. So far the vast majority of research on pigments application in aquatics have carried out about nuptial and flesh coloration; whereas, in this trial we focused on rearing parameters and survival rate which both are impacted by provitamin A and antioxidant functions of pigments [9], [12], [17], [20], [27], [29], [31], [33], [34], [51]. Thus, Vitaton as an organic and cheaper source of pigment was compared with synthetic and expensive astaxanthin. For each pigment two different doses (50 and 80 (ppm)) were utilized in order to define more efficient dose of the superior and more economic pigment.

Results of the experiment showed that none of the treatments significantly influenced the growth response and survival, also no gross deficiency signs were observed in any of the fish. This result is in agreement with some previous studies on different sources of astaxanthin in rainbow trout, *Oncorhynchus mykiss* [2], [13], [15], [42], and Atlantic salmon *Salmo salar* (Baker et al., 2006; Bjerkeng et al., 2006) and Black tiger Shrimp, *Penaeus mondon* (Barclay, 2006) which in most of them rearing factors were not the ultimate target of research, and they were designed as an adjunct to the main cause. On the other hand our finding disagrees with the result of some other investigations in which positive effects of astaxanthin on the growth and survival of salmonid fry demonstrated [38], [27], [30], [28]. Also, other adverse results have been reported about positive effects of dietary carotenoids on the growth and survival of Red tilapia, *Oreochromis nilticus*, and Indian carp [22], [32], Lobster, *Hommarus americanus* [23] and, kruma prawn, *Penaeus japonicus* [24]. More recently, similar positive impacts have been reported about sturgeon hybrids treated with different doses of Vitaton (Kolman and Sidorov, 2005).

The likely reasons that may interpret the obtained result about growth parameters are the effects of fish size, environmental conditions and aquatic species characteristics. Larval fish display a high growth and metabolic rate so could deplete the storage of vitamin A (as a pro-growth and antioxidant factor) faster. However, larger fish has probably stored more Vitamin A and its precursors in body from previous diets. So it takes a long period to deplete these sources when fed with vitamin A and carotenoid-deficient diet. A literature survey about the effect of age or size on vitamin deficiency has revealed that fish size correlates positively with the time of appearance of the first vitamin deficiency symptoms (Dabrowski, 1989). In connection with, effect of

environmental conditions such as; ambient temperature, photoperiod (season) and water flow should not be ignored which in the current trial all were relatively low. Regarding the fact that decreasing the mentioned factors may result in decreased metabolic rate and; therefore, slower depletion, this can also account for not observing any deficiency symptoms within the fish and consequently no remarkable growth promotion in pigmented treatments.

Aquatic species have distinctive nutritional physiology as well as different regimes, so they may rely on various sources for obtaining vital nutrients and vitamins. Even the Teleosts show great diversity in the form and function of their digestive tracts [10] so poor utilization of carotenoids in Salmonids, may be explained in general terms by poor uptake from the intestinal tract [51], [19], [18]. Also, some other surveys have demonstrated that the apparent digestibility coefficients (ADC) of carotenoids in salmonid fishes are quite low compared that of essential nutrients [8], [6], [7]. It may occur because salmonids have carnivorous regime and feed on higher levels of food chain relative to herbivorous and omnivorous fish so they should be innately capable of utilizing major nutritional substances in flesh rather than plants. There for the above findings may obviously illustrate the conflict between our results and the previous mentioned studies in other finfish which most of them were among herbivorers and omnivores such as Red tilapia [22], Indian crap [32], and Sturgeon hybrids (Kolman and Sidorov, 2005).

Overall, the results confirmed that large trout fish under the given environmental condition depleted vitamin A relatively slower. Correspondingly, the required vitamin A was lower for large rainbow trout in the present study, which could account for no differences in growth and survival. Consequently a longer experimental duration and smaller trout fish Should be adopted for further evaluations especially about Vitaton. Also, about Vitaton it is recommended to carry out more research in herbivorous and omnivorous fish which naturally feed on ß-carotene sources in water ecosystems.

Besides, the main finding the sensory assessment of color showed a significant difference between fish fed on astaxanthin and the control fish. Though, no considerable difference was observed between Vitaton treatments and control. Our finding about astaxanthin is supported by previous findings which has shown positive effect of astaxanthin on flesh pigmentation [49], [40], [25], [19], [15]. Bjerkeng, 1990.

Overall, it has been already proved that astaxanthin is the most effective carotenoid for all salmonids except Atlantic salmon in which canthaxanthin can be equally as effective as astaxanthin.

Furthermore, increased visual color score as a result of increased flesh pigment concentration occurred due to increasing the dietary astaxanthin dose which is in agreement with Torrissen's findings in 1995. To our knowledge so far no studies have been reported on the use of Vitaton as a pigment source in trout fish diet, though ß-carotene have been utilized in some other aquatics mainly Omnivores and herbivores, like black tiger shrimp. A literature survey in this aquatic have shown that doubling the amount of dietary ß-carotene makes it as effective as astaxanthin in pigmentation (Barclay, 2006). But it should be considered that shrimp has an especial metabolic ability for exchanging any kind of cartenoid to astaxanthin.

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