Journal of Animal and Veterinary Advances 10 (14): 1875-1882, 2011
ISSN: 1680-5593

Effects of Oleoresin Paprika (*Capsicum annum*) and Synthetic Carotenoids (Canthaxantin and Astaxanthin) on Pigmentation Levels and Growth in Rainbow Trout *Oncorhynchus mykiss* W.

[1]Nihat Yesilayer and [2]Muammer Erdem
[1]Department of Aquaculture, Faculty of Agriculture, Gaziosmanpasa University, Tokat, Turkey
[2]Departments of Aquaculture, Sinop Faculty of Fisheries, University of Sinop, Sinop, Turkey

Abstract: The effects of different carotenoid sources on pigmentation of rainbow trout were investigated. The fish (154.35±24.02 g) were fed with diets containing 50 mg kg^{-1} astaxanthin, 70 mg kg^{-1} canthaxantin and 765 mg kg^{-1} oleoresin paprika and compared with a control group for 60 days. Rainbow trout were captured in fresh water in triplicate groups (25 fish $tank^{-1}$) at water temperature of 14.74°C. There was no significant difference among experiment groups in the average weights, specific growth rates and feed conversion ratios at the end of the experiment. Feed supplemented with oleoresin paprika, canthaxantin and astaxanthin caused >6 mg kg^{-1} carotenoid concentration in the muscle tissues of the fish. The canthaxantin group (10.07 mg kg^{-1}) developed more of an accumulation than rainbow trout fillets in the other groups. L* a* b* values were determined with a Minolta Chroma Meter. While carotenoid concentration in the trout fillet and red intensity (a*) increased proportionally in the fillet, the brightness (L*) decreased. Tristimulus Chrometer a* values ranged between 0.40 and 9.55; b* values from 11.11-22.14 and L* values from 54.40-45.21. Meanwhile, Chroma (C*ab) and Hue (H_{ab}°) angle values, respectively varied 11.13-23.73 and from 87.35-62.43. In the Roche Salmofan™ card score, the increase in the feed containing oleoresin paprika, canthaxantin and astaxanthin was proportional to the increase in the fillet. While the increase of pigmentation in the fish fillet depended on the increase of carotenoid concentration in the feed, there was a decrease in the carotenoid retention rate. As observed, there is a direct proportional relationship between the color parameters in the muscle and carotenoid concentrations.

Key words: Rainbow trout, oleoresin paprika, canthaxantin, astaxanthin, muscle colour, Turkey

INTRODUCTION

The aquaculture sector will grow an estimated >50%, the next 20 years and the demand will constantly increase to provide an additional 40 million ton to the present production by 2030. One of the sectors of rapid development in Turkey is aquaculture whose growth is also parallel to a global increase in aquaculture (FAO, 2007).

The leading species of fish in this industry is rainbow trout (*Oncorhynchus mykiss*) whose increase in production has its own specific problems. One of the main problems is marketing because of the physical characteristics influencing consumer preferences such as external appearance, color and freshness, all of which are of major importance.

The flesh colors ranging from pink to red of fish in the Salmonidae family, the migrating salmonid species (*Salmo* sp., *Oncorhynchus* sp. *and Salvelinus* sp.) are one of the distinguished features found in these fish imparting a selective appearance. Thus, it has great importance economically that these fish are tinted to meet consumer demands whether grown naturally or through aquaculture (Torrissen *et al*., 1989).

Since, pigmentation is a major factor not only for salmon species but also in aquarium fish, sea fish, shrimp farming and other areas in the food sector. Many scientists continue to conduct studies and analyses to enhance color of fish. Problems in coloring, significant in marketing aquaculture products, may be overcome by adding proper coloring substances to fish diets.

Salmonids are attractive for their pink color obtained under natural conditions by the dietary intake of oxygenated carotenoid, particularly from astaxanthin, a pigment obtained from a diet of crustacean and insects. Salmonids cannot synthesise carotenoid pigments so these pigments must be added in the feed during the production cycle (Storebakken and Nome, 1992). Canthaxantin is absorbed by rainbow trout through their feed and they are able to store it in their tissues,

Corresponding Author: Nihat Yesilayer, Department of Aquaculture, Faculty of Agriculture, Gaziosmanpasa University, Tokat, Turkey

especially in their muscles at rather high concentration levels (Choubert, 1985). Astaxanthin and canthaxantin alone or in combination are most extensively used for salmon species pigmentation carotenoid while the carotenoids added to fish diets have increased the price of feed by at least 20% (Torrissen *et al*., 1989; Storebakken and Nome, 1992). Research has also been conducted on natural sources of pigments such as shrimp residues, krill, spirulina, green algae (*Haematococcus pluvialis*), crayfish extracts, red yeast, *Rhodotorula sanneri* yeast, *Tagetes erecta* and *Adonis aestivalis* flowers, red pepper, paprica and extracts. Production of cost-effective nutritionally-balanced fish diets is the main factor affecting intensive aquaculture because of its influence on growth, health and production cost.

This situation has led to searching for cheaper and natural alternative sources of carotenoids such as crustacean waste, certain plants, yeast and micro algae. An interesting alternative is esterified and saponified extracts from plants such as red chilli bells (*Capsicum annuum*) (Vernon-Carter *et al*., 1996).

Paprika contains more potent carotenoids, predominantly as capsanthin and capsorubin. Capsantin and capsorubin have a better radical scavenging ability than astaxanthin. Consequently, it is important that carotenoids quench these free radicals to produce harmless end products (Hirayama *et al*., 1994). The esterified and/or saponified extracts of red chili or red pepper have been studied for their ability to pigment rainbow trout and Atlantic salmon and the results indicate good potential for the pigmentation of skin and muscle and for stimulating gonad development in rainbow trout (Vernon-Carter *et al*., 1994).

Among vegetable feed materials, red pepper is one of the sources especially rich in carotenoids. Powdered red pepper contains 800-1500 mg kg^{-1} total carotenoids (Yanar *et al*., 1997). Using red pepper meal and extract in pigmenting trout has not been sufficiently researched.

Using these types of products in high quantities as sources of carotenoids in addition to using them for human food is believed to bring good results. Therefore, the main objective of this experiment is to compare the effects of natural carotenoid (oleoresin paprika) and synthetic carotenoids astaxanthin and canthaxantin on live-weight gain, specific growth rate and feed conversion ratio as well as the color and carotenoid concentration and retention rates.

MATERIALS AND METHODS

The experiment was conducted at the Fresh Water Fish Farming Unit of the Sinop Faculty of Fisheries in Sinop University (Sinop, Turkey).

Fish and experimental rearing conditions: Rainbow trout (*O. mykiss*) of 154.35±24.02 g mean weight obtained from a private facility in Samsun, Turkey then were transported to the facilities at the faculty of fisheries. Prior to the experiment, the fish were fed a commercial trout diet to satiation twice a day for 15 days in stocking tanks for acclimatization to the new environment. Experimental fish were then randomly distributed into twelve identical 330 L circular tanks filled with 300 L fresh water (25 fish $tank^{-1}$ with three replicates of each treatment). Throughout the experiment, the temperature, dissolved oxygen level and pH of the rearing water were 14.74±0.09°C, 5.84±0.12 mg L^{-1} and pH 7.99±0.02 (WTW 340i/Set, Weilheim, Germany). Fish were exposed to natural light regime (35°09'E, 42°01'N).

The experimental tanks were cleaned daily to remove uneaten feed and faecal material. Fish were fed on 54 of a total of 60 days. Fish were fed by hand *ad libitum* twice daily at 8:00 and 17:00 h. Feeding activity was monitored carefully to ensure an even distribution of the feed offered among all experimental fish in each tank. Fish were individually weighed at the start of the experiment after 20 and 40 days of experiment and at the end of the experiment on 60th day. Before weighing, fish were deprived of feed for 1 day. Feed consumption was recorded everyday. After weighing the sampled fish, fillets were prepared using a knife.

During feeding times, the tanks were inspected for dead fish and if found their weights and the weights of the samples with flesh color determinations were taken and the values were recorded. Because weight gain and feed conversion rates were affected both the weights of dead fish and sample fish were taken into consideration in the calculations. Weight Gain (WG), Specific Growth Rate (SGR) and the Feed Conversion Rate (FCR) were calculated as described by Yigit *et al*. (2002).

Experimental diets and preparation: Four practical diets were formulated with commercially available ingredients and were produced at the fisheries faculty in Sinop, Turkey. The four different peletted feeds used in the experiment were as fallows:

- Diet without carotenoid (control or basal diet)
- Diet supplemented with 750 mg kg^{-1} (765 mg kg^{-1}) oleoresin paprika
- Diet supplemented with 50 mg kg^{-1} (46 mg kg^{-1}) synthetic astaxanthin (Carophyll® pink (8%), DSM, Basel, Switzerland)
- Diet supplemented with 70 mg kg^{-1} (76.49 mg kg^{-1}) synthetic canthaxantin (Carophyll® red (10%), DSM, Basel, Switzerland)

We have not used equal concentration of astaxanthin and canthaxanthin because of the fact that astaxanthin is

more efficiently utilized by rainbow trout than canthaxanthin. The studies showed that mean retention coefficient for astaxanthin was 1.3 times higher than for canthaxanthin (Choubert and Storebakken, 1989). The corresponding concentration of canthaxanthin was calculated as 70 mg kg^{-1}. The concentration of oleoresin paprika was used higher than the concentration of astaxanthin (50 mg kg^{-1}) and canthaxanthin (70 mg kg^{-1}). The previous studies indicated that 350 mg kg^{-1} of oleoresin paprika did not result in highly desirable pink to red color (Akhtar *et al*., 1999). Therefore in order to get desirable color 750 mg kg^{-1} of oleoresin paprika was added to experimental diet. In the experiment, a basal diet consisting of control group containing 424 g kg^{-1} crude protein, 266 g kg^{-1} crude fat and 5.68 kcal g^{-1} gross energy was used (Table 1).

The dry feed materials were screened through a 500 μm sieve, weighed separately with a scale of 0.01 g sensitivity and mixed with the oil in a blender for 15 min. Oleoresin paprika was blended together with the dry ingredients and the oil. The synthetic carotenoids were dissolved in water at 60-70°C and added to the mixture after blending. The diets were dried to a moisture content of 92-95 g kg^{-1} in an oven and stored at -20°C, protected from light throughout the experiment.

Table 1: Proximate composition of the diets for the experiment

Ingredients (g kg^{-1})	Control	Oleoresin paprika	Astaxanthin	Canthaxantin
Proximate composition (g kg^{-1} dry matter)				
Moisture	22.7	45	35.8	24.1
Crude lipid	266.4	257.1	252.5	258.5
Crude ash	89.3	89.5	87.6	87.1
Crude protein	424.0	427.3	430.7	431.3
Nitrogen-free extracts*	187.6	193	198.5	192.6
Crude cellulose	32.7	33.1	30.7	30.5
GE (kcal g^{-1})**	5.68	5.64	5.63	5.67
P:E (mg $kcal^{-1}$)	74.65	75.76	76.50	76.06
Oleoresin paprika[1]	-	750 mg kg^{-1}	-	-
Astaxanthin[2]	-	-	50 mg kg^{-1}	-
Canthaxantin[3]	-	-	-	70 mg kg^{-1}
Amino acid content (g kg^{-1})[4]				
Lys	33.1	33.3	33.1	33.1
Met+Cys	16.1	16.3	16.1	16.1
Tyr	5.3	5.3	5.3	5.3
Thr	18.8	18.9	18.8	18.8
Arg	30.4	30.7	30.4	30.4
His	14.5	14.6	14.5	14.5
Ilea	20.3	20.3	20.3	20.3
Leu	34.2	34.4	34.2	34.2
Phen	19.7	19.9	19.7	19.7
Val	23.7	23.7	23.7	23.7

[1]Oleoresin paprika extract: Aromeks Ltd. sti., Istanbul, Turkey.[2]Astaxanthin: Carophyll ®pink (8%), DSM, Basel, Switzerland. [3]Canthaxantin: Carophyll® red (%10), DSM, Basel, Switzerland. [4]Essential amino acid contents calculated from feed raw materials. *Nitrogen Free Extract (NFE) = 100- (Crude protein% + Crude lipids% + Crude ash% + Crude cellulose%). **Calculated according to 5.65 kcal g^{-1} protein, 9.45 kcal g^{-1} lipid, 4.1 kcal g^{-1} nitrogen free extract

Proximate composition analysis: The chemical composition of the diets was determined according to AOAC (1984) guidelines as follows: dry matter after drying at 105°C for 24 h, ash by combustion at 550°C for 12 h, crude protein (N×6.25) by the Kjeldahl method after acid digestion and crude lipid by ethyl ether extraction in a Soxhlet system; nitrogen free extracts was calculated by difference.

Color measurements and chemical determination of carotenoids: During the experiment, trout were sampled four times. The 1st sampling was done at the beginning of the experiment and subsequent samplings were carried out 20, 40 and 60 days. Three fish were randomly sampled from each diet treatment group per sampling period. The fish were filleted and cut anterior to the dorsal and adipose fins. Visual flesh colors assessments were carried out by use of Roche Salmo Fan™ card (Hoffmann-La Roche, Basel, Switzerland) in sampling periods. The scale on the fan ranged from 11-18 where, 10 indicate no visible pigmentation (Foss *et al*., 1984). Panelists were selected to avoid color blindness. After visual analysis; the same samples stored in polyethylene bags and frozen (-30°C) for Colorimetric measurements and carotenoid analysis.

Colorimetric measurements were conducted at the Food Engineering Lab. of the Faculty of Agriculture at Gaziosmanpasa University. Fillet samples were assessed instrumentally using a Tristimulus colorimeter (Minolta Chroma Meter, CR-300 Minolta, Osaka, Japan) which measures light reflection from the flesh of fish in comparison to a standard calibration plate. The measured color parameters were lightness (L*), red-green chromaticity (a*) and yellow-blue chromaticity (b*) as recommended by the International Commission on Illumination. The right side of each fish fillet was used for Minolta Chroma Meter analysis.

The instrument was placed on the flesh and triplicate measurements were taken at the filleted area. The a* value represented the redness and the b* value, the yellowness of the flesh. From the a* and b* values, the Chroma (C_{ab}*) and Hue ($H°_{ab}$) were calculated. The chroma expression of the intensity and clarity of the colour and is expressed by the equation; $C_{ab}^* = (a^{*2}+b^{*2})^{½}$ (Hunt, 1977) whereas the Hue is expressed the relationship between the redness and the yellowness of the fillet and is calculated by the equation; if $a^*>0°$ then $H_{ab}° = \tan^{-1}(b^*/a^*)$ if $a^*<0°$ then $H_{ab}° = 180+\tan^{-1}(b^*/a^*)$ (Hunt, 1977). The Hue is an angular measurement where, 0° indicates a red Hue and 90° indicates a yellow Hue (Nickell and Bromage, 1998; Yesilayer *et al*., 2011).

All samples taken for carotenoid analysis were stored at -30°C until analyzed. The skin and bone of the fish fillets were removed. The removed muscle samples were

homogenized with a homogenizer and samples of approximately 20 g were taken for analysis and then transferred to 200 mL volumetric flasks. The total carotenoid in the flesh of the fish was determined by a method modified after Foss *et al*. (1984) and Skrede and Storebakken (1986a). Muscle samples were extracted three times with acetone (50+50+50 mL). Extracts were centrifuged at 5000 rpm for 3 min and then stored for 24 h at 4°C in a refrigerator. Extracts were measured by spectrophotometer (Jasco-V-530 UV/VIS spectrophotometer) using extinction coefficients (E 1.1% cm) of 1900 for astaxanthin and canthaxantin and extinction coefficients (E 1.1% cm) of 1922 for oleoresin paprika at 474 nm in acetone (Saito and Regier, 1970; Foss *et al*., 1984; Skrede and Storebakken, 1986a). For the total carotenoid analysis of diets with added natural and synthetic coloring materials, the AOAC spectrophotometer analysis method suggested by Akhtar *et al*. (1999) was utilized. To minimize losses that could occur within the extract due to isomerization or auto-oxidation, the absorbance measurements were performed as quickly as possible in a spectrophotometer (Jasco-V-530 UV/VIS spectrophotometer) whose wavelength was adjusted to 460 nm.

The total percentage of carotenoid in the analyzed feed was calculated according to the formula below. In calculating the total carotenoid percentage of oleoresin paprika extract samples, the extinction coefficient of acetone was taken as (E 1.1% cm = 1922) (Lai *et al*., 1996). For astaxanthin and canthaxantin, the value was used as (E 1.1% cm = 1900) (Foss *et al*., 1984; Skrede and Storebakken 1986a). The following equation was used to calculate carotenoid retention rates for each group at the initial and end of the experiment: In the equation flesh weight was found by multiplying the fish weight by the coefficient 0.61 (Wathne *et al*., 1998):

$$\text{Retention rate (\%)} = \frac{100\times 0.61\times\{(W_S\times FC_S)-(W_B\times FC_B)\}}{\{(W_S-W_B)\times FCR\times F_{CC}\}}$$

W_S = Fish weight at the end of trial (g)
W_B = Fish weight at the start of trial (g)
FC_S = Concentration of carotenoid in the flesh at the end of trial (mg kg^{-1})
FC_B = Concentration of carotenoid in the flesh at the start of trial (mg kg^{-1})
FCR = Feed conversion ratio
F_{CC} = Carotenoid concentration in feed (mg kg^{-1})

Statistical analyses of data: The data was presented as mean±Standard Error (SE). The Anderson-Darling-Levene statistic tests were applied to test normality and homogeneity of variance, respectively. ANOVA and the Tukey multiple-range test were used to detect significant differences ($p<0.05$) in final body weight, SGR, FCR, total carotenoid concentration, color parameters and the Roche Salmo Fan™ card score. All statistical analyses were performed using the MINITAB Release 13.1 statistical analysis software program for windows, Version 10.0.1 (Minitab Inc., Chicago, Illinois, USA).

RESULTS

Findings on growth performance: Growth and feed utilization efficiency indicated that there were no significant differences in Weight Gains (WG%), Specific Growth Rates (SGR%), Feed Conversion Ration (FCR) and Survival Rate (SR %) (Table 2). There were no signs of disease during the experiment. The weight gain in fish fed the control diet was higher than in fish fed the experimental diets but the differences were not significant (Table 2).

Visual color scores and color parameters measured in the fillet: The average values of the colour cards (Roche Salmo Fan™) recorded for the fresh fillet of the rainbow trout are shown in Table 3. Roche Salmo Fan™ card scores increased in fish fed with feed containing carotenoid during the experiment. The best visual score results were obtained in the canthaxantin, oleoresin paprika extract and astaxanthin groups, respectively ($p<0.05$). Roche Salmo Fan™ scores ranged from 15-13 in experimental groups. The average values of color parameters recorded for the fillet of the rainbow trout were shown in Table 4. Generally, all the color parameters increased regardless of the carotenoid diet at the end of the 60 days period. While the L* (brightness) value was constantly reduced

Table 2: Weight gains among groups (G), weight gains (WG%) and specific growth rates (SGR%), feed conversion ration (FCR), survival rate (SR%) at the initial of the experiment and the end of the experiment

	Weight (g)						
Group no.	Initial	Final	Increase	WG (%)	SGR (% day^{-1})	FCR	SR (%)
Control	155.23±2.96[a]	320.73±7.74[a]	165.30±7.86[a]	106.47±4.97[a]	1.18±0.04[a]	1.14±0.0[a]	97.33
Oleoresin paprika	154.47±2.72[a]	306.86±7.21[a]	152.35±8.49[a]	98.63±5,48[a]	1.14±0.05[a]	1.14±0.0[a]	98.70
Astaxanthin	153.69±2.70[a]	298.07±6.47[a]	144.27±3.67[a]	93.87±2.49[a]	1.13±0.12[a]	1.14±0.0[a]	98.70
Canthaxantin	154.01±2.76[a]	305.73±7.79[a]	151.72±23.0[a]	98.30±15.0[a]	1.11±0.02[a]	1.21±0.0[a]	98.70

Value (mean±standard error of data for triplicate groups) with different superscript in the same column is significantly different (one way ANOVA and Tukey's multiple-range test, $p<0.05$)

throughout the experiment in all groups fed with natural and synthetic feed with containing carotenoids in comparison with those at the start of the experiment, it remained constant in the control group. At the end of the experiment, the L* value in the fish fillet was found to be the lowest in the group fed with the feed containing canthaxantin compared to the control group as shown in Table 4 ($p>0.05$).

The a* (red pigment) values of groups fed with feed containing canthaxantin, oleoresin paprika extract and astaxanthin were found to be significantly different in comparison to the control group ($p>0.05$). There was a significant increase in the a* values of fish fed with feed containing carotenoid on day 60th.

The best numerical results were obtained in the group canthaxantin, astaxanthin and oleoresin paprika extract, respectively. Maximum a* was achieved after 60 days in the fillet with an increase in a* values observed in fish from canthaxantin supplemented diets.The yellow pigment values (b*) increased in all of the groups fed with feed containing carotenoid and it was determined at the end of the experiment that the differences between these groups and the control group were significant ($p>0.05$). On day 60th, the highest b* values were obtained in fish fed with feed containing oleoresin paprika extract.

The Hue (H°ab) values of the experiment groups on the average after 60 days of feeding were found to be best in the canthaxantin group then respectively in the astaxanthin and oleoresin paprika extract groups. At the end of the experiment, the H°ab values in groups fed with feed containing carotenoid were reduced in comparison to those at the initial of the experiment. The lowest H°ab values were obtained in canthaxantin group. Although, the highest Chroma (C_{ab}*) value was found in the oleoresin paprika extract group, the difference between fed groups containing synthetic astaxanthin and canthaxantin were insignificant but the differences between the carotenoid groups and the control group were significant. C_{ab}* increased throughout the experiment in the groups fed with pigmented feed.

Table 3: Colour card values determined in the fillet of the fish fed with the diets of the experiment groups through visual colour determinations (Roche Salmo Fan™)

	Groups			
Periods (days)	Control	Oleoresin paprika	Astaxanthin	Canthaxantin
0	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}
20	10.05±0.02^{a}	11.37±0.08^{b}	10.98±0.07^{c}	11.82±0.09^{d}
40	10.04±0.02^{a}	13.05±0.07^{b}	12.94±0.09^{b}	13.61±0.07^{c}
60	10.05±0.02^{a}	13.47±0.09^{b}	13.39±0.11^{b}	15.12±0.12^{c}

Superscript letters indicate statistical differences. Mean±standard error of data for triplicate groups with no common letter significantly different (one way ANOVA and Tukey's multiple-range test ($p<0.05$)

Total carotenoid concentration: Initial and final carotenoid concentrations of the fish fillets are shown in Table 5. The Total Carotenoid Concentration (TCC) in the fillets increased significantly ($p<0.05$) for all the diets and significant differences existed between diets with carotenoids at the end of the experiment. The highest TCC was obtained in the group fed, the diet containing canthaxanthin after 60 days and then oleoresin paprika and astaxanthin groups, respectively.

Findings of total carotenoid quantities in fish feed using spectrophotometric methods: The quantities of carotenoids added to experiment feed based on

Table 4: Colour parameters measurements of rainbow trout fillets in the experiment groups during periods

		Fillet			
Colour parameters	Experiment periods (days)	Control	Oleoresin paprika	Astaxanthin	Canthaxantin
Brightness (L*)	0	54.40±1.88^{a}	54.40±1.88^{a}	54.40±1.88^{a}	54.40±1.88
	20	53.10±2.08^{ab}	50.39±1.06^{a}	52.35±0.78^{ab}	49.52±1.28^{a}
	40	54.56±2.58^{a}	50.23±2.86^{a}	49.08±0.88^{a}	46.77±0.90^{a}
	60	55.14±2.17^{a}	48.59±1.44^{ab}	49.25±1.15^{ab}	45.21±1.35^{b}
Red pigments (a*)	0	0.40±0.19^{a}	0.40±0.19^{a}	0.40±0.19^{a}	0.40±0.19^{a}
	20	0.26±0.31^{a}	2.46±0.37^{ab}	3.45±0.47^{bc}	5.56±0.52^{c}
	40	1.25±0.88^{a}	5.81±0.35^{b}	6.97±0.73^{b}	8.24±0.35^{b}
	60	1.02±0.13^{a}	8.41±1.27^{b}	9.35±0.77^{b}	9.55±1.03^{b}
Yellow pigments (b*)	0	11.11±2.05^{a}	11.11±2.05^{a}	11.11±2.05^{a}	11.11±2.05^{a}
	20	15.34±1.33^{a}	11.99±1.10^{a}	15.37±1.27^{a}	18.27±2.02^{a}
	40	11.85±1.23^{a}	19.30±2.62^{b}	14.17±0.96^{ab}	19.71±0.35^{b}
	60	9.79±1.36^{a}	22.14±1.54^{b}	19.40±0.57^{b}	18.18±1.13^{b}
Hue (H°ab)	0	87.35±1.54^{a}	87.35±1.54^{a}	87.35±1.54^{a}	87.35±1.54^{a}
	20	89.09±1.05^{a}	78.53±0.10^{b}	77.33±1.34^{bc}	73.28±0.91^{c}
	40	83.98±4.22^{a}	72.72±2.46^{ab}	63.89±1.20^{b}	67.33±0.58^{b}
	60	83.98±0.57^{a}	69.25±2.46^{b}	64.36±1.14^{bc}	62.43±1.12^{c}
Chroma (C_{ab}*)	0	11.13±2.04^{a}	11.13±2.04^{a}	11.13±2.04^{a}	11.13±2.04^{a}
	20	15.35±1.34^{a}	12.24±1.14^{a}	15.76±1.30^{a}	19.10±2.19^{a}
	40	11.98±1.24^{a}	20.19±2.50^{b}	15.80±1.16^{ab}	21.37±0.44^{b}
	60	9.85±1.36^{a}	23.73±1.70^{b}	21.55±0.85^{b}	20.55±1.47^{b}

Mean±standard error of data in the same row with different superscript letters are significantly different ($p<0.05$)

Table 5: Total carotenoid concentrations determined in the flesh of the fish throughout the experiment using spectrophotometric methods (mg carotenoid kg fish flesh^{-1})

Periods	Groups			
(days)	Control	Oleoresin paprika	Astaxanthin	Canthaxantin
0	0.86±0.01^{a}	0.86±0.01^{a}	0.86±0.01^{a}	0.86±0.01^{a}
20	0.83±0.01^{a}	3.79±0.03^{bc}	3.54±0.18^{b}	4.06±0.02^{c}
40	0.90±0.03^{a}	5.77±0.06^{b}	4.71±0.07^{c}	8.36±0.02^{d}
60	1.08±0.06^{a}	6.19±0.02^{b}	6.11±0.01^{b}	10.07±0.22^{c}

Mean±standard error of data in the same row with different superscript letters are significantly different ($p<0.05$)

Table 6: Added and determined carotenoid quantities in the experiment diets

Groups	Control	Oleoresin paprika	Astaxanthin	Canthaxantin
A. quantity*	0.00	1% (750)	0.06% (50)	0.07% (70)
D. quantity**	0.00	765±0.09	46.63±0.29	76.49±0.13

*Quantity of carotenoid added to the diet (mg kg^{-1}).**Determined carotenoid quantity (mg kg^{-1}) in the diet

Table 7: Carotenoid retention rates in the experiment groups

Groups	Oleoresin paprika	Astaxanthin	Canthaxantin
FCC (mg kg^{-1})	765	46.63	76.49
TCC (mg kg^{-1})	6.19	6.11	10.07
CRR (%)	0.78	12.97	12.49

FCC: Feed Carotenoid Concentration, TCC: Total Carotenoid Concentration in the fillet; CRR: Carotenoid Retention Rate

calculations were determined following the extraction procedure as shown in Table 6. They were found to be higher than calculated in the oleoresin paprika with 765±0.09 mg kg^{-1} and the canthaxantin groups with 76.49±0.13 mg kg^{-1} while lower in the astaxanthin group at 46.63±0.29 mg kg^{-1}.

Retention rates (%): The carotenoid retention rates were calculated in feed groups with natural and synthetic carotenoid additives. The carotenoid retention rates in the groups were determined as shown in Table 7 during the experiment. At the end of the experiment, the retention rates in the oleoresin paprika extract, astaxanthin and canthaxantin groups were determined 0.78, 12.97 and 12.49, respectively. The rate of carotenoid retention was much higher in feed groups with synthetic carotenoid additives than the feed group with natural carotenoid. Especially, the group with high carotenoid containing oleoresin paprika (765 mg kg^{-1}) was found much lower in retention when compared to the other groups. It appears that fish retain synthetic carotenoid sources better than oleoresin paprika. Retention rate of canthaxantin was obtained as high as astaxanthin.

DISCUSSION

The growth of the rainbow trout fed experimental diets was acceptable under Turkey's cultural conditions at the end of the feeding experiment an increase of at least 93% of the initial weight of the fish was achieved (Table 2). Similar to previous studies (Rehulka, 2000), the results also demonstrated that growth was not affected by carotenoid sources in salmonids. Other studies (Vernon-Carter *et al*., 1994; Yanar *et al*., 1997) have suggested that capsantin pungency can increase the segregation of gastric juice, causing an improvement in nutrient assimilation and increased growth.

During the research, the TCC in the fish fillet was determined and coloration was initiated in the fish after a 20 days period with the highest carotenoid concentration at the end of the experiment found in the canthaxantin group (10.07 mg kg^{-1}). The TCC in rainbow trout fillet with oleoresin paprika and asthaxanthin containing diets obtained 6.19 mg kg^{-1} and 6.11 mg kg^{-1}, respectively (Table 5). In the study, the TCC quantity found with the oleoresin paprika was similar to the values found by another study where red chilli oleoresin was added at 5.6 and 6.3 mg kg^{-1} concentration (Ingle *et al*., 2006). In other studies carried out on red pepper, TCC found in the fish fillet ranged from 1.5-5.6 mg kg^{-1}, depending on application time and percentage of red pepper (Akhtar *et al*., 1999; Yanar *et al*., 1997).

Torrissen *et al*. (1989) showed that experimental groups were adequate for a desired coloration in rainbow trout because of a level of 4 mg kg^{-1} in the fish fillet is regarded as a minimum acceptable carotenoid concentration in marketed farmed salmon. The carotenoid composition of salmon flesh is not only due to species, size and gender but also depends on physiological and environmental conditions (Arai *et al*., 1987). In addition, it was reported by Bjerkeng that not only the effect of the carotenoid source but also the quantity of the carotenoid source added to the feed, feeding time and the structure of the feed would be effective in the carotenoid pigmentation.

Due to the inherent variability that occurs when measuring flesh carotenoid concentrations using a color card, the value in comparing results from one study with those of another is limited. In the study, the Roche Salmo Fan^{TM} color card scale 11-18 was used for fillets as specified. A visual score of ≥13 was considered to be an intensity of color suitable for marketable fish (Smith *et al*., 1992). The visual color scores obtained in all the carotenoid additive groups were >13 indicating that the required conditions for marketability were realized (Table 3). Visual score was increased the 1st period (20 days) of feeding. These findings agree with that of Foss *et al*. (1984) and Smith *et al*. (1992) for salmonids. Although, the use of color cards is inexpensive and simple, instrumental assays are preferred due to higher objectivity in differing results because of fluctuating lighting conditions and they are less subjective (Hatlen *et al*., 1998). According to the results

of the analyses, the colour parameters, L*, a*, b* and H°ab and C_{ab}* values were determined (Table 4). The variations of the average values of colour parameters measured in the fillet of rainbow trout can be attributed to three main factors: a non-uniform distribution of carotenoid in the flesh; the use of few discrete measurement points on the fillet surface and the incident beam reflectance (Ingle *et al*., 2006; Skrede and Storebakken, 1986b; Hatlen *et al*., 1998). Parallel to the increase in carotenoid concentration in the trout muscle, while increases in a*, b* and C_{ab}* values were determined as in many other studies that decreases in the L* and H°ab values were also observed (Smith *et al*., 1992; Ingle *et al*., 2006). The a* value is the colour parameter generally showing the best relationship with the increase in the carotenoid level. Because this is the a* value colour parameter that indicates the red colour. As observed since, the beginning of the experiment, the a* value increased in the 1st sampling (Table 4). At the end of the experiment, although the oleoresin paprika was the carotenoid added at the highest rate to the feed (765 mg kg^{-1}), its retention rate was the lowest (0.78%) (Table 6). The retention rate in canthaxantin and astaxanthin groups after 60 days was about 13% for fish fed the synthetic carotenoids (Table 7). The results for retention rate showed that synthetic carotenoids are efficiently utilized by rainbow trout. This result is similar to those reported by Storebakken and Nome (1992) and Ingle *et al*. (2006). Pigment deposition rate in muscle with diets including astaxanthin and canthaxanthin were affected by the dietary carotenoid concentration and the duration of the feeding experiment (Storebakken *et al*., 1986).

Several researchers have proposed reasons for difference in pigment deposition rate: the limited absorption capacity of the intestine; the catabolism process (Foss *et al*., 1984); the absorption by different tissues or the limited capacity of the chemical bonding with the muscle. Astaxanthin is more efficiently absorbed and bio-accumulated in the muscle than canthaxantin (Foss *et al*., 1984) and both pigments are independently absorbed and metabolised in different ways (Torrissen *et al*., 1989). Torrissen *et al*. (1989) reported that salmonids absorb astaxanthin 10-20 times as much as lutein and zeaxanthin.

The absorption of dietary carotenoid is probably a result of a selective process dominated by the carotenoid in a carotenoid blend (e.g., capsanthin in oleoresin paprika) (Ingle *et al*., 2006). Ingle *et al*. (2006) reported that whatever the predominate mechanism in carotenoid absorption by trout, it seems they are metabolised and deposited with different efficiency in the muscle of the rainbow trout. The results in the retention rate of flesh were similar to other researchers who also show that there is an increase in the carotenoid percentage of the feed while the retention rate in the flesh reduces (Storebakken and Choubert, 1991; Akhtar *et al*., 1999). As reported, the absorption, storage and therefore retention of carotenoids in the flesh of salmonid fish are affected by factors such as pigment origin, feed composition, physiological phases of the fish and size of the fish, sexual maturation period and genetic make-up (Kamata and Simpson, 1992). Another researcher has found that rather than feeding with pigmented feed throughout the entire production process, feeding with feed containing carotenoid at the end of the production process increased the accumulation of carotenoids (Torrissen *et al*., 1989). The retention rates are important, because carotenoids increase feed costs by 15-20% and the final product is affected accordingly (Torrissen *et al*., 1995).

CONCLUSION

Another results from this study is that in addition to synthetic carotenoids, the oleoresin paprika, a natural carotenoid may be used for pigmentation of rainbow trout and does not cause any negative effects in the growth of the fish. In addition to its lower price, compared to fish feed containing synthetic carotenoids, oleoresin paprika may be added to the feed in future studies at lower rates and the carotenoid retention rates might be increased even further providing even more positive development for the global salmon industry.

ACKNOWLEDGEMENTS

This study is a part of Nihat Yesilayer's Ph.D. thesis. This study was supported by department of the Scientific Research Project Commission, University of Gaziosmanpasa (Project No: 2005/038). The researchers would like to thank DSM Nutritional products Turkey for providing Carophyll® pink (8%) and Carophyll® red (10%). We would also like to thank Aromeks Ltd. sti., Istanbul, Turkey for providing the oleoresin paprika and Sibal Feed Industry in Sinop for providing the raw materials for the feed.

REFERENCES

AOAC, 1984. Official Methods for Analysis. 14th Edn., Association of Official Analytical Chemists, Arlington, VA., USA.

Akhtar, P., J.I. Gray, T.H. Cooper, D.L. Garling and A.M. Booren, 1999. Dietary pigmentation and deposition of tocopherol and carotenoids in rainbow trout muscle and liver tissue. J. Food Sci., 64: 234-239.

Arai, S., T. Mori, W. Miki, K.Yamaguchi, S. Konosu, M. Satake and T. Fujita, 1987. Pigmentation of juvenile Coho Salmon with carotenoid oil extracted from Antarctic krill. Aquaculture, 66: 255-264.

Choubert, G. and T. Storebakken, 1989. Dose response to astaxanthin and canthaxanthin pigmentation of rainbow trout fed various dietary carotenoid concentrations. Aquaculture, 81: 69-77.

Choubert, G., 1985. Effects on starvation and feeding on canthaxanthin depletion in the muscle of rainbow trout *Salmo gairdneri* (R.). Aquaculture, 46: 293-298.

FAO, 2007. FAO fisheries and aquaculture statistical database. FAO, USA.

Foss, P., T. Storebakken, K. Schiedt, S. Liaaen-Jensen, E. Austreng and K. S treiff, 1984. Carotenoids in diets for s almonids. I. Pigmentation of rainbow trout with the individual optical isomers of astaxanthin in comparison with canthaxanthin. Aquaculture, 41: 213-226.

Hatlen, B., M. Jobling and B. Bjerkeng, 1998. Relationship between carotenoids concentrations and colour of fillets of Arctic charr, *Salvelinus alpinus* (L.), fed astaxanthin. Aquacult. Res., 29: 191-202.

Hirayama, O., K. Nakamura, S. Hamada and Y. Kobayasi, 1994. Singlet oxygen quenching ability of naturally occuring carotenoid. Lipids, 29: 149-150.

Hunt, R.W.G., 1977. The specification of colour appearance. 1. Concepts and terms. Colour Res. Applic., 2: 55-68.

Ingle, M.G., I.G.J.L.A. Figueroa, J.T.P. Polofox, I.D.A.B. Soca and J.E.V. Carter, 2006. Comparison of red chili (*Capsicum annuum*) oleoresin and astaxanthin on rainbow trout (*Oncorhynchus mykiss*) fillet pigmentation. Aquaculture, 258: 487-495.

Kamata, T. and K.L. Simpson, 1992. A study of astaxanthin its application for the pigmentation of salmonid fish. Bull. Kagoshima Pref. College, 43: 11-39.

Lai, S.M., J.I. Gray, C.J. Flegal and T. Cooper, 1996. Deposition of carotenoids in eggs from hens fed diets containing saponified and unsaponified *O. paprika*. J. Sci. Food Agric., 72: 166-170.

Nickell, D.C. and N.R. Bromage, 1998. The effect of dietary lipid level on variation of flesh pigmentation in rainbow trout. Aquaculture, 161: 237-251.

Rehulka, J., 2000. Influence of astaxanthin on growth rate condition amd some blood indices of rainbow trout *Oncorhynchus mykiss*. Aquaculture, 190: 27-47.

Saito, A. and L.W. Regier, 1970. Determination of total carotenoid pigments in trout and salmon flesh. J. Fish. Res. Board Canada, 36: 1-3.

Skrede, G. and T. Storebakken, 1986a. Characteristics of color in raw, baked and smoked wild and pen-reared *Atlantic salmon*. J. Food Sci., 51: 804-808.

Skrede, G. and T. Storebakken, 1986b. Instrumental colour analysis of farmed and wild atlantic salmon, when raw, baked and smoked. Aquaculture, 53: 279-286.

Smith, B.E., R.W. Hardy and O.J. Torrissen, 1992. Synthetic astaxanthin deposition in pan-size coho salmon (*Oncorhynchus kisutch*). Aquaculture, 104: 105-119.

Storebakken, T. and G. Choubert, 1991. Flesh pigmentation of rainbow trout fed astaxanthin or canthaxanthin at different feeding rates in freshwater and saltwater. Aquaculture, 95: 289-295.

Storebakken, T. and H.K. Nome, 1992. Pigmentation of rainbow trout. Aquaculture, 100: 209-229.

Storebakken, T., P. Foss, I. Huse, A. Wandsvik and T.B. Lea, 1986. Carotenoid in diets for salmonids. III. Utilization of canthaxanthin from dry and wet diets by *Atlantic salmon*, rainbow trout and sea trout. Aquaculture, 51: 245-255.

Torrissen, O.J., R. Christiansen, G. Struksnaes and R. Estermann, 1995. Astaxanthin deposition in the flesh of atlantic salmon (*Salmo salar* L.) in relation to dietary astaxanthin concentration and feeding period. Aquacul. Nutr., 1: 77-84.

Torrissen, O.J., R.W. Hardy and K.D. Shearer, 1989. Pigmentation of salmonids-carotenoid deposition and metabolism. CRC Crit. Rev. Aquat. Sci., 1: 209-225.

Vernon-Carter, E.J., J. Ponce-Palafox and R. Pedroza-Islas, 1994. Bioensayo de Pigmentación de Trucha Arcoiris (*Oncorhynchus mykiss*) con extractos de Chile Ancho (*Capsicum annuum*). Arch. Latinoam. Nutr., 44: 252-255.

Vernon-Carter, E.J., J.T. Ponce-Palafox and R. Pedroza-Islas, 1996. Pigmentation of Pacific white shrimp (*Penaeus vannamei*) using Aztec marigold (*Tagetes erecta*) extracts as carotenoid source. Arch. Latinoam. Nutri., 46: 243-246.

Wathne, E., B. Bjerkeng, T. Storebakken, V. Vassvik and A.B. Odland, 1998. Pigmentation of *Atlantic salmon* (Salmo salar) fed astaxanthin in all meals or in alternating meals. Aquaculture, 159: 217-231.

Yanar, M., M. Kumlu, M. Celik, Y. Yanar and N. Tekelioglu, 1997. Pigmentation of rainbow trout (*Oncorhynchus mykiss*) with carotenoids from red pepper. Isr. J. Aquacul. Bamidgeh, 49: 193-198.

Yesilayer, N., O. Aral, Z. Karsli, M. Oz, A. Karacuha and F. Yagci, 2011. The effects of different carotenoid sources on skin pigmentation of Goldfish (*Carassius auratus*). The Israeli J. Aquacult. Bamidgeh, 63: 523-532.

Yigit, M., O. Yardim and S. Koshio, 2002. The protein sparing effects of high lipid levels in diets for rainbow trout (*Oncorhynchus mykiss*, W. 1792) with special reference to reduction of total nitrogen excretion. Isr. J. Aquac. Bamidgeh, 54: 79-88.