EFFECTS OF THE ß-CAROTENE ON THE GROWTH PERFORMANCE AND SKIN PIGMENTATION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, W. 1792)

Gülüzar TUNA KELEŞTEMUR* , **Özlem EMİR ÇOBAN**

Fisheries Faculty, Firat University, Department of Aquaculture, Elazig, Turkey

*gkelestemur@hotmail.com

Aim of This Study

Skin color is important factor in aquaculture influencing the commercial value of fish, mostly in those species sold live or fresh. Most studies of fish skin color in aquaculture have focused particularly on the effects of diet. Synthetic carotenoids usage are increasing importance for the pigmentation of fish farmed . Animals are able to absorb carotenoids from their diet, and deposit them in the unesterified from. They are absorbed intake by the mucosal cells and appear unchanged in the circulation and tissues. Carotenoids are absorbed differentially by different tissues. Little is known about the mechanisms of tissue absorption of carotenoids at this time. The major site of tissue storage of carotenoids is the adipose tissue. However, the pigmentation of rainbow trout change throughout life. Generally, fingerlings have limited capacity for carotenoid deposition in the flesh, while significant amounts are deposited in the skin. Beta carotene one of the carotenoids responsible for the orange and red pigmentation of fish. Aquatic animals cannot synthesize carotene and therefore it must be supplemented in the diet. As well as being a pigment, beta-carotene has been shown to have other biological and nutritional functions essential for fish growth and health. This study was therefore undertaken to find out if synthetic beta carotene at two different dietary concentrations would affect survival, growth, and skin pigmentation of juvenile rainbow trout.

Materials and Methods

- *Experimental Disayn*

This trial was conducted in Keban Dam Lake (Elazig, Keban) third hunting ground in special facility.

Keban Dam Lake

This lake is the biggest artificial lake of Turkey. The activities of fishing and fish production are executed in the Keban Dam Lake. There are numerous promenade places and fish houses at the shores of the Keban Dam Lake where the public can rest and enjoy.


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Three cages were used in the research. Each cage was stocked 300 rainbow trout (*Oncorhynchus mykiss*). Fish (initial average weight, 60.3±0.27 g; initial length, 17.24±0.04 cm were distributed into three round cage (mesh size: 1.8 mm, bag depth: 4 m, diameter: 9 m). Cages were placed approximately away from 100 m from shore.

Composition of the experimental diets are shown in Table 1. Experimental diets supplemented with 30 and 70 mg / kg beta-carotene (respectively; β30, β70), and basal diet (C) (not supplemented diet of beta carotene) were prepared.

Table 1. Composition and proximate analysis of the experimental diets

Ingredient	Percent of dry weigth
Fish (anchovy) meal	50
Soybean meal	23.1
Wheat flour	19.8
Sunflower oil	6
Antibiotic[a]	0,10
Vitamin premix[b]	0.90
Mineral premix[c]	0.10
Proximate composition	
Crude protein	44.55
Crude fat	8.46
Crude fibre	4.12
Crude ash	13.43

[a]Antioxidant (mg/kg dry diet): Butilen Hydroxytoluene (BHT); 125.000 mg/ kg

[b]Vitamin premix (IU or mg/kg dry diet): Menadion 3.000, Riboflavin 6.000, Pridoxin 5.000, Cobalamin 15, Niasin 25.000, Biotin 40, Folic asid 1.000, Colin Chloride 300, Calcium, D-pantothenat 8.000, Calsiferol 2.000.000, Vitamin A 1000, Vitamin E 1.000.000 IU. Ascorbic Asid, 150.000

[c]Mineral premix (mg/kg dry diet): Mn 80.000, Fe 35.000, Zn 50.000, Cu 5.000, I 2.000, Co 400, Se 150.

- All experimental fish were acclimated to the basal diet for two week prior to start of the trial. The experimental fish were fed three times a day. Daily feed allowance was 3% body weight per day. The feeding trial was conducted for 12 weeks. Before the fish were anesthesia (Quinaldin), these body weights were measured one every 2 weeks. Growth and survival performances were estimated by the following formulas:
- Weigth gain (WG): (final w_t - initial w_t)
- Specific growth rate (SGR) (%): [($\log_e$ final w_t - $\log_e$ initial w_t) / duration in days] x 100
- Feed conservation ratio (FCR) : duration in days consumed feed / (final w_t - initial w_t)
- Protein efficiency ratio (PER) : (final w_t - initial w_t) / feed consumption (g) × feed in the diet
- Survival Rate (SUR): 100 x Final fish number /Initial fish number
- ($w_{t:}$ Weigth)

Carotenoid Determination

A 1.5-2 g sample (diet and tissue) was homogenized in the presence of Na_2SO_4 and extracted with acetone. The exctract was filtred with chloroform into a round-bottomed flask and evaporeted to dryness with a rotary evaporator at bath temparatures between 30-35 ℃. The residue was saponified with 1ml 50% KOH and 10 ml methanol in a flask that fillet with nitrogen before being left for 2 h in the dark. Thereafter, the mixture was transferred to a seperating funnel with 40 ml each of ether and distilled water and extracted twices. The pooled ether extract were filtered through Na_2SO_4 to remove residual moisture, and evaporated to dryness under vacum. The residue was dissolve in hexan (Amara et al., 2004; Metusalach, 1997). Jast after the pruduction of solution was determined in 472 nm. Carotenoid concantration was calculated according to this formula:

C = Absorbance ×10000/2100

where C is concentration (ug/g for tissue) 2100 is E (1%, 1 cm)= the extinction coefficent of the carotenoids in hexan at 472 nm; 10000 is the scale factor.

- *Statistical methods*

Differences between group means were assessed by a one-way analysis of variance (ANOVA) and post-hoc Duncan test used by SPSS/PC computer program (SPSS, 1999). Results with $P<0.05$ were considered statistically significant.

Results

WG, SGR, FCR, PER and SUR values of rainbow trout at the end of the experiment are presented in Table 2. The highest WG, SGR and SUR were obtained in the β-carotene supplemented groups ($p<0.05$), while the lowest WG, SGR and SUR were obtained in the C group ($p<0.05$). The FCR improved in β-carotene supplemented diet groups compared to without supplemented control diet group. On the other hand, PER values were not different among all diets groups. Additionally, crude protein values were found significantly higher in the β-carotene supplemented diet groups than C groups ($p<0.05$). However, crude lipid and ash were not statistically different among the groups ($p>0.05$) (Table 2.)

Table 2: Experimental groups of WG, SGR (specific growth rate), FCR (feed conversation ratio), PER (protein efficiency ratio), SUR (live rate) values and body composition of fish.

Growth Parameters	C	β_{30}	β_{70}
WG	23.82±0.24 [b]	37.29±0.45 [a]	39.13±0.19 [a]
SGR	1.43±0.04[b]	1.76± 0.02[a]	1.79±0.02[a]
FCR	3.87±0.24[a]	2.12±0.11[b]	2.17±0.21[b]
PER	1.56±0.09	1.60± 0.13	1.59±0.15
SUR	82.72 [b]	90.15[a]	92.34[a]
Body Composition (%)			
Crude protein	47.46 [b]	55.67[a]	54.83[a]
Crude lipid	9.32	10.45	10.67
Crude Ash	25.67	27.34	26.26

[a-b] Means in the same column with different supscript are significantly (ANOVA, $P<0.05$).

Skin Carotenoid Concentration

Skin carotene concentrations lateral and tail region of fish fed with the diets are presented in Table 3.

The results of one-way ANOVA test showed that carotene concentration of fish skin were positively affected by dietary supplementation of beta carotene. β-carotene levels in lateral region of fish were found to be significantly higher in the β_{70} group than other groups ($p<0.01$). The lowest carotenoid concentrations in the lateral and tail regions was obtained in the C group.

Skin Carotenoid Concentration

Table 3. Carotenoid concentrations of lateral and tail regions of the experimental groups determined with the spectrophotometric method (ug/g)

Skin Carotene Concentrations (ug/g)	C	β_{30}	β_{70}	P
Tail Region	0.009 ± 0.0038^{c}	0.055 ± 0.009^{b}	0.106 ± 0.015^{a}	**
Lateral Region	0.223 ± 0.078^{c}	0.476 ± 0.122^{b}	0.843 ± 0.113^{a}	*

$^{a-c}$ Means in the same column with different supscript are significantly. (ANOVA, *: $P<0.05$, **$P<0.01$)

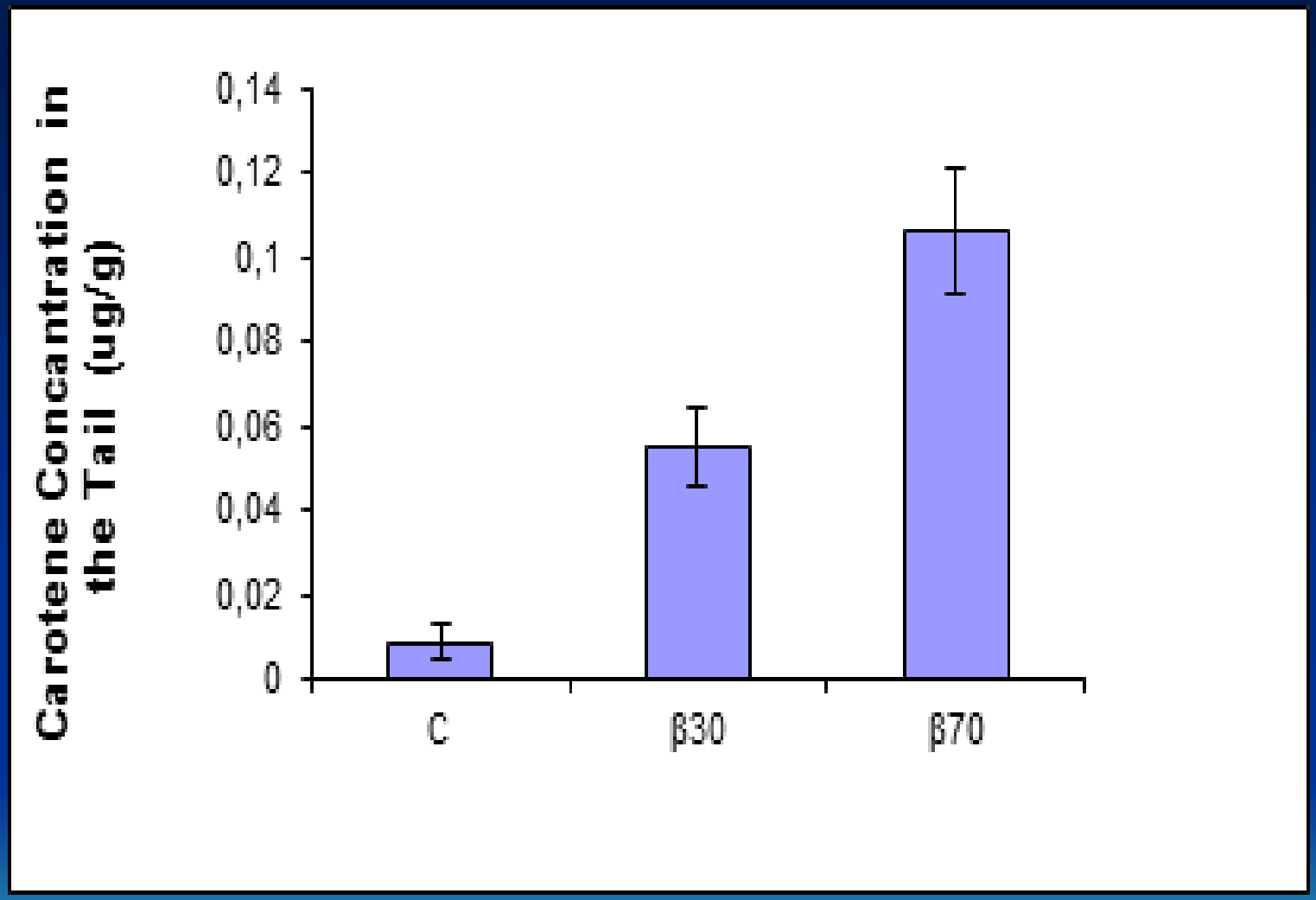

Fig. 1. Carotenoid concentraions of tail region of the control, β_{30} and β_{70} groups

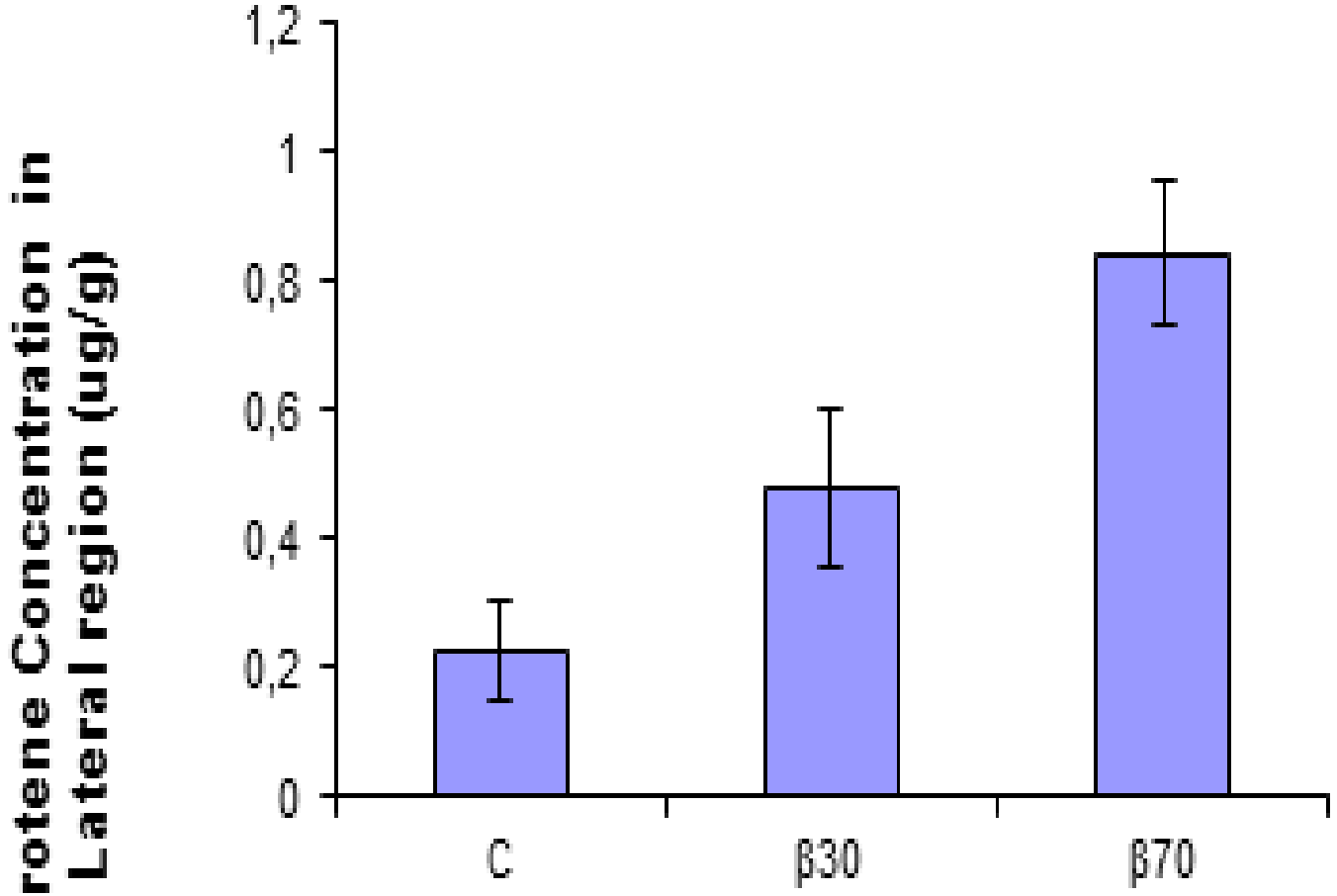

Fig. 2. Carotenoid concentrations of lateral ragion of the control, β_{30} and β_{70} groups

Discussion and Conclusion

For salmonids and trouts, increasing worldwide production and pricing pressures have focused attention on flesh quality issues to satisfy market preferences. Although there is no simple definition of flesh quality factors, of particular importance are the nutritional value, safety, flavour, colour, preservation and processing characteristics of the fillets (Jhonston *et al.*, 2000). Carotenoids, particularly those that are vitamin A precursors, have received increasing attention in recent years due to their reported health benefits. Already, the effects of carotenoids on aquatic animals are multi-faceted: they enhance larval growth and survival, improve the performance of broodstock and nauplii quality, as well as increase resistance to diseases (Amara *et al.,* 2004). Carotenoids also have excellent antioxidative characteristics. Cold-water fishes, like salmon, have a high level of polyunsaturated fat in their membranes, and protection of lipid tissue from peroxidation seems to be a metabolic function (Bell *et al.,* 2000).

Metusalach et al. *(*1997) and Torrissen (1989) found that asta and canta supplementation in diet had a growth promoting effect in Atlantic salmon fry. Hu et. al. 2006, growth of fish fed highest level (200 mg) of β-carotene reduced to similar WG as those fed diet supplemented with 15 mg β-carotene migth be harmful to tilapia. High beta-carotene thus migth build up relatively high amount of oxidized producs in animal body. Hu et al. (2006), reported that beta-carotene needed for normal growth of tilapia was 26.6-44.3 mg/kg. In our study, it was observed that growth performance of fish were positively affected by dietary supplementation of beta carotene. These results indicated that beta carotene supplementation in diet has the growth stimulating action for juvenile rainbow trout. But, in contrast another previous experiments reported that various sources of did not affect growth and survival of various fish species (Wang *et al.,* 2006; Page & Davis, 2002). Exting differences in water temparature, feeding regime, diet formulation and size of fish migth be responsible for the observed differences.

Conclusion

- In this study, β-carotene dietary supplementations increased regional coloration in juvenile rainbow trout. Similarly, previous experiments reported that there was increased in the concantration of carotenoids in fish flesh as the duration of feeding of fish on pigmented diets was increased (Merhabi *et al.,* 2010).
- In conclusion, using β-carotene supplementation in juvenile rainbow trout diets improved growth performance and skin carotene concentration positively affected. However, 30 mg/kg β-carotene supplementation in juvenile rainbow trout diet was determined to be sufficent on growth performance. In addition it was determined that 70 mg/kg β-carotene supplementation was more effective on skin carotene concentration.