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Caspian Sea gammarus (*Pontogammarus maeoticus*) as a carotenoid source for muscle pigmentation of rainbow trout (*Oncorhynchus mykiss*)

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Abstracts: The red coloration of rainbow trout muscle is an important quality criterion. Duplicate groups of rainbow trout (*Oncorhynchus mykiss*; initial weight of 200±10g) were fed diets either supplemented 0 (control), 0.5, 2, or 4%, of the Caspian Sea gammarus (*Pontogammarus maeoticus*) respectively as a prospective alternative carotenoid source. The feeding trial lasted for 39 days. No significant differences ($P>0.05$) in specific growth rate (SGR), or condition factor (CF) were found between treatments. Carotenoid concentrations were determined in the dorsal region and the Norwegian Quality Cut (NQC). The correlation between mean total carotenoid content of the Norwegian quality cut per treatment and dietary inclusion level of *P. maeoticus* was high ($R^2=0.99$; $P<0.01$). Less correlation was found

between total carotenoid content of the dorsal cut and inclusion level of *P. maeoticus* (R^2=0.80 ; P=0.10). The total carotenoid concentrations were significantly higher (P<0.05) in male (4.26±0.39 mg/kg) than in female (3.38±0.23 mg/kg) rainbow trout. In conclusion, it was found that dietary supplementation of the Caspian Sea gammarus at levels of 2 and 4% effectively improved muscle pigmentation of rainbow trout.

Keywords: Rainbow trout, Pigmentation, Gammarus, Caspian Sea, Iran

Introduction

Consumers have preferences for red-coloured products of salmonid fishes in many countries (Ostrander *et al.*, 1976 ; Hatano *et al.*, 1987 ; Gormely, 1992 ; Sigurisladottir *et al.*, 1994 ; Skonberg, *et al.*, 1998), and pigment feeding is considered a major success criterion for marketing of those fishes (Moe, 1990). Salmonid fishes are incapable of either *de novo* synthesis of carotenoids or conversion of canthaxanthin into astaxanthin, and therefore rely on the diet to acquire carotenoid pigments (Torrissen *et al.*, 1989). The red flesh color is primarily caused by ingestion of the ketocarotenoids astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) and/or canthaxanthin (β,β-carotene-4,4′-dione) that are manufactured predominantly by chemical synthesis. Supplementing diets with carotenoid pigments, therefore, is standard practice in the aquaculture industry.

Addition of carotenoids to animal diets is expensive, and usually less than 20% of the dietary carotenoids contribute to flesh pigmentation in salmonid fishes (Torrissen *et al.*, ; Storebakken & No, 1992). Investigations on alternative astaxanthin sources have mainly focused on crustaceans and microbial sources (Shahidi & Metusalach, 1998). Certain sources may be of special interest for farmers in various countries due to their local abundance. Thus, the red crab langostilla (*Pleuroncodes planipes*), abundant in the southwest Baja California (Mexico), has been shown to provide adequate pigmentation to rainbow trout (Spinelli & Mahnken, 1978 ; Coral *et al.*, 1998). Ensilage was found to be a realistic way of preserving by-products from processing of shrimp (*Pandalus borealis*) for pigmentation of rainbow trout in Norway (Torrissen *et al.*, 1981-1982). The Caspian Sea gammarus (*Pontogammarus maeoticus*) is the dominant

species of gammaridae in the Caspian Sea (Mirzajani, 1994). Since this species is abundant at the southen coasts of the Caspian Sea, it may provide a valuable alternative to synthetic carotenoids in this region. The aim of this study was to evaluate the utilization of the Caspian Sea gammarus for muscle pigmentation of rainbow trout.

Material and Methods

Diets and feeding

The experiment was carried out in triplicate in rectangular concrete tanks (12m ×1m × 0.6 m), for 39 days, at Jajroud Trout Farming Company, Tehran, Iran. Each tank contained 30 rainbow trout with an initial weight of 200±10g. The composition of the control diet without supplemented gammarus is presented in Table 1 and 2. Three pelleted experimental feeds were supplemented with 0.5%, 2% and 4% gammarus, respectively, and compared to a control diet without supplemented carotenoids. Diets were supplied by the Research Center for Natural Resources and Animals of Jihad, Khojir, Tehran, Iran. The pellets were 3mm in diameter, and were stored in a cool dry place (~ 20°C), until fed to the fish. The fish were hand fed twice, daily, according to a feeding table based on water temperature and body weight. The average water temperature was 15±0.5°C, the water contained 7±0.5mg/l dissolved oxygen at the inlet and the average water pH was 7.5. On days 15, 30, and 39 the biomass of each tank were measured and the average weight of fish was calculated. Five individuals from each tank were chosen at random for biometry. Four fish were sampled from each tank for carotenoid analysis. Specific growth rate (SGR) and condition factor (CF) were calculated according to the following fromulae:

$SGR = 100 \times (\ln (Wt_2) - \ln (Wt_1)) / \text{Days of feeding}$

where Wt_2 and Wt_1 are the final and initial mean body weights of the fish respectively.

$CF = (\text{Weight (g)}) / (\text{Length (cm)})^3$

Table 1: Formulation of the base diet

Ingredients	g/kg
Fish meal	360.0
Soybean meal	196.3
Wheat	170.0
Corn	118.7
Gelatin	10.0
Fish oil	30.0
Soybean oil	40.0
Wheat bran	50.0
Lysine	0.5
DCP	9.5
Vitamin and Mineral premix*	15

* Supplied per kg of feed: 25000 IU Vit. A; 2000 IU Vit D_3 ; 100 IU Vit E; 150mg Vit C; 15 mg Vit K; 20mg thiamine; 30 mg Vit.B_2 ; 10 mg panthothenic acid; 20mg pyridoxin; 210 mg inositol; 2000mg choline; 0.05mg Vit B_{12} ; 220mg niacin; 0.5mg biotin; 5.0mg acid folic; 70mg Zn; 60mg Mg; 60mg Mn; 4.0mg Fe; 1.5mg I; 20mg Cu; 0.5mg C0; 0.05mg Se.

Table 2 : Proximate composition of the base diet

Composition	(g/kg DM)
Dry matter (DM)	900
Crude protein (N×6.25, g)	380
Crude fat (g)	106
Calcium (g)	16
Phosphorus (g)	11
Digestible energy (MJ/kg)	16.1
Crude fiber (g)	22

Sampling and carotenoid analyses

Four fish from each tank (12 fish per treatment) were used for carotenoid measurements at the Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran. Each of the four fishes were placed in a polyethylene bag and sent to IBB, and stored at –18°C until carotenoid analysis. The frozen fish were thawed overnight, cleaned, washed and the head and entrails removed. The NQC and dorsal regions were cut. The NQC region was a complete cut of the fillet from the dorsal to the ventral surface, posterior to the dorsal fin, but anterior of the adipose fin and consisted of epaxial and hypaxial muscle (Norwegian Standard Association, 1994). Each flesh sample was minced to a paste, and duplicate aliquotes (0.3g) were weighed into laboratory tubes along with anhydrous magnesium sulfate (0.1g) and acetone (3ml). The tubes were sealed and covered with an aluminium sheet, stored in the dark in a refrigerator (4°C) for two days to allow acetone to penetrate into the flesh and to extract the carotenoids. The mixture was homogenized, and centrifuged at 4000g for 4 min (Yanar *et al.*, 1997). An aliquot (1ml) of the supernatant was directly subjected to spectrophotometric determination of total carotenoids at 450nm, in a spectrophotometer (Lambda-2, Perkin-Elmer). An extinction coefficient of $E_{1\%,\ 1cm}$=1900, was chosen to calculate the total carotenoid concentration.

Statistical analyses

Data on specific growth rate (SGR), condition factor (CF) and carotenoid levels, were subjected to analysis of variance (ANOVA), using GLM procedure, non parametric Kruskal-Wallis test, and regression analysis for total carotenoid concentration to obtain response surface. Significant differences were ranked with LSD test (Least significant difference) at the 5% level of significance, using Minitab Statistical Package, version 13.1 (Minitab Inc., PA, USA). Paired Student's t-test was used to compare the total carotenoid concentrations in the NQC and dorsal regions. To reveal the effects of inclusion level of the Caspian Sea gammarus on the mean concentration of carotenoids in the NQC and dorsal cuts regression, analyses of the data were performed using the SAS Software Release

8.2 for Windows (SAS Institute Inc., Cary, NC, USA). Significance was indicated when $P<0.05$.

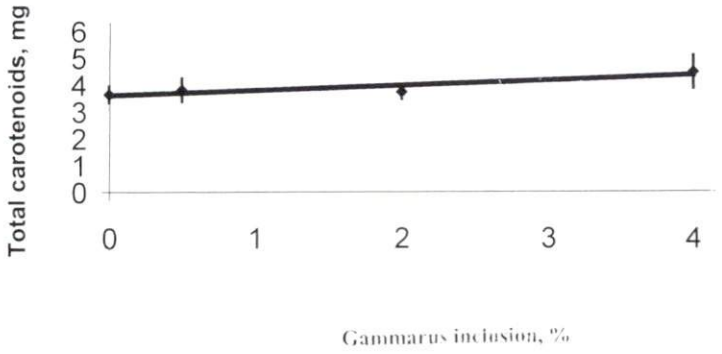
Results

The specific growth rate (SGR), and condition factor (CF) were similar for all treatments ($P>0.05$), (Table 3). The total carotenoid concentrations ranged from 3.54±0.30 to 4.37±0.62 mg/kg in the dorsal flesh samples, and from 2.43±0.36 to 5.75±1.20mg/kg in the Norwegian Quality Cut (NQC) samples for diets supplemented with 0 to 4% of *P. maeoticus*. The coefficients of determination (R^2) between total carotenoid content of the Norwegian Quality Cut and dorsal cut, and inclusion level of *P. maeoticus* was 0.99 ($P<0.01$) and 0.80 ($P=0.10$), respectively (Fig. 1). The total carotenoid concentrations in the Norwegian Quality Cut were significantly higher than ($P<0.05$) in the dorsal region in the group fed the diet supplemented with 0.5% *P. maeoticus*, but in other groups no significant difference were found. Carotenoid deposition in the muscles of male fish (4.26±0.39mg/kg) was significantly higher than ($P<0.05$) that of female fish (3.38±0.23mg/kg).

Table 3: Specific growth rate (% /day) and condition factor (g/cm^3), for rainbow trout fed diets supplemented with 0, 0.5, 2 or 4% of the Caspian Sea gammarus as carotenoid source; (Mean ± SE, n=3 tanks)

Gammarus inclusion level, %	0	0.5	2	4
SGR	0.43± 0.09	0.34 ± 0.05	0.41 ± 0.04	0.24 ± 0.05
CF	1.32± 0.04	1.17 ± 0.04	1.32 ± 0.07	1.22 ± 0.05

a



b

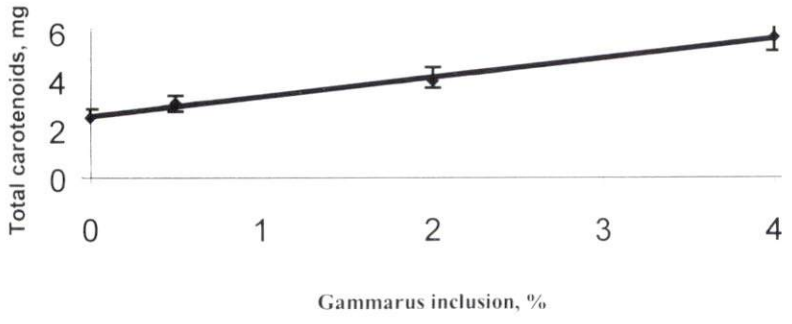


Figure 1:Deposition of total carotenoids in: (a) dorsal muscle sample, and (b) NQC sample of rainbow trout fed diets with increasing levels of the Caspian Sea gammarus (0-4%). a: Regression line for dorsal sample, b: Total carotenoid (mg/kg) = 0.188 (inclusion level, %) + 3.51, R^2 = 0.8018, P=0.1 ; Regression line for NQC sample: Total carotenoid (mg/kg) = 0.800 (inclusion level, %) + 2.49, R^2 = 0.9907.

Discussion

Various carotenoid sources have been tested for efficiency of muscle pigmentation of rainbow trout, including astaxanthin and canthaxanthin (Foss *et al.*, 1984 ; Storebakken *et al.*, 1985 ; Choubert & Storebakken, 1989 ; Torrissen, 1989 ; Bjerkeng, 1990 ; Nickell & Bromage, 1998), shrimp meal (Choubert & Luquet, 1983), red pepper (Yanar *et al.*, 1997), red crab (Spinelli & Mahnken, 1978 ; Coral *et al.*, 1998), krill meal (Kotik *et al.*, 1979), crustacean processing wastes (Ibrahim *et al.*, 1984). However, red pepper (Carter *et al.*, 1994) containing high levels of capsanthin and capsorubin, and various plant sources such as spirulina and marigold petals (Boonyaratpalin & Unprasert, 1989) were reported to be effective in the pigmentation of rainbow trout, but these carotenoids are not part of the natural diet of salmonid fishes. The carotenoid concentration in Gammaridae is about 20% higher than that in Daphnia or Chironomidae. On the other hand, digestibility of carotenoids from Gammaridae is three times higher than that for Chironomidae (Choubert & de la Noüe, 1987). Choubert and de la Noüe reported that a feed supplemented with 30% of gammarus can be used for feeding rainbow trout. In a study by Mathias *et al.*, (1982) rainbow trout fed with either commercial feed or feed supplemented with *Gammarus lacustris* had similar weight gains as that of control fish. In the present study a significant difference ($P<0.01$) was found in total flesh carotenoid concentrations in fish fed with diet supplemented with 4% gammarus, compared to fish fed with the control diet. This finding is in agreement with that of Choubert and Luquet, 1983. The response to higher dietary inclusion level of the Caspian Sea gammarus was higher in the NQC than that of the dorsal cut, therefore, NQC cut is more suitable to monitor reponse in carotenoid deposition in the muscle. The Caspian Sea gammarus is the dominant amphipoda species in the area and is abundant in the south coast of Caspian Sea, Iran. The present study has demonstrated that the Caspian Sea gammarus can be utilized efficiently for pigmentation of rainbow trout flesh, and may provide a valuable source of carotenoids in salmonid fish farming in Iran. Feeds containing 2% or 4% of gammarus can be used in this regard for about 40 days depending on water

temperature, other important environmental factors, and desired level of flesh pigmentation.

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