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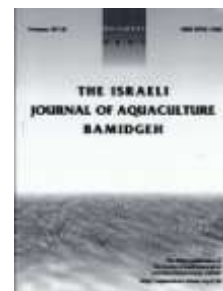
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## Effect of Citric Acid on Growth and Red Coloration of Red Porgy (*Pagrus pagrus*)

Stavros Chatzifotis\* and Lara Gomez Martinez

*Institute of Marine Biology, Biotechnology and Aquaculture (IMBBA),  
Hellenic Center for Marine Research (HCMR) Thalassocosmos, Gournes  
Pediados, 715 00 Heraklion, Crete, Greece*

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### Abstract

This study investigates the effect of dietary citric acid supplementation on the growth and red coloration of red porgy (*Pagrus pagrus*). Red porgy (~0.5 g) were fed one of four diets: (a) an unsupplemented control, (b) a diet containing astaxanthin at 0.06 g/kg, (c) a diet containing citric acid at 30 g/kg, or (d) a diet containing both astaxanthin at 0.06 g/kg and citric acid at 30 g/kg. After two months, fish reached ~6 g with no differences in growth between groups. Astaxanthin increased the total carotenoid concentration in the skin from 3.16 to 45.15 µg/g in fish fed the astaxanthin-supplemented diet and from 2.61 to 50.07 µg/g in fish fed the diet supplemented by both astaxanthin and citric acid, producing a distinctive red skin color. Diet composition did not affect the melanin concentration of the skin, which ranged 57.60-68.18 µg/g and did not differ between groups. Dietary supplementation of citric acid did not affect growth or coloration in red porgy, thus alternative means of administering citric acid should be sought, for example, adding citric acid to the rearing water or injecting it into the fish body.

\* Corresponding author. Mailing address: HCMR, P.O. Box 2214, 71003 Heraklion, Greece, e-mail: [stavros@hcmr.gr](mailto:stavros@hcmr.gr)

## Introduction

Organic acids are used in animal nutrition as feed preservatives and for lowering stomach pH and thereby improving protein digestibility by stimulating the conversion of inactive pepsinogen to active pepsin (Luckstad and Mellor, 2011). In addition, organic acids act as chelating agents for minerals, improving their digestibility (Ravindran and Kornegay, 1993). In this context, the application of citric acid in aquafeeds has been evaluated in a number of species. In rainbow trout *Oncorhynchus mykiss*, dietary citric acid increased the apparent availability of calcium, phosphorus, magnesium, iron manganese, and strontium due to acidification that solubilized the bone minerals in fishmeal (Sugiura et al., 1998). In red sea bream *Pagrus major*, citric acid reduced the use of inorganic phosphorus in feeds (Alam Sarker et al., 2005; Hossain et al., 2007). In *Tilapia zillii*, citric acid supplementation of a sub-optimal diet increased feed consumption (Adams et al., 1988). On the other hand, citric acid cannot be used as a substitute for protein in rainbow trout diets (Fauconneau, 1988).

Apart from aquafeeds, citric acid is used to prevent browning in foods as it inhibits tyrosinase by lowering pH and chelating copper at effective sites of the enzyme (Richardson and Hyslop, 1985; Langdon, 1987; Iyengar and McEvily, 1992; Pizzocaro et al., 1993). This action may have an implication in the culture of red porgy which, when grown in cages, develop a darker coloration than their wild counterparts due to hypermelanosis (Pavlidis et al., 2011).

Reduction of skin melanin content by inhibiting melanin synthesis is a challenge to the successful culture of red porgy. A wide range of environmental factors (light intensity and spectrum, background color, water temperature) can be manipulated to control skin melanin concentration and melanophore proliferation. Rearing in shaded or submersible cages (Papandroulakis et al., 2008) and avoiding extreme water temperatures (Adachi et al. 2006) ameliorate the problem and improve the skin color of cultured red porgy. In the present investigation, we took a nutritional approach: citric acid was included in the feed in an effort to suppress melanin synthesis by inhibiting the activity of tyrosinase, a copper-containing enzyme that catalyzes the production of melanin from tyrosine. A second objective of the study was to investigate the effect of dietary citric acid supplementation on the growth of red porgy.

## Materials and Methods

Red porgies were produced by mesocom hatchery technology using eggs from a single broodfish population and totally weaned to dry feed. The fish (0.5 g) were divided in four groups and fed one of four diets: (a) control, (b) astaxanthin supplemented, (c) citric acid supplemented, or (d) astaxanthin+citric acid supplemented (Table 1). The astaxanthin originated from *Haematococcus pluvialis* algae supplied by Cyanotech Corporation, Hawaii, USA. The citric acid (citric acid monohydrate; Cat. No. 33114) was supplied by Sigma-Aldrich O.M. Ltd., Athens, Greece. The desired dietary concentrations of astaxanthin and citric acid were derived from earlier investigations (Alam Sarker et al., 2005; Chatzifotis et al., 2005). All diet ingredients were thoroughly mixed, moistened by the addition of 75% (w/v) water, and minced into small pellets. The diets were dried overnight at 35°C and stored at -18°C until use.

The fish were allocated to twelve circular 50-l tanks at 17 fish per tank with three tanks per treatment. The tanks were supplied with borehole seawater of 40 ppt salinity, oxygenated to about 70% saturation by air supply, and held indoors under the natural photoperiod (35°20'22.99'N, 025°10'49.07'E). Water temperature was stable at 20°C. The fish had constant access to the feeds which were supplied by mechanical self-feeders that released a couple of pellets per activation. Before starting the experiment, all groups of fish were acclimatized to the control diet for 1 week. The feeding trial lasted 60 days and at the end of the trial all the fish in every tank were weighed to determine specific growth rate (SGR) as  $100(\ln_{\text{final wt}} - \ln_{\text{initial wt}})/\text{days of growth}$ .

Crude protein and crude lipid contents of the diets were measured by the Kjeldahl (factor 6.25) and Folch methods (Folch et al., 1957), respectively. At the termination of the experiment, skin was dissected from the dorsal area to determine melanin and

Table 1. Compositions of experimental diets fed to red porgy (g/kg).

Ingredient	Diet			
	Control	Astaxanthin	Citric acid	Astaxanthin + citric acid
Fishmeal	686	686	686	686
Starch	150	150	150	150
Fish oil	100	100	100	100
Vitamin/mineral mix	20	20	20	20
Alginate	10	10	10	10
Cellulose	34	30.7	4	0.7
Heamatococcus algae	0	3.3	0	3.3
Citric acid	0	0	30	30
<i>Chemical analysis (%)</i>				
Protein	50	49	50	51
Lipid	14	16	16	16
Ash	11	11	11	11

total carotenoid concentration in the skin from 3.16 to 45.15 µg/g in the astaxanthin diet and from 2.61 to 50.07 µg/g in the astaxanthin+citric acid diet, producing a distinctive red skin coloration. On the other hand, diet composition did not significantly affect the melanin concentration. Unfortunately, the small size of the fish did not allow the use of a color meter to measure  $L^*$ ,  $a^*$ , and  $b^*$ , which could have provided a more accurate indication of the dietary effects on coloration.

Table 2. Effect of dietary astaxanthin and citric acid on the growth, skin carotenoid concentration and skin melanin concentration of red porgy after 60 days of feeding (mean±sd).

	Diet			
	Control	Astaxanthin	Citric acid	Astaxanthin + citric acid
Initial wt (g)	0.56±0.002	0.56±0.01	0.55±0.03	0.53±0.01
Final wt (g)	6.07±1.11	6.39±0.33	6.47±0.77	5.99±0.82
SGR (%/day)	4.13±0.08	3.76±0.15	4.04±0.11	4.23±0.25
<i>Content in skin</i>				
Carotenoid (µg/g)	3.16±1.23 <sup>a</sup>	45.15±12.62 <sup>b</sup>	2.61±1.53 <sup>a</sup>	50.07±16.01 <sup>b</sup>
Melanin (µg/g)	67.33±15.55	57.6±8.28	66.46±14.29	68.18±13.11

## Discussion

Citric acid can inhibit tyrosinase and reduce melanogenesis, acting as a reducing agent by chelating metal ions such as copper and iron that are necessary for activating tyrosinase (Parvez et al., 2006, 2007). However, in the present investigation, dietary citric acid did not reduce the melanin concentration in the skin of red porgy. We cannot provide any concrete explanation for this, other than the fact that the alimentary channel may not have been an effective route for supplying citric acid to act on skin tyrosinase. The process of absorption and metabolism of citric acid in the body (Krebs and Johnson, 1980) may have prevented the citric acid from acting on the tyrosinase. Thus, alternative routes of administering citric acid should be explored. These may include diluting the citric acid in the rearing water or injecting it into the fish body, approaches which have proved effective for kojic acid (Mishima et al., 1988) and for hydroquinone and 2,3-dihydroxybenzoic acid in black goldfish (*Carassius auratus*; Chavin and Schlesinger, 1966; Chavin, 1971).

Similar to our results, dietary citric acid supplementation at 1% did not improve the growth rate of red sea bream *Pagrus major* (Hossain et al., 2007). It appears that citric acid supplements are beneficial to fish when they are given a diet of sub-optimal composition, e.g., citric acid significantly increased growth and feed conversion efficiency in *Labeo rohita* fed a sub-optimal protein diet (Baruah et al., 2007). Similarly, citric acid improved the growth rate of red sea bream fed a phosphorus deficient diet (Alam Sarker et al., 2005). Citric acid enhances the mineral utilization of fishmeal and increases

carotenoid contents. Melanin concentration was determined using a modified procedure (Wilson and Dodd, 1973), as described in Szisch et al. (2002). Total carotenoids were extracted with acetone and quantified spectrophotometrically using the extinction coefficient  $E_{1\%}^{1\text{cm}} = 2100$  for astaxanthin in acetone. Data were tested for significance at  $p < 0.05$  using the statistical package SigmaStat 3.5, San Jose, California, USA.

## Results

All groups grew to an average weight of about 6 g without significant mortality (Table 2). The composition of the diet did not affect the growth rate. However, astaxanthin increased the

phosphorus absorption due to an acidifying effect that leads to solubilization of bone minerals (Alam Sarker et al., 2005). This finding is also in line with the observation that citric acid increases bone ash content in *Labeo rohita*, apparently due to better mineralization of the bone (Baruah et al., 2005). Similarly, dietary citric acid increased whole body ash content and whole body iron in rainbow trout (Vielma et al., 1999).

Although the present study was not designed to monitor feed consumption, we did not observe any feed stimulator action of citric acid in red porgy. This is in contrast to findings in *Tilapia zillii*, an herbivorous species, where a stimulator action of citric acid was noted (Adams et al., 1988). On the contrary, in a study of protein substitution by citric acid, citric acid significantly decreased the feed intake of rainbow trout (Fauconneau, 1988).

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