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Spirulina as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheus acei*

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Abstract A feeding trial on yellow tail cichlid *Pseudotropheus acei* (Regan 1922) was undertaken to asses the effect of dietary *Spirulina* meal as a natural carotenoid source. Four experimental diets were formulated to progressively replace 0% (C), 2.5% (SP2.5), 5% (SP5) and 10% (SP10) of fish meal weight. Ten fish per tank (initial weight 3.75 ± 0.02 g) were randomly distributed into twelve 80 l fiberglass tanks connected to a closed recirculation system (temperature 26.7 ± 0.06°C). The diets were tested in triplicate for 12 weeks. The specific growth rate of fish fed all *Spirulina* diets were significantly higher compared to diet C. Feed intake (FI) tended to increase with dietary *Spirulina* level, and fish fed diet SP10 had significantly higher FI values compared to diet C. No significant differences in feed conversion ratio were observed among these groups. Although the protein efficiency ratio of fish fed diet C was lower than that of all *Spirulina* diets, no significant differences were observed among these groups ($P > 0.05$). The total egg production and hatching rate (%) of eggs derived from all fish fed with *Spirulina* diets was significantly higher compared those from fish fed diet C. The yellow and blue coloration of yellow tail cichlid fed the diet containing *Spirulina* meal was enhanced and inclusion of dietary *Spirulina* meal was elevated carotenoids in skin. Results of the present study indicated that *Spirulina* meal has the potential to enhance the growth, reproductive performance and coloration on yellow tail cichlid.

Keywords Yellow tail cichlid · *Pseudotropheus acei* · *Spirulina* · Reproduction · Pigmentation

Introduction

In ornamental high-value species, such as yellow tail cichlid *Pseudotropheus acei*, discus *Symphysodon aequifasciata*, electric blue cichlid *Sciaenochromis fryerii* and blue streak hap *Labidochromis caeruleus*, emphasis is placed on achieving high levels of skin

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pigmentation as this is one of the most important quality criteria dictating their market value (Gouveia et al. 2003; Harpaz and Padowicz 2007). High fry quality and survival in the production of ornamental fish is also important. The age and nutritional status of broodstock are the major factors that effect productive and quality of fish eggs (Izquierdo et al. 2001); it is reported that the essential fatty acids, alfa tocopherol, ascorbic acid and carotenoids in fish feeds affect on the stimulation of sexual maturation, enhancement of fertility, egg quality and viability of the juveniles (Scabini et al. 2011). Among these components, carotenoid sources have an important role on the coloration and reproduction performance of fish, and studies have shown that dietary supplementation of carotenoid sources improves the reproduction performance of fish (Vilchez et al. 2011; Watanabe and Vassallo-Agius 2003). Commercial aquafeeds typically contain the natural components like carotenogenic microalgae and krill (Gouveia et al. 2002, 2003; Verakunpiriya et al. 1997) as well as astaxanthin and cantaxin (de la Mora et al. 2006; Kalinowski et al. 2005; Lakeh et al. 2010). Carotenoids may have various biological effects in fish such as provitamin A, immunoenhancement or antioxidation (Izquierdo et al. 2005). The primary sources of color in the skin of aquarium fish are from dietary microalgae, phytoplankton and higher plants. Additionally, micro algae have been used as dietary supplements to enhance health and nutritional performance of a range of fish species. Improvements of growth, feed utilization, lipid metabolism, physiological activity, stress response, disease resistance and carcass quality have been reported with dietary algal inclusion levels of 1–5% (Güroy et al. 2011; Mustafa and Nakagawa 1995). *Spirulina* is one of the most commonly used micro algae in aquafeeds because of its rich source of protein, vitamins, essential amino acids, minerals, essential fatty acids and carotenoids such as β-carotene, xanthophylls, zeaxanthin, echinenone and cryptoxanthin (Nakagawa and Montgomery 2007).

The effects of *Spirulina* on fish growth, feed intake, pigmentation and nutrient utilization have been investigated for several fish species (Mustafa et al. 1997; Nandeesha et al. 1998; Palmegiano et al. 2008; Takeuchi et al. 2002). The yellow tail cichlid is popular due to its brilliant colors and because it may be kept in small aquaria (Axelrod et al. 2007). There is no information in the literature on the effects of dietary *Spirulina* levels on growth, coloration and reproductive performance in yellow tail cichlid. Therefore, the aim of the present study was to determine the effect of dietary *Spirulina* levels on the coloration, reproductive performance and growth of yellow tail cichlid.

Materials and methods

Fish and rearing conditions

Yellow tail cichlids were obtained from a local commercial aquarium (Zonguldak, Turkey) and transported to the Ornamental Fish Unit of Armutlu Community College, University of Yalova, Turkey. Yellow tail cichlids were randomly distributed among 12 tanks at a density of 10 (2 male and 8 female) fish per tank with three replicate tanks for each dietary treatment. Experimental groups were fed twice daily (0830 and 1630) by hand to visual satiation. The tank flow rate was 3 l min^{-1}, and water quality was monitored daily. Water temperature was maintained at 26.7 ± 0.0°C, dissolved oxygen at 8.1 ± 0.06 mg l^{-1} and pH at 8.14 ± 0.06. The system was housed in a climate-controlled laboratory with controlled photoperiod (12 h light:12 h dark).

Experimental diets

Spirulina meal was provided by Egert Doğal Ürünler Limited (İzmir, Turkey) (Table 1). Four isonitrogenous (350 g kg^{-1} crude protein) and isoenergetic (21 MJ kg^{-1}) experimental diets were formulated according to Güroy et al. (2012). A diet with fish meal as the main protein source was used as the control diet (C). The other three diets were formulated incorporating *Spirulina* at 2.5% (SP2.5), 5% (SP5) and 10% (SP10) of fish meal weight. Fish oil and vegetable oil were used at a 1:1 ratio. The proximate composition of the experimental diets is shown in Table 2.

Dietary ingredients were mixed in a food mixer (Dirmak Food Equipment, Turkey, model no: IBT-22) with warm water until a soft slightly moist consistency was achieved. This was then cold-press extruded (PTM P6 extruder, Yalova, Turkey) to produce a 1-mm pellet. The moist pellets were then fan-dried and stored frozen at −20°C until use.

Sampling and chemical analyses

Analysis of crude protein, moisture, lipid and ash in diets and ingredients was performed according to standard AOAC (2000) procedures. Dry matter was determined by drying at 105°C until a constant weight was obtained. Ash content was determined by burning in a muffle furnace at 525°C for 12 h. Crude protein (N*6.25) was analyzed by the Kjeldahl method after acid digestion. Crude lipid was analyzed by the Soxhlet method. Crude fiber was determined by acid/alkali hydrolysis recovering filtered residue and ignition of the dried sample for 3 h. Nitrogen-free extract (NFE) was calculated by taking the sum values for crude protein, lipid, ash and crude fiber and then subtracting this from 100. Dietary gross energy was calculated using the conversion factors of 23.7 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17.2 kJ g^{-1} for carbohydrate (Brett and Groves 1979).

Reproductive performance

The reproductive performance of yellow tail fed their respective diet was monitored over 12 weeks. Brooding females were checked for spawning activity daily. Whenever present, eggs were gently removed from the buccal cavity of brooding fish, and the hatching rate monitored over 6–10 days.

Table 1 Chemical composition (%) of fish meal, *Spirulina* meal and dehulled soybean meal

(%)	Fish meal	*Spirulina* meal	Dehulled soybean meal
Crude protein	69.36	63.00	52.00
Crude lipid	9.33	6.71	1.00
Crude ash	14.00	10.00	3.00
Crude fiber	0.68	3.20	6.00
Nitrogen-free extract[a]	6.63	17.09	38.00

[a] Nitrogen-free extract (%) = 100 − (protein + lipid + ash + fiber)

Table 2 Formulation and proximate composition of experimental diets

	C	SP2.5	SP5	SP10
Fish meal[a]	39.0	36.5	34.5	30
Spirulina meal[b]	0	2.5	5	10
Dehulled soybean meal[c]	16	16	16	16
Corn starch[d]	35.1	34.7	34.0	33.1
Fish oil[e]	3.0	3.2	3.3	3.5
Vegetable oil[f]	3.0	3.2	3.3	3.5
Vitamin mix[g]	1.8	1.8	1.8	1.8
Mineral mix[h]	1.1	1.1	1.1	1.1
Vitamin C[i]	0.2	0.2	0.2	0.2
Choline chloride (60%)[j]	0.6	0.6	0.6	0.6
Binder[k]	0.2	0.2	0.2	0.2
Chemical analyses				
Crude protein (%)	35.97	35.78	35.65	35.77
Crude lipid (%)	10.48	10.73	10.03	10.12
Crude ash (%)	5.70	5.30	5.10	4.40
Crude fiber (%)	0.85	0.78	0.70	0.85
Nitrogen-free extract (%)	53.00	52.59	51.48	51.14
Gross energy (MJ/kg)	21.78	21.76	21.27	21.27

[a] Anchovy fishmeal. Agromey Feed Mill Company, İzmir, Turkey

[b] Egert Doğal Ürünler Ltd., İzmir, Turkey

[c] Agromey Feed Mill Company, İzmir, Turkey

[d] Cargill, İstanbul, Turkey

[e] Anchovy fish oil. Agromey Feed Mill Company, İzmir, Turkey

[f] Soybean oil. Zade Natural, İstanbul, Turkey

[g] Per g mixture: vitamin A: 342 IU; vitamin D_3: 329 IU; vitamin E: 0.0274 IU; vitamin K_3: 48 mg; vitamin B_1: 2.05 mg; vitamin B_2: 3.42 mg; vitamin B_3: 20.5 mg; vitamin B_5: 5.48 mg; vitamin B_6: 2.05 mg; vitamin B_{12}: 2.74 mg; vitamin C: 24.0 mg, biotin: 0.411 mg; folic acid: 0.685 Agromey Feed Mill Company, İzmir, Turkey

[h] Per g mixture: mg; Zn: 12.3 mg; Mn: 4.80 mg; Cu: 1.64 mg; I: 0.274 mg; Se: 0.0274 mg; Ca: 125 mg; K: 189 mg, Agromey Feed Mill Company, İzmir, Turkey

[i] Rovimix® Stay-C® 35, DSM Nutritional Products, Turkey

[j] DSA Agrifood Products, Ankara, Turkey

[k] Guar gum, Kartal Chemical Incorporated, Kocaeli, Turkey

Color measurement

Fish were individually weighed and measured for skin color using a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Asaka, Japan) before commencement of the feeding trial to establish baseline measurements (week 0) and then every 2 weeks for the 12-week period. The measurements were performed on left flanks of body area and caudal region of each fish. The Chroma Meter was set to take absolute measurements in the L* a* b* measuring mode (CIE 1976) using D65 illuminant. L* is the lightness variable (where white = 100 L* and black = 0 L*), a* is the red chromaticity coordinates where +a* stands for red, and −a* stands for green, and +b* is the yellow chromaticity coordinates where +b* stands for yellow, and −b* stands for blue.

Carotenoid analyses

The skin was dissected from the dorsal body area for carotenoid content determination. Total carotenoids were extracted with acetone and quantified spectrophotometrically using the extinction coefficient ($E_{1\%1\text{cm}}$) of 2,100 (Gouveia et al. 1997).

Calculations

Growth performance, in terms of specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were determined using the following formulae:

$$\text{Feed Conversion Ratio(FCR)} = \text{FI}/\text{WG}$$
$$\text{Specific Growth Rate(SGR)}(\%\ \text{day}) = 100 \times ((\ln \text{FBW} - \ln\text{IBW})/\text{T})$$
$$\text{Protein Efficiency Ratio(PER)} = 100 \times (\text{WG}/\text{PI})$$

where FBW is the final body weight (g), IBW is initial body weight (g), FI = feed intake (g)WG = weight gain (g), T = time (days) and PI = dietary protein intake (g).

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA), when a significant difference was found among treatments; Duncan's multiple range test was performed to rank the groups using Statgraphics 4.0 (Manugistics Incorporated, Rockville MD, USA) statistical software (Zar 2001). All values were considered significant at the 5% level.

Results

The growth and reproduction parameters for yellow tail cichlid fed increasing levels of *Spirulina* are shown in Table 3. The survival rate was 100% for all treatments. Mean final weight ranged from 7.31 g (C) to 8.56 g (SP10) and was significantly lower in fish fed diet C than all *Spirulina* diets. Furthermore, the final mean weight (FMW) and specific growth rate (SGR) of fish fed diet SP10 were not significantly different compared to fish fed SP2.5 and SP5 diets. All the experimental diets were well accepted by fish; furthermore, feed intake (FI) tended to elevate with increasing dietary *Spirulina* level, and fish fed diet SP10 (12.50 g fish^{-1}) had significantly higher FI values compared to fish fed diet C (9.63 g fish^{-1}). No significant differences in feed conversion ratio (FCR) were observed among these groups. Although the protein efficiency ratio (PER) of group C was lower than that of all *Spirulina* diets, these differences were not significant ($P > 0.05$).

Dietary *Spirulina* significantly affected the total egg production (TEP) of the yellow tail cichlid and the subsequent hatching rate (%) of the eggs (Table 3). The TEP of yellow tail varied from 101 to 346, with the all *Spirulina* groups superior ($P < 0.05$) than those fed the diet C. Additionally, the TEP was higher in fish fed with the SP2.5 (346) diet than in diets SP5 (231) and SP10 (191). The eggs from fish fed all *Spirulina* diets displayed significantly higher hatching rates compared to the eggs from fish fed diet C ($P < 0.05$). Although hatching rate tended to decrease with increasing dietary *Spirulina* level, no significant differences were observed among these groups ($P > 0.05$).

There were significant effects of experimental diets on lightness (*L*) values in the body area (Table 4) and in the caudal region (Table 5). The skin of fish fed diets containing

Table 3 Growth and reproduction performance of yellow tail cichlids after 12 weeks of feeding on experimental diets

	C	SP2.5	SP5	SP10
Initial mean weight (g)	3.75 ± 0.02	3.74 ± 0.01	3.75 ± 0.01	3.76 ± 0.01
Final mean weight (g)	7.31 ± 0.13[a]	8.26 ± 0.23[b]	8.39 ± 0.04[b]	8.56 ± 0.02[b]
Specific growth rate (SGR; % day^{-1})	0.80 ± 0.02[a]	0.94 ± 0.03[b]	0.96 ± 0.02[b]	0.98 ± 0.01[b]
Feed Intake (FI; g $fish^{-1}$)	9.63 ± 1.08[a]	11.65 ± 0.09[ab]	12.27 ± 0.56[ab]	12.50 ± 0.48[b]
Feed conversion ratio (FCR)	2.69 ± 0.22	2.53 ± 0.21	2.62 ± 0.17	2.60 ± 0.08
Protein efficiency ratio (PER)	1.05 ± 0.08	1.11 ± 0.09	1.07 ± 0.06	1.08 ± 0.04
Total egg production (TEP)	101 ± 4.36[a]	346 ± 4.12[c]	231 ± 5.45[b]	192 ± 6.34[b]
Hatching rate (%)	71.28 ± 1.59[a]	97.68 ± 2.61[b]	91.79 ± 4.18[b]	87.12 ± 3.59[b]

In the same line, values with different superscript letters are significantly different ($P < 0.05$)

Feed Conversion Ratio (FCR) = FI/WG

Specific Growth Rate (SGR) (% day) = 100 × ((ln FBW − lnIBW)/T)

Protein Efficiency Ratio (PER) = WG/PI

where FBW is the final body weight (g), IBW is initial body weight (g), FI = feed intake (g)

WG weight gain (g), *T* time (days) and *PI* dietary protein intake (g)

Table 4 Color parameters (*L, a, b*) in body of yellow tail cichlid fed experimental diets for 12 weeks

	Body			
	C	SP2.5	SP5	SP10
L (lightness)	37.04 ± 1.75[b]	32.63 ± 0.68[a]	33.23 ± 1.56[a]	33.12 ± 2.22[a]
a (red/green tonality)	1.56 ± 0.32[a]	2.05 ± 0.19[b]	2.27 ± 0.40[b]	2.06 ± 0.29[b]
b (yellow/blue tonality)	−6.88 ± 0.43	−7.31 ± 0.15	−7.74 ± 0.72	−8.17 ± 0.26

In the same line, values with different superscript letters are significantly different ($P < 0.05$)

Spirulina had significantly lower lightness values than fish fed the control diet in the both caudal and body regions. Red/green tonality (*a*) of skin from the body varied from 1.56 (C) to 2.27 (SP5), with the highest values being found in fish fed the *Spirulina* diets. The red/green tonality (*a*) of skin from the body of fish fed diet SP2.5 (2.05) was not significantly different compared to fish fed the SP5 diet (2.27). The yellow/blue tonality (*b*) in the body was not affected by the concentration of *Spirulina* in the diet ($P > 0.05$). However, the yellow/blue tonality (*b*) in the caudal region was elevated with increasing dietary *Spirulina* levels, and fish fed diet SP10 (−8.17) had significantly higher the yellow/blue tonality (b) values compared to fish fed diet C (−6.88). The highest carotenoid concentrations in skin were obtained in diets supplemented with *Spirulina* (1.47–2.30 μg g^{-1}) (Fig. 1) ($P < 0.05$).

Discussion

The present study is the first study to evaluate *Spirulina* in diets for yellow tail cichlid. The current investigations were performed to test the feasibility of including dietary *Spirulina* meal in practical diets for yellow tail cichlid. In the nutrition trial, supplementation of

Table 5 Color parameters (*L, a, b*) in caudal region of yellow tail cichlid fed experimental diets for 12 weeks

	Caudal			
	Control	SP2.5	SP5	SP10
L (lightness)	40.40 ± 1.35[b]	37.29 ± 1.15[a]	36.29 ± 1.87[a]	35.87 ± 1.70[a]
a (red/green tonality)	−0.10 ± 0.02[a]	0.33 ± 0.01[b]	0.59 ± 0.02[bc]	0.90 ± 0.02[c]
b (yellow/blue tonality)	−0.67 ± 0.18[a]	−1.15 ± 0.23[b]	−1.84 ± 0.39[b]	−2.32 ± 0.36[c]

In the same line, values with different superscript letters are significantly different ($P < 0.05$)

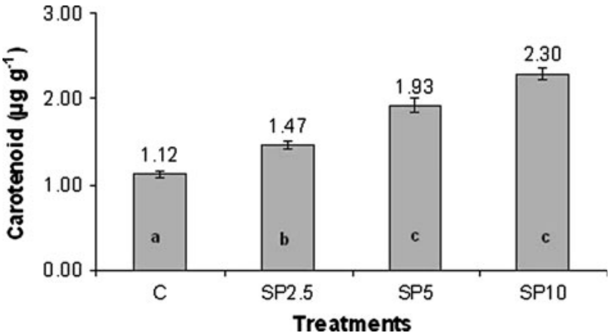

Fig. 1 Total carotenoid of skin of yellow tail cichlids after 12 weeks of feeding on experimental diets

Spirulina had clear beneficial effects on the growth, skin coloration and reproduction performance of yellow tail cichlid. The SGR of fish fed all *Spirulina* diets were significantly higher compared to the non-*Spirulina* supplemented diet (C). Because *Spirulina* is rich in proteins, vitamins, minerals, essential amino acids and fatty acids (Nakagawa and Montgomery 2007), it has been identified as a potential feed ingredient for cichlids and ornamental fish and appears to be a promising dietary ingredient. Numerous studies have shown that dietary *Spirulina* can affect the growth performance of various cichlid and non-cichlid fish species. For example, in cichlids, it has previously been reported that 20% (Ungsethaphand et al. 2010) and 40% (Olvera-Novoa et al. 1998) of FM can be replaced with *Spirulina* without detrimentally affecting the growth performance of tilapia *Oreochromis mossambicus*. Furthermore, James et al. (2006) reported that dietary inclusion of 8% *Spirulina* significantly elevated growth performance of the ornamental red swordtail *Xiphophorus helleri*. The present study is in agreement with the prior reported cichlid and ornamental studies and illustrates the possible use of *Spirulina* as a partial substitute for FM in yellow tail cichlid diets.

To the authors' knowledge, there is no information about the effects of dietary *Spirulina* meal on reproductive performance of cichlids; however, James et al. (2006) reported that dietary *Spirulina* elevated red swordtail reproductive performance. In the present study, reproductive performance of yellow tail cichlid also increased with the dietary supplementation of *Spirulina* meal; the highest TEP and hatching rate was observed in the SP2.5 diet, but there were no significant differences among the *Spirulina* diets in terms of hatching rate. The reason for this elevation in reproductive performance is not fully understood, but previous studies have shown that reproductive performance can be modulated by dietary carotenoids (Watanabe and Vassallo-Agius 2003), which may be mediated through various biological responses to the elevated dietary provision of provitamin A, protection from adverse lighting conditions, promoting chemotaxis of spermatozoa or antioxidant functions (Izquierdo et al. 2005). An improvement in reproductive performance in response to elevated inclusion of dietary carotenoid sources has been previously reported in gilthead seabream *Sparus aurata* (Scabini et al. 2011), yellow tail *Seriola quinqueradiata* (Vassallo-Agius et al. 2001, 2002) and rainbow trout *Oncorhynchus mykiss* (Dabrowski et al. 1987).

Dietary carotenoids do not only have functional roles in modulating reproductive processes, but also, as is well documented, carotenoids are utilized in diets to induce coloration. In the present study, the diet containing the *Spirulina* meal was the most successful in inducing the yellow and blue coloration in the skin of the yellow tail cichlid. *Spirulina* meal has also been used successfully to increase the skin coloration of red tilapia (Matsuno et al. 1980), red sword tail (James et al. 2006), blue gourami, *Trichogaster trichopterus* (Alagappan et al. 2004) and goldfish, *Carassius auratus* (Gouveia et al. 2003; James et al. 2009). Moreover, Ako et al. (2000) reported diets containing 1.5–2.0% of a carotenoid-rich strain of *Spirulina platensis,* and 1% of *Haematococcus pluvialis* significantly enhanced coloration of red velvet sword tail, rainbow fish *Pseudomugil furcatus* and topaz cichlids *Cichlasoma myrnae*. Indeed, *Spirulina* is a well-recognized carotenoid source, containing high levels of xanthophyll, β-carotene and zeaxanthin, and dietary inclusion of *Spirulina* has been reported to affect fish pigmentation (Gouveia et al. 2003; Nandeesha et al. 2001). The skin coloration changes observed in the present study were confirmed by the observed elevated total carotenoid levels ($\mu g\ g^{-1}$) in the skin of yellow tail cichlids fed dietary *Spirulina* meal.

The results clearly show that *Spirulina* meal could be used in yellow tail cichlid diets to replace up to 10% of the dietary fish meal without causing any adverse effects on growth, reproductive performance or coloration. Indeed, all fish fed with *Spirulina* diets exhibited higher growth values and broodstock performance. *Spirulina* meal clearly offers much potential for inclusion in diets for yellow tail cichlids, and this preliminary study confirms a basis for more extensive investigations.

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References

Ako H, Tamaru CS, Asano L, Yuen B, Yamamoto M (2000) Achieving natural coloration in fish under culture. In: Tamaru CCT, Tamaru CS, Mcvey JP, Ikuta K (eds) Spawning and maturation of aquatic species. United States-Japan cooperative program in natural resources technical report no 28. University of Hawaii, Sea Grant College Program, Honolulu, Hawaii

Alagappan M, Vijula K, Sinha A (2004) Utilization of spirulina algae as a source of carotenoid pigment for blue gouramis (*Trichogaster trichopterus*, Pallas). J Aquaricult Aquat Sci 10:1–11

AOAC (2000) Official methods of analysis of the association of official analytical chemists, 19th edn. Association of Official Analytical Chemists, Arlington

Axelrod GS, Axelrod HR, Burgess WE (2007) Dr. Axelrod's atlas of freshwater aquarium fishes. TFH Publications, USA

Brett JR, Groves TDD (1979) Physiological energetics. In: Hoar S, Randall DJ, Brett JR (eds) Fish physiology: bioenergetics and growth. Academic Press, New York, pp 279–352

Dabrowski K, Luczynski M, Czeczuga L, Falkowski S (1987) Relationships among corregonid fresh reproductive effort, carotenoid content in eggs and survival of embryons. Arch Hydrobiol 79:29–48 de la Mora GI, Arredondo-Figueroa JL, Ponce-Palafox JT, Barriga-Soca ID, Vernon-Carter JE (2006) Comparison of red chilli (*Capsicum annuum*) oleoresin and astaxanthin on rainbow trout (*Oncorhyncus mykiss*) fillet pigmentation. Aquaculture 258:487–495

Gouveia L, Gomes E, Empis J (1997) Use of *Chlorella vulgaris* for rainbow trout, *Oncorhynchus mykiss*, diets to enhance muscle pigmentation. J Appl Aquacult 7:61–70

Gouveia L, Choubert G, Gomes E, Pereira N, Santinha J, Empis J (2002) Pigmentation of gilthead seabream, *Sparus aurata* (Lin 1875) using *Chlorella vulgaris* microalga. Aquac Res 33:987–993

Gouveia L, Rema P, Pereira O, Empis J (2003) Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. Aquac Nutr 9:123–129

Güroy D, Sahin I, Güroy B, Altin A, Merrifield DL (2012) Effect of dietary protein level on growth performance and nitrogen excretion of yellow tail cichlid *Pseudotropheus acei.* Isr J Aquacult/Bamidgeh IIC:64.2012.684

Güroy D, Güroy B, Merrifield DL, Ergun S, Tekinay AA, Yigit M (2011) Effect of dietary Ulva and Spirulina on weight loss and body composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), during a starvation period. J Anim Physiol Anim Nutr 95:320–327

Harpaz S, Padowicz D (2007) Color enhancement in the ornamental dwarf cichlid *Microgeophagus ramirezi* by addition of plant carotenoids to the fish diet. Isr J Aquacult Bamid 59:195–200

Izquierdo MS, Fernandez-Palacios H, Tacon AGJ (2001) Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197:25–42

Izquierdo MS, Kalinowski CT, Thongrod S, Robaina L (2005) Nutritional needs for correct pigmentation in european red porgy *Pagrus pagrus*. In: Lyons TP, Jacques KA (eds) Nutritional biotechnology in the feed and food industries. Nottingham University, UK, pp 307–313

James R, Sampath K, Thangarathinam R, Vasudevan I (2006) Effect of dietary spirulina level on growth, fertility, coloration and leucocyte count in red swordtail, *Xiphophorus helleri*. Isr J Aquacult Bamidgeh 58:97–104

James R, Vasudhevan I, Sampath K (2009) Interaction of Spirulina with different levels of vitamin E on growth, reproduction, and coloration in goldfish (*Carassius auratus*). Isr J Aquacult Bamidgeh 61:330–338

Kalinowski CT, Robaina LE, Fernandez-Palacios H, Schuchardt D, Izquierdo MS (2005) Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. Aquaculture 244:223–231

Lakeh AAB, Ahmadi MR, Safi S, Ytrestoyl T, Bjerkeng B (2010) Growth performance, mortality and carotenoid pigmentation of fry offspring as affected by dietary supplementation of astaxanthin to female rainbow trout (*Oncorhynchus mykiss*) broodstock. J Appl Ichthyol 26:35–39

Matsuno T, Katsuyama M, Iwahashi M, Koike T, Okada M (1980) Intensification of color of red tilapia with lutein, rhodoxanthin and spirulina. Bull Jpn Soc Sci Fish 46:479–482

Mustafa MG, Nakagawa H (1995) A review: dietary benefits of algae as an additive in fish feed. Isr J Aquacult Bamidgeh 47:155–162

Mustafa MG, Umino T, Nakagawa H (1997) Limited synergistic effect of dietary Spirulina on vitamin C nutrition of red sea bream *Pagrus major*. J Mar Biotechnol 5:129–132

Nakagawa H, Montgomery WL (2007) Algae. In: Gatlin DM III, Nakagawa H, Sato M (eds) Dietary supplements for the health and quality of cultured fish. Cabi International, Cambridge, pp 133–167

Nandeesha MC, Gangadhar B, Varghese TJ, Keshavanath P (1998) Effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio* L. Aquac Res 29:305–312

Nandeesha MC, Gangadhara B, Manissery JK, Venkataraman LV (2001) Growth performance of two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels of *Spirulina platensis*. Bioresour Technol 80:117–120

Olvera-Novoa MA, Dominguez-Cen LJ, Olivera-Castillo L (1998) Effect of the use of the microalga *Spirulina maxima* as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Aquac Res 29:709–715

Palmegiano GB, Gai F, Dapra F, Gasco L, Pazzaglia M, Peiretti PG (2008) Effects of Spirulina and plant oil on the growth and lipid traits of white sturgeon (*Acipenser transmontanus*) fingerlings. Aquac Res 39:587–595

Scabini V, Fernandez-Palacios H, Robaina L, Kalinowski T, Izquierdo MS (2011) Reproductive performance of gilthead seabream (*Sparus aurata* L., 1758) fed two combined levels of carotenoids from paprika oleoresin and essential fatty acids. Aquac Nutr 17:304–312

Takeuchi T, Lu J, Yoshizaki G, Satoh S (2002) Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw Spirulina. Fish Sci 68:34–40

Ungsethaphand T, Peerapornpisal Y, Whangchai N, Sardsud U (2010) Effect of feeding *Spirulina platensis* on growth and carcass composition of hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*). Maejo Int J Sci Technol 4:331–336

Vassallo-Agius R, Watanabe T, Satoh S, Kiron V, Imaizumi H, Yamazaki T, Kawano K (2001) Supplementation of paprika as a carotenoid source ın soft-dry pellets for broodstock yellowtail *Seriola quinqueradiata* (Temminck & Schlegel). Aquac Res 32:263–272

Vassallo-Agius R, Watanabe T, Imaizumi H, Yamazaki T (2002) Spawning performance of yellowtail *Seriola quinqueradiata* fed dry pellets containing paprika and squid meal. Fish Sci 68:230–232

Verakunpiriya V, Watanabe K, Mushiake K, Kawano K, Kobayashi T, Hasegawa I, Kiron V, Satoh S, Watanabe T (1997) Effect of krill meal supplementation in soft-dry pellets on spawning and quality of egg of yellowtail. Fish Sci 63:433–439

Vilchez C, Forjan E, Cuaresma M, Bedmar F, Garbayo I, Vega J (2011) Marine carotenoids: biological functions and commercial applications. Mar Drugs 9:319–333

Watanabe T, Vassallo-Agius R (2003) Broodstock nutrition research on marine finfish in Japan. Aquaculture 227:35–61

Zar JH (2001) Biostatistical analysis, 4th edn. Prentice-Hall Inc., Upper Saddle River