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THE EFFECTS OF DIETS SUPPLEMENTED WITH *SPIRULINA PLATENSIS* IN DIFFERENT QUANTITIES ON PIGMENTATION AND GROWTH PERFORMANCE OF GOLDFISH *(CARASSIUS AURATUS*)

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Spirulina sp., a blue - green algae, is in the form of a spiral ring with microscopic cells. S. platensis contains 60% vegetable protein, essential vitamins and β-carotene which is an important antioxidant. Spirulina's dark green colour is due to the pigments of carotenoid (orange), phycocyanin (blue) and chlorophyll (green). Green chlorophyll is masked by blue phycocyanin, which is an auxiliary pigment, alloficocyanin and red coloured phycoerythrin. The only chlorophyllin that Spirulina sp. contain is chlorophyll a and its amount varies between 0.8-1.5% in dry weight. Xanthophyll content of freeze-dried Spirulina sp is quite high and it accounts 6.9 g.kg-1. Other major carotenoids are mycoxanthophyll (37%), β-carotene (28%) and zeaxanthin (17%). Due to its pigment composition, Spirulina sp. is utilized for pigmentation of ornamental fish used as a feed additive particularly in Goldfish.

If the colour formation of Carassius auratus cannot be achieved at the desired level, it leads to decrease in market demand and value of such a fish significantly. Even though fish feeds containing carotenoids are available in the market, these feeds are quite expensive. For this reason, we investigated the effects of Spirulina platensis on pigmentation and growth of the Goldfish in the study. In this context, Specific Growth Rate (SGR), the Feed Conversion Ratio (FCR), Condition Factor (CF) and Survival Rate (SR) were investigated to evaluate the growth performance of C. auratus species through usage of 3 different feed quantities (25 mg.kg-1 diet, 50 mg.kg-1 diet, 75 mg.kg-1 diet). This study demonstrated that S. platensis added to fish-feed in various quantities had no significant effect on the growth of Goldfish however it contributed to skin pigmentation. The best carotenoid ratio was achieved in the feeding group in which 75 mg.kg-1 of S. platensis was supplemented to the diet.

The goal in this study, the effects of the addition of Spirulina sp. as a carotenoid source at different levels to the fish- feed on the growth, development and pigmentation of Goldfish (Carassius auratus) were investigated.

Keywords: *Spirulina platensis; Carassius auratus; feeding; growth rate; pigmentation.*

Introduction

A bluegreen algae *Spirulina* sp. is in the form of a spiral ring with microscopic cells [1]. *Spirulina* (*Arthrospira platensis*) is a filamentous and multicellular blue-green alga capable of reducing inflammation and also manifesting antioxidant effects. It is a rich source of vitamins, especially vitamin B12, minerals, protein, and carotenoids [2]. The multicellular filamentous, alkaliphilic cyanobacterium *Arthrospira platensis* is widely cultured around the world as both a source of health food and as a source of the blue pigment cyanophycin which is used in cosmetics and food [3].

S. platensis contains 60% vegetable protein, essential vitamins and β-carotene which is an important antioxidant and a rarely found essential fatty acid gamma linolenic acid (GLA), as well as phytonutrients such as sulfolipids, glycolipids and polysaccharides [2]. *Spirulina*'s dark green colour is due to the pigments of carotenoid (orange), phycocyanin (blue) and chlorophyll (green). Green chlorophyll is masked by blue phycocyanin, which is an auxiliary pigment, alloficocyanin and red coloured phycoerythrin [2]. The only chlorophyllin that *Spirulina* sp. contains is chlorophyll a and its amount varies between 0.8–1.5% in dry weight. Xanthophyll content of freeze-dried *Spirulina* sp. is quite high and it accounts 6.9 g kg-1. Other major carotenoids are mycoxanthophyll (37%), β-carotene (28%), and zeaxanthin (17%) [3]. Due to its pigment composition, *Spirulina* sp. is utilized for pigmentation of ornamental fish used as a feed additive, particularly, in Goldfish [4]. These are flavins, melanin, guanine, and carotenoids which give the yellow, brown, grey, and black; metallic shining and silver; and the yellow-red colors, respectively [5]. Carotenoids belong to the terpene group, and the double bond they have (keto = oxo) has an important role in pigmentation [6].

The skin pigmentation of fancy fish is the most important quality parameter determining the market value and consumer acceptability. The fish tend to lose their color as if fading when maintained in captivity, and this decreases their market value [7].

Therefore, several studies have been focused on improving fish skin coloration. Various factors were contributed to the color intensity of aquatic animals,

such as the source and type of pigments, water temperature, brightness, feeding rate, diet composition, species, size, and physiological conditions [8].

The colour formation in the fish occurs partly because of the physical breakdown and reflection of the light, and predominantly due to the pigments existing under the skin.

The skin pigmentation of fancy fish is the most important quality parameter determining the market value and consumer acceptability. The fish tend to lose their color as if fading when maintained in captivity, and this decreases their market value [7].

Therefore, several studies have been focused on improving fish skin coloration. Various factors were contributed to the color intensity of aquatic animals, such as the source and type of pigments, water temperature, brightness, feeding rate, diet composition, species, size, and physiological conditions [8]. The colour formation in the fish occurs partly because of the physical breakdown and reflection of the light, and predominantly due to the pigments existing under the skin. However, carotenoids that are lipid-soluble pigments are the most effective and consistent means to enhance the skin coloration in ornamental fishes [9].

Four kinds of colour materials were determined in fish. These are flavinas, melanin, guanine, and carotenoids which give the yellow, brown, grey, and black; metallic shining and silver; and the yellow-red colours, respectively [10]. Carotenoids belong to the terpene group, and the double bond they have (keto = oxo) has an important role in pigmentation [11]. Birds and fish, in general, prefer oxidized carotenoids (astaxanthin, cantaxantin, zeaxanthin and lutein) [12]. Mainly, lutein and zeaxanthin are the carotenoids which are effective in Goldfish and absorbed three times more efficiently compared to astaxanthin [13]. Carotenoids which are taken into the body are able to accumulate in various tissues and organs (skin, scale, fin, operculum, liver, bile, eggs, blood and fat) in different amounts. The differences in accumulation is, mainly, related to the fish age, size, sexual maturity status and gender. Pigmentation should be considered within the duo of environmental conditions and genetic structure**.** Fish can obtain the carotenoids they need only from the exogenous feed resources. These foods are primarily phytoplankton, zooplankton and various crustaceans. Since fishes are incapable of synthesizing carotenoids de novo in their body, so these pigments must be supplied in the diet [14].

A variety of carotenoids from both natural and synthetic sources, such as astaxanthin, cantaxanthin, β-carotene, lutein and xanthophylls, have been incorporated into fish diets for color enhancement [15;16]. However, the use of syntetic carotenoids may have negative impacts, such as environmental deterioration and carcinogenic effects, and they tend to have a high cost [17], so recent efforts

have emphasized the potential use of coloring agents from alternative natural sources, such as fairy shrimps**,** beetroots [18] to replace the synthetic chemicals.

In prior studies the natural compounds derived from red yeast, marine bacteria, and green algae, were efficient as synthetic carotenoids for improving the skin pigmentation in several ornamental fish species, including goldfish (*Carassius auratus*) [19], Kenyi cichlids (*Maylandia lombardo*) and tomato clownfish (*Amphiprion frenatus*) [20].

Carotenoids are primary class of compounds that can affect the skin coloration of fish. There are effects of natural carotenoid supplemented diets on growth and feed utilization efficiency. Besides their beneficial effects on pigmentation, carotenoids also play a significant role in enhancing nutrient utilization that may contribute to survival and growth performance**.** Carotenoids, in addition to their effect on fish pigmentation, have also functions such as accelerating growth and development, as well as increasing the fish tolerance to environmental conditions [21].

In the present study, fish fed with carotenoid supplemented diets did not significantly differ from the control group in the growth, feed utilization efficiency or survival rate. These results are in accordance with previous studies carried out with gilt-head seabream (*Sparus aurata*)**,** red porgy (*Pagrus pagrus*)**,** goldfish and large yellow croaker (*Larimichthys croceus*) [22]. However, in some studies a dietary carotenoid supplement improved the growth and feed utilization efficiency in fish**.** However, in some studies a dietary carotenoid supplement improved the growth and feed utilization efficiency in fish [23].

Ornamental fish farming and aquarium industry have developed quickly and become an important business sector in Turkey in recent years because the countries surrounding the Mediterranean Sea have favorable ecological conditions for farming goldfish. However, pigmentation of a part of goldfish becomes late or it is not possible to achieve the desired level of color. This situation decreases the market value of fish at a significant rate [24].

In this study, we investigated the effects of the addition of *Spirulina* sp. as a carotenoid source at different levels to the fish- feed on the growth, development and pigmentation of Goldfish (*Carassius auratus*).

Samples and Research Methods

The experiment was carried out at an ornamental fish culture unit of Mersin University. Unpigmented Goldfish (*C. auratus*) similar size (5.152 ± 0.098 cm) and weight (3.434 ± 0.159 g) were used in the experiment. Nutrient content of the *S. platensis* powder used in the experiment is provided in Table 1.

Table 1.

Nutrient content of *S. platensis*

Nutrient content	Rate%
Protein	65.50
Fat	7.20
Humidity	6.42
Ash	6.60
Phycocyanin	13.30
Carotenoid	100 mg.100g^{-1}

Juvenile trout feed (2-3 mm) was used as feed material due to its appropriate ingredients and availability on the market [13]. Feed nutrient values are provided in Table 2.

Table 2.

Nutrient values of the feed used in the experiment

Nutrient values	Rate%
Protein	45
Fat	20
Cellulose	2
Humidity	10
Ash	11

Experiment Design

A total of 12 aquariums with about 95 L volume and in dimensions of 60 × 45 ×35 cm were used in experiment. Tap water from a reservoir tank was used in the experiment after being dechlorinized. Each dietary treatment was replicated three times. 25 fish were randomly allocated in each of the aquariums. The difference between the initial average weights of the fish were assured to be statistically insignificant.

Preparation of Feeds Used in the Experiment

S. platensis powder mixed in distilled water was sprayed to the trout pellets used in the experiment and subsequently dried in dark environment. Feed groups utilized in the experiment are given in Table 3.

Table 3.

Feed groups utilized in the experiment

Groups	Content
Group 1 feed (Control)	Commercial trout pellet
Group II feed	Trout pellet + 25 mg.kg^{-1} *S. platensis*
Group III feed	Trout pellet + 50 mg.kg^{-1} *S. platensis*
Group IV feed	Trout pellet+ 75 mg.kg^{-1} S. *platensis*

Feeding Regime

After the amount of feed to be given to fish weighed every 15 ds was determined, the feeding was done by means of an automatic feeding machine as 4 meals a day. The average amount of feed given to fish according to body weight is provided in Table 4.

Table 4.

The average amount of feed to be provided according to body weight

Body Weight (g)	Feed Amount (%)
0.2-5	4
5-20	3
20<	2

Daily maintenance of Aquariums Employed in the Experiment and Water Quality

The temperature of water in the aquariums was fixed at 28 ± 2°C using thermostat heaters. The oxygenation of the waters was performed by means of a filter air pump. Approximately1/2 of the aquarium water were siphoned from the bottom once a day and feed residues and fish excrement were removed from the environment. Photoperiod time was set as12:12 [14]. The chemical composition of the water utilized in the experiment is provided in Table 5.

Table 5.

The chemical composition of the water utilized in the experiment

Dissolved Oxygen	8.±0.37 mg.L^{-1}
pH	8.32±0.20
Total Alkalinity	325±0.80 mg.L^{-1}
Total Hardness	230±6.36 mg.$L^{-1}CaCO_3$

Determining of the Pigmentation

The colours of fish were measured using spectrophotometric methods (UV-Visible Spectrophotometer Shimadzu UV 1208). Measurements were performed at the start and at the end of the experimental period. The extraction of carotenoids was performed according to the method of Renstrom et al. [25] modified by Torrissen and Naevdal [26]. Total carotenoid content in fish skin was measured by spectrophotometric method at the beginning and end of the trial (60 days). In order to measure this, 4 fish were randomly taken from each weight group and two parallel analyses were performed. Acetone was used as the control solution for reading the samples. The maximum absorbance of the

test solutions in the spectrophotometer was determined as 475 nm. In the calculation of total carotenoids in the skin, theoretical extraction of 1% solution of astaxanthin in acetone at 474 nm and 1 cm force was taken 2000 [27].

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Evaluation of Growth Performance

Live weight, total length, standard length, specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF) and survival rate (SR)were evaluated in determining the growth performance. Specific growth rate was calculated in the following formula [28].

$$SGR(\%\text{division}.day) = \frac{Final\ Weight\ (g) - \ln Initial\ Weight\ (g)}{Farming\ period\ (day)} \times 100$$

The following formula was used to determinate the feed conversion ratio [29].

$$FCR = \frac{Amount\ of\ given\ feed\ (g)}{Weight\ gain\ (g)}$$

The following formula was used to determine the condition factor [30].

$$CF\left(g/cm^3\right) = \frac{w}{L^3} \times 100 \quad CF\left(g/cm^3\right) = \frac{w}{L^3} \times 100$$

The following formula was used to determine survival rate [31].

$$SR = \frac{Number\ of\ fish\ at\ the\ end\ of\ experiment}{Number\ of\ fish\ in\ each\ experiment} \times 100$$

Statistics Analysis

Statistical analysis of the data obtained from the experiment was performed by using statistical package, SPSS (v17) for Windows (2008) [22]. The signif-

icance of treatment effects on the different parameters measured were determined by one-way ANOVA. After the variance homogeneity test was applied to all data, Duncan multiple comparison test was performed if the data in the ANOVA analysis showed homogeneous distribution, and Tamhane's T2 test if it was not homogeneous distribution ($p < 0.05$). Results Mean ± Standard Deviation (Avg. ± SD) is given in the form.

Research Results and Discussion

Growth Parameters

Growth parameters live weight gain, total height, standard length, condition factor (CF), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR) and obtained from the experimental fish in different dietary groups in every 30 days are given in Tables (6,7,8) and Fig. 1 below.

Table 6.

Average live weight of the juvenile fish in the experiment (LW)

GROUPS	TIME			
	Initial	30th day	60th day	90th day
Group I	3.440±0.226[a]	4.130±0.157[a]	5.767±0.438[a]	6.850±0.592[a]
Group II	3.460±0.207[a]	4.197±0.119[a]	5.640±0.466[a]	6.437±0.7908[a]
Group III	3.360±0.090[a]	3.967±0.320[a]	6.397±0.189[a]	7.637±0.100[a]
Group IV	3.477±0.112[a]	4.197±0.179[a]	5.930±0.654[a]	6.617±0.780[a]

*: There difference is a statistical between the data shown with different letters ($p<0.05$)

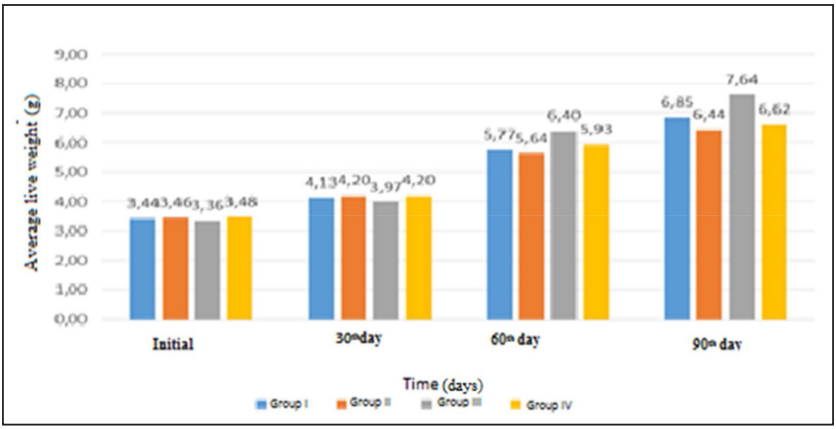

Fig. 1. Average live weight of the juvenile fish in the experiment (LW)

Table 7.

Average standard length of the juvenile fish in the experiment

GROUPS	TIME			
	Initial	30th day	60th day	90th day
Group I	3.460±0.125[a]	3.637±0.074[a]	4.013±0.148[a]	4.280±0.131[a]
Group II	3.470±0.085[a]	3.623±0.080[a]	4.003±0.052[a]	4.180±0.164[a]
Group III	3.397±0.071[a]	3.550±0.121[a]	4. 127±0.112[a]	4.403±0.055[a]
Group IV	3.507±0.071[a]	3.647±0.076[a]	4.003±0.140[a]	4. 236±0.152[a]

*: There is a statistical difference between the data shown with different letters ($p<0.05$)

Table 8.

Average total length of the juvenile fish in the experiment

GROUPS	TIME			
	Initial	30th day	60th day	90th day
Group I	5.167±0.124[a]	5.783±0.137[a]	6.567±0.472[a]	7.330±0.132[a]
Group II	5.177±0.127[a]	5.557±0.481[a]	6.737±0.150[a]	7.157±0.158[a]
Group III	5.080±0.095[a]	5.530±0.069[a]	6.913±0.201[a]	7.377±0.153[a]
Group IV	5.183±0.045[a]	5.780±0.069[a]	6.873±0.187[a]	7.323±0.240[a]

*: There is a statistical difference between the data shown with different letters ($p<0.05$)

No statistically significant difference was determined in initial live weight, average total length and average standard length of fish between the dietary groups and depending on the period of time in the experiment ($p> 0.05$). However, at the end of the 60th and 90th days, the highest average live weight and standard length, total length were observed in Group III (50 mg.kg^{-1}) ($p>0.05$).

There was no statistically significant difference between the initial condition factor values of the fishes in the experiment groups ($p> 0.05$). There were also no significant differences between the groups on the 30th and 60th days. There was a significant difference in Group III (50 mg.kg^{-1}) and Group IV (75 mg.kg^{-1}) on the 90th day ($p <0.05$) Table 9.

Table 9.

Condition factor of juvenile fish in the experiment (g.(cm^3)$^{-1}$)

GROUPS	TIME			
	Initial	30th day	60th day	90th day
Group I	2.494±0.124[a]	2.139±0.136[a]	2.060±0.323[a]	1.737±0.132[ab]
Group II	2.495±0.035[a]	2.512±0.622[a]	1.845±0.105[a]	1.751±0.158[ab]
Group III	2.565±0.090[a]	2. 348±0.207[a]	1.939±0.118[a]	1.907±0.153[b]
Group IV	2.495±0.020[a]	2.177±0.029[a]	1.822±0.057[a]	1.681±0.240[a]

*: There is a statistical difference between the data shown with different letters ($p<0.05$)

There was no statistically significant difference between groups on the 30th day according to Group I (Control) in the specific growth rate, ($p > 0.05$). There was a significant difference in Group III (50 mg.kg^{-1}) on the 60th and 90th day according to all groups ($p < 0.05$) Table 10.

Table 10.

Specific growth rate of juvenile fish in the experiment (division.day^{-1})

GROUPS	TIME		
	30th day	60th day	90th day
Group I	0.613±0.094^{a}	0.860±0.078^{a}	0.765±0.081^{ab}
Group II	0.643±0.134^{a}	1.811±0.074^{a}	0.6851±0.115^{a}
Group III	0.550±0.357^{a}	1.073±0.190^{b}	0.913±0.016^{b}
Group IV	0.623±0.125^{a}	0.881±0.181^{ab}	0.709±0.113^{a}

*:There is a statistical difference between the data shown with different letters ($p<0.05$)

The survival rate of fry fish in the experiment is given in Table 11.

Table 11.

The survival rate of juvenile fish in the experiment (%)

GROUPS	TIME		
	30th day	60th day	90th day
Group I	100.0	98.70	96.00
Group II	100.0	97.30	97.30
Group III	100.0	98.70	97.30
Group IV	100.0	98.70	96.00

Feed conversion ratio of juvenile fish in the experiment (FCR) is given in Table 12.

Table 12.

Feed conversion ratio of juvenile fish in the experiment (FCR)

GROUPS	FCR
Group I	2.367 ± 0.060
Group II	2.368 ± 0.173
Group III	2.341 ± 0.105
Group IV	2.474 ± 0.226

Amount of carotenoid

The amount of carotenoid measured at the end of 90th day in experiment groups is given in Table 13.

Table 13.

The amount of carotenoid of juvenile fish in the experiment (mg.kg^{-1})

GROUPS	CAROTENOID AMOUNT
Initial	2.229[a]
Group I	1.924[b]
Group II	2.009[c]
Group III	4.321[d]
Group IV	4.713[e]

*: There is a statistical difference between the data shown with different letters (p<0.05)

The difference in carotenoid amount between groups was found significant (p <0.05). The highest pigmentation was observed in fish in Group IV (75 mg.kg^{-1}). The Second-high value was found in fish fed dietary Group III (50 mg.kg) Fig. 2.

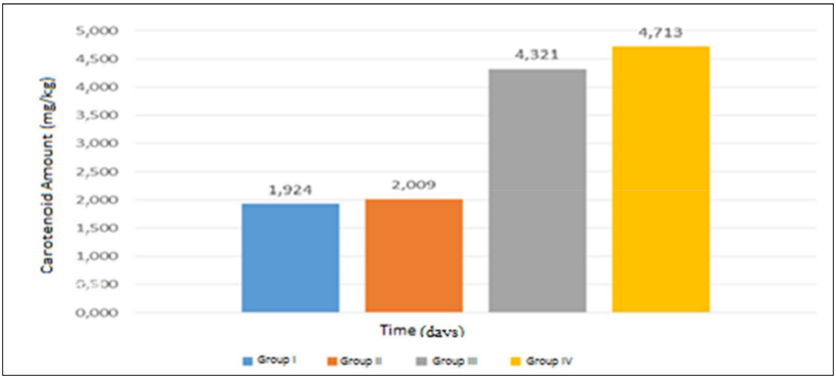

Fig. 2. The amount of carotenoid of juvenile fish in the experiment

Although carotenoids are known to have positive effects on intermediate metabolism in fish [21], the debate about their role in fish growth continues. Some researchers report that carotenoids accumulate in tissues cause pigmentation in fish in addition to their positive effects on growth while others have mentioned that they do not have a positive effect [33]. The presence of other constituents in the feeds in which natural carotenoid sources are used and their interactions may also increase growth [34]. It has been reported that when Goldfish *(C. auratus)* larvae and juveniles fed for 12 weeks with five different feeds (45 mg.kg^{-1} *H. pluvialis, C. vulgaris, S. platensis,* synthetic pigments astaxanthin and control group), there was no positive effect on the rate of growth

and survival**.** The differences between the groups was not significant in terms of average live weight gains, standard height and total height values for 12 weeks (90 days) in this study and results are in line with what was reported for previous studies.

The condition factor (CF) is the best formula to control the morphological structure of fish and is one of the criteria for indicating nutritional status and development [35]. Generally, condition factor should be close to 1 in fish. Condition factor in a trout with good nutritional requirements is optimum 1:37, a trout with condition factor above 1.53 is accepted very fatty while a trout with condition factor under 1.14 is considered to be too lean [36]. In our study, the condition factor was 1.74, 1.75, 1.91 and 1.68 respectively. These results reveal that conditions of the fish used in the study were low while they had high fat ratios. The reason for this is thought to be because of the high fat content in the given pellets.

The weight and the period for weight gain must be related in order to be able to determine growth. For this purpose, specific growth rate (SGR) is often used as a growth rate in fish. SGR was found to be 1.1 in the group fed with *S. platensis* when Goldfish *(C. auratus)* larvae and juveniles were fed for 12 weeks, with five different feed (45 $mg.kg^{-1}$ *H. pluvialis, C. vulgaris, S. platensis,* synthetic pigments astaxanthin and control group) [34]. SGR was found to be an average of 1.24 in goldfish fed with *Tagetes erecta* (marigolds) in different doses in another study done**.** Average SGR values of the experimental group in our study were 0.77, 0.69, 0.91 and 0.71respectively. We think that the SGR results which are found lower compared to other studies is due to our use of fish in the juvenile period instead of fish in the larval stage.

Larval and juvenile survival rates are also discussed in addition to the positive effect of carotenoids on fish pigmentation [37]. It has been reported that addition of green water culture into larvae tanks in marine fish farming increases the survival rate of juvenile fish, effects growth and increases feed consumption rate**.** It was determined in our experiment that the rate of survival is ideal and does not constitute any issue.

The formula named FCR worldwide is known as the conversion rate of feed to meat. Generally, conversion of fish feed to meat increases when FCR is about 1 or gets closer to 1**.** This ratio is highly variable in aquarium fish. It is known in the studies conducted with Goldfish that it varies between 2.0-5.5 [33, 36]. FCR has been found as 2.37, 2.37, 2.34 and 2.47 respectively in our study and it shows compatibility with previous studies.

Fish have a specific carotenoid metabolism, and the efficiency of carotenoids stored as a pigment source varies from species to species [38]. *Spirulina sp.* is

a good source of carotenoids with its high content of xanthophylls, β-carotene and zeaxanthin and affects the pigmentation of fish significantly [39]. In previous studies, *Spirulina sp*. has caused a successful pigmentation in red tilapia**,** swordtail**,** blue gourami (*Trichogaster trichopterus*) and goldfish *(C. auratus*) species [39]. Although best results were obtained with *H. pluvialis* and *C. vulgaris* in juvenile *C. auratus* fed with five different feeds (*H. pluvialis, C. vulgaris, S. platensis,* synthetic astaxanthin and control), effect of *S. platensis* on the pigmentation was quite high [39]. The highest pigmentation was observed in groups IV and III and the amount of carotenoid detected in groups I and II was found to be lower than the initial values in our study. The reason for this is thought to be the feeding of the fish with diets with high carotenoid content before being received from the farm.

Conclusions

In conclusion, it has been determined that *S. platensis* added to feed in different levels did not have a significant effect on the growth of goldfish but contributed to the skin pigmentation in our study. The best carotenoid rate was achieved in the feeding group in which 75 $mg.kg^{-1}$ of S. *platensis* was added into the diets.

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