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Research Article

The Effects of Red Pepper, Marigold Flower, and Synthetic Astaxanthin on Pigmentation, Growth, and Proximate Composition of *Penaeus semisulcatus*

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Abstract: Shrimps with an initial weight of 11.10 ± 0.26 g were fed diets supplemented with 6.6% red pepper (RP), 2.4% marigold flower (MF), and 100 mg/kg synthetic astaxanthin (SA), each of which contained 100 mg/kg total carotenoid, and a control diet (BD), without carotenoid supplement for 60 days.

Dietary carotenoid sources did not significantly affect the growth of the shrimps ($P > 0.05$). SA supplementation yielded the highest survival rate (92%), followed by RP (82%) and the other diets (75%) ($P < 0.05$). All dietary carotenoid sources provided higher carotenoid accumulation compared to the control group ($P < 0.05$), but the difference between them was not significant ($P > 0.05$).

RP and SA resulted in a greater protein increase in shrimp muscle than MF ($P < 0.05$); however, lipid, ash, and water content were similar in all diet groups ($P > 0.05$).

Key Words: Red pepper, marigold flower, astaxanthin, pigmentation, flesh composition, growth, *Penaeus semisulcatus*

Kırmızı Biber, Kadife Çiçeği ve Sentetik Astaksantin'in *Penaeus semisulcatus*'un Pigmentasyon, Büyüme ve Besin Bileşenleri Üzerine Etkisi

Özet: Başlangıç ağırlığı 11,10 ± 0,26 g olan karidesler her biri 100 mg/kg karotenoyit içeren % 6,6 kırmızı biber (RP), % 2,4 kadife çiçeği (MF), 100 mg/kg sentetik astaksantin (SA) ve karotenoyit içermeyen kontrol grubu (BD) diyetleriyle 60 gün beslenmişlerdir.

Karotenoyit kaynaklarının karideslerin büyümesi üzerine bir etkisi olmamıştır ($P > 0,05$). En yüksek yaşama oranını SA diyeti (% 92) göstermiş ve bunu sırasıyla RP (% 82) ve diğer diyetler (% 75) izlemiştir ($P < 0,05$). Tüm karotenoyit katkılı gruplar kontrol grubuna göre daha fazla karotenoyit birikimi sağlamış ($P < 0,05$), ancak kendi aralarında önemli bir farklılık bulunmamıştır ($P > 0,05$).

RP ve SA karideslerin kas dokusunda MF' ye göre daha fazla bir protein artışına neden olmuştur ($P < 0,05$). Lipit, ham kül ve su miktarları ise tüm diyet gruplarında benzer bulunmuştur ($P > 0,05$).

Anahtar Sözcükler: Kırmızı biber, kadife çiçeği, astaksantin, pigmentasyon, et kompozisyonu, büyüme, *Penaeus semisulcatus*

Introduction

The pigmentation level of an aquatic animal may be an important factor affecting its market value and may also directly indicate its healthiness and quality (1). The most effective carotenoid responsible for pigmentation in shrimps is astaxanthin (2). It is well known that carotenoids are synthesised by algae, some micro-organisms, and fungi, as well as higher plants. Animals, including those of aquatic origin, cannot synthesise these pigments, but can convert them into other forms (3). As a result, all animals have to rely on carotenoid producing organisms for their requirement (4,5).

In intensive systems, shrimps cannot efficiently utilise natural productivity and have to rely on artificial food, which in most instances contains limited amounts of carotenoids. Artificial carotenoids, such as astaxanthin or canthaxanthin, are generally included in shrimp diets to enhance pigmentation. Studies on the pigmentation of

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Marsupenaeus japonicus juveniles showed that dietary carotenoids may improve survival and growth (1,6); however, Yamada et al. (7) found no notable increase in growth, feed efficiency, or daily feed intake of *M. japonicus* reared on diets with and without carotenoids.

Studies of *Marsupenaeus japonicus* have shown that diets containing 50-400 mg/kg carotenoids can cause a carotenoid deposition of 15-40 mg/kg in abdomen muscle, depending on the duration of application (1-2 months) (6, 7). A positive linear relationship has been established between the level of dietary carotenoids and survival of shrimps (1,8). Yamada et al. (7) observed a 91% survival rate when *M. japonicus* were fed a diet supplemented with 100 mg/kg synthetic astaxanthin (SA) for 4-8 weeks in comparison to a 57% survival rate among the controls. SA has been found to increase moulting and accelerate growth in post-larval shrimp (9). In addition, carotenoids have been suggested to a play role similar to vitamin A (10). It has been suggested that carotenoids are essential and must be included in the diets of aquatic animals at a minimum level of 5-10 mg/kg (11).

The most promising alternatives to SA for crustacean pigmentation today are apparently Spirulina spp. (12,13), red pepper (RP) (14), *Phaffia rhodozyma* and krill oil (12), *Dunaliella salina* (1,15), and alfalfa (15). RP is a good source of carotenoid (800-1500 mg/kg) and is abundant in Turkey (16-18). Most studies concerning RP have been carried out on salmonids and only one study has dealt with the effects of RP on pigmentation of shrimp, *Litopenaeus vannamei* (14). Marigold flower (MF) (*Tagetes erecta*), which has been commonly used in the pigmentation of poultry, is also abundant in Turkey (19). This plant contains total carotenoid of between 1320 and 5700 mg/kg (20). To the best of our knowledge, no previous study has been conducted on the pigmentation potential of this plant in penaeid shrimps.

In the developing world, public interest in the consumption of more natural foods is increasing. A rise in health problems and unidentified diseases has been attributed to the use of synthetic products in human foods. Therefore, the main aim of the present study was to assess 2 inexpensive, natural carotenoid sources (RP and MF) on pigmentation, growth, and body composition of the green tiger shrimp, *Penaeus semisulcatus*, one of the most commercially important species in the north-eastern Mediterranean Sea (21,22), as potential replacements for astaxanthin in their diet.

Materials and Methods

Experimental Procedure

The effects of RP, MF, and SA on growth and survival (first experiment), and pigmentation (second experiment) of *Penaeus semisulcatus* juveniles (initial weight of 11.10 ± 0.26 g) were measured. Both experiments were performed in 3 replicates, each for 60 days. The shrimps were obtained from Yumurtalık Marine Research Station, University of Çukurova, Adana, Turkey.

The first experiment was carried out in 100-l round concrete tanks with a diameter of 90 cm. Fifteen shrimps per tank were stocked randomly for each experimental feed. All the shrimps from each tank were weighed every 10 days to determine growth. Growth performance of shrimps was computed in terms of specific growth rate (%/day), daily growth rate (g/day), and average weight (g) on sampling days.

The second experiment was performed in 250-l round plastic tanks with a diameter of 55 cm. Eight shrimps per tank were stocked randomly for each experimental feed. Two shrimps from each tank were sampled every 20 days for total carotenoid analysis.

Shrimps were fed ad libitum 4 times a day. Continuous aeration was supplied to each tank by a blower. Following removal of excess water with tissue paper, the individual weight of each animal was measured to the nearest 0.001 g. Salinity (YSI-30, YSI Industries, Yellow Springs, OH, USA), pH, temperature, and dissolved oxygen (WTW pH-meter and oxygen-meter, Wilhelm, Germany) levels in each tank were monitored daily throughout the study. Daily water exchange rate in culture tanks was maintained at 150%-200% per day.

Feeding Trial

Four different diets, described below, were used in the experiment.

BD: a control diet, or Basal diet (no carotenoid supplementation), containing 45% crude protein, 10% crude fat, 3% crude fibre, 13% crude ash, 88% dry matter, and 4100 ME kg/cal energy (obtained from Çamlı Yem A.Ş., İzmir, Turkey).

SA: a diet of 100 mg/kg SA (supplied by Hoffman-La Roche, Switzerland) in the form of carophyll-pink containing 80,000 mg/kg SA. This pigment source was dissolved in water at 50 °C prior to adding to BD.

RP: a diet containing 6.6% RP to obtain a total carotenoid content of 100 mg/kg.

MF: a diet containing 2.4% MF to obtain a total carotenoid content of 100 mg/kg.

Both vegetable carotenoid sources supplied by local producers were dried at 40 °C and ground into powder before being added to BD. Protein levels of the experimental diets were equalised by adding fish meal containing 71% protein. The pellets for all 4 diets were produced in the same way. The ingredients were first turned into a homogeneous doughy consistency by adding water; and then converted into pellet form by being pressed through a sieve with 3 mm holes in a grinding machine. The pellets were stored in refrigerator containers at -20 °C. Pellets were thawed before they were given to the fish.

Carotenoid Analysis

The carotenoid content of the samples was determined as described by Torrissen and Naevdal (23). Two shrimp samples were used for carotenoid analysis and the analyses were run in triplicate.

Abdomen samples of 0.5-1 g, viscera-free, were collected from the abdominal muscle and homogenised with a homogeniser and then transferred to 10-ml pre-weighed glass tubes. First, 10 ml of dry acetone was added to the samples, which was followed by about 1-1.5 g of anhydrous sodium sulphate. The solutions were centrifuged at 5000 rpm for 5 min and then stored in a refrigerator at 4 °C. After 3 days of extraction in sealed glass tubes, the absorption of the extracts was measured at 476 nm in a spectrophotometer. A similar method was used for the total carotenoid analysis of RP and MF meal; but anhydrous sodium sulphate was not used in these plants, as it was already in dry form, and measured at 450 nm.

Total carotenoid concentration in the samples was calculated spectrophotometrically in acetone using $E_{(1\%, 1cm)}$ = 1900 (24) at 474 nm for muscle samples and $E_{(1\%, 1cm)}$ = 2500 (25) at 450 nm for both plants. The total carotenoid concentration of dried RP and MF was 1500 and 4200 mg/kg, respectively, and these amounts were taken into account while adding them to the diets (Table 1).

Proximate Composition Analysis

Crude protein, fat, ash, and moisture content of samples were determined according to AOAC (1980) methods as 7.057, 7.056, 7.009, and 7.003, respectively. Three samples were used for each analysis and all analyses were run in duplicate (26) (Table 1).

Statistical Analysis

The data were analysed with one-way ANOVA and significant differences were determined at 0.05 probability by Scheffé's multiple comparison test after the normality and homogeneity (Bartlett's test) of the data were checked with Minitab Statistical Package.

Results

In the first experiment, although RP supplementation resulted in the highest weight gain, followed by the group fed the SA fortified diet, no significant difference was observed among the diet groups in terms of specific growth rate and average final weight ($P > 0.05$, see Figure and Table 2). Daily growth rates ranged between 0.04 and 0.05 g/day, while specific growth rate ranged between 0.28% and 0.37%/day. On the other hand, survival rate was significantly different among the diet groups ($P < 0.05$). The shrimps fed SA had the highest survival rate (92%), followed by RP (82%), MF (75%), and the control group (75%) ($P < 0.05$) (Table 2).

Table 1. Proximate composition and carotenoid content of natural carotenoid sources.

Carotenoid sources	Proximate composition of samples (%)				Carotenoid content (mg/kg)
	crude protein	crude fat	crude ash	moisture	
Marigold flower	12.2 ± 0.35	9.2 ± 0.12	8.24 ± 0.53	6.18 ± 0.15	4200 ± 0.10
Red pepper	12.3 ± 0.23	16.1 ± 0.67	6.15 ± 0.39	7.23 ± 0.12	1500 ± 0.15

Each value is a mean ± sd (n = 3 replicates). Each replicate was run in duplicate

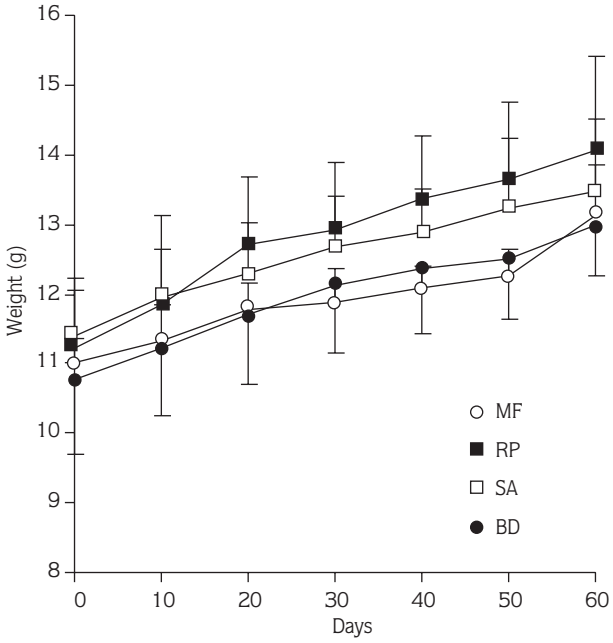

Figure. Final mean weight of *Penaeus semisulcatus* fed different diets for 60 days. Each symbol represents a mean (n = 3 replicates).

In the second experiment, total carotenoid content of the flesh of the shrimps started to vary among the groups on day 20 of the culture period ($P < 0.05$) (Table 3). The carotenoid accumulation rate in the flesh in the second and third part of the experiment was considerably lower compared to the first 20-day culture period. The shrimps had the greatest carotenoid accumulation with SA, followed by RP and the other groups until the 40th day of the culture period ($P < 0.05$); however, final total carotenoid accumulation in shrimp flesh was similar in all the dietary carotenoid groups ($P > 0.05$). On the other hand, all dietary carotenoid groups provided carotenoid accumulation higher than the control group at the end of the experiment ($P < 0.05$).

Carotenoid content of the shrimps fed the control diet remained stable (between 13.07 and 13.76 mg/kg), while the carotenoid level rose from 13.07 to 22 mg/kg in those fed the RP and SA diets. The shrimps fed MF also accumulated carotenoids at a level of up to 20 mg/kg.

Meat yield did not differ (49.94%-50.99%) among the groups (Table 4). Flesh protein content of the shrimps fed the experimental diets ranged from 20.69% (MF) to 22.16% (SA) ($P < 0.05$) (Table 4). Flesh lipid content was the lowest in the group fed the MF diet (1.24%) as compared to the other experimental diets (1.30%-1.46%). Ash and water content ranged from 1.61% to 1.77% and from 74.76% to 75.19%, respectively (Table 4).

Discussion

In the present study, growth of *P. semisulcatus* was not affected by the addition of synthetic or natural carotenoids into their diet, but survival was. The effects of carotenoids on growth and survival rate of aquatic organisms have been controversial. Harpaz and Schmalbach (28) stated that dietary plant matter, which can also serve as a pigment source, had a beneficial effect on crustacean moulting and growth. Similarly, Chien and

Table 2. Daily and specific growth rates, final weight, and final survival of *Penaeus semisulcatus* fed different diets.

Diets	Daily growth rate (g/day)	Specific growth rate (%/day)	Final weight (g)	Final survival (%)
MF	0.04	0.29 ± 0.13^{a}	13.12 ± 0.72^{a}	75.0 ± 4.50^{c}
RP	0.05	0.37 ± 0.19^{a}	14.06 ± 1.35^{a}	83.0 ± 3.26^{b}
SA	0.04	0.28 ± 0.04^{a}	13.46 ± 1.05^{a}	91.7 ± 6.25^{a}
BD	0.04	0.32 ± 0.11^{a}	13.00 ± 0.75^{a}	75.0 ± 2.86^{c}

The means with different letters in each column denote a significant difference ($P < 0.05$). Each value is a mean ± sd (n = 3 replicates) and each replicate consists of the measurement of 11-15 shrimps (for weight data).
Specific Growth Rate (%/day) = 100 x (In W_t-In W_o)/t; Daily Growth Rate (g/day) = W_t-W_o/t; Wt and W0 are final and initial weights (g), respectively; t is the feeding duration (day) (27).

Table 3. Total carotenoid accumulation in the flesh of *Penaeus semisulcatus* (mg/kg) fed different diets.

Diets	Total Carotenoid content (mg/kg)			
	Day 0	Day 20	Day 40	Day 60
MF	13.07 ± 0.58[a]	15.96 ± 1.85b	18.09 ± 0.53[c]	19.74 ± 1.76[a]
RP	13.07 ± 0.58[a]	17.09 ± 1.53[ab]	19.89 ± 1.35[b]	21.29 ± 1.04[a]
SA	13.07 ± 0.58[a]	18.63 ± 0.78[a]	21.52 ± 1.57[a]	22.08 ± 2.09[a]
BD	13.07 ± 0.58[a]	13.13 ± 0.57[c]	13.30 ± 0.79d	13.76 ± 2.36[b]

The means with different letters in each column denote a significant difference ($P < 0.05$). Each value is a mean ± sd (n = 3 replicates) and each replicate consists of 2 shrimps. Each carotenoid analysis was run in triplicate.

Table 4. Meat proximate composition (% wet weight) and edible meat of *Penaeus semisulcatus* (mg/kg) fed different diets.

Diets	Proximate Composition (% wet weight)				
	Protein	Lipid	Ash	Water	Edible Meat
MF	20.69 ± 0.33b	1.24 ± 0.09[ab]	1.70 ± 0.04[ab]	75.19 ± 0.33[a]	50.99 ± 0.19[a]
RP	21.95 ± 0.18[a]	1.46 ± 0.06[a]	1.61 ± 0.05[b]	75.01 ± 0.23[a]	50.70 ± 0.70[a]
SA	22.16 ± 0.68[a]	1.42 ± 0.05[a]	1.75 ± 0.02[a]	74.92 ± 0.22[a]	50.60 ± 0.70[a]
BD	21.42 ± 0.57[ab]	1.30 ± 0.19[ab]	1.77 ± 0.10[a]	74.76 ± 0.24[a]	49.94 ± 0.63[a]

The means with different letters in each column denote a significant difference ($P < 0.05$).
Each value is a mean ± sd (n = 3 replicates) and each replicate consists on measurement of 3 shrimps (for proximate analyse data) or 11-15 shrimps (for edible meat data). Each proximate analysis was run in duplicate.

Jeng (1) reported a higher survival rate of animals (*P. japonicus*) fed astaxanthin-supplemented diets compared to animals fed a supplement of beta carotene or algal meal; yet, the supplementation of diet with β-carotene or astaxanthin had no significant effect on growth, survival rate, or feed conversion ratio in *P. monodon* (29). Other studies carried out with crustaceans have also reported non-significant effects of dietary carotenoids on both growth and survival (7,15).

In the present study, carotenoid accumulation in the flesh was faster during the first 20 days of the culture as compared to the remainder of the rearing period. This may be because the shrimp muscle reached its maximum carotenoid accumulation level (satiation point) during the first 20 days. The satiation point for rainbow trout fillet was stated to be between 6 and 8 mg/kg (30); however, this satiation point has yet to be studied in shrimps. Nonetheless, Arredondo-Figueroa et al. (14) pointed out that carotenoid accumulation in the abdomen muscle of *Litopenaeus vannamei* reached a level of 17 mg/kg. The results of our study showed that the satiation point may be close to 20-22 mg/kg for *P. semisulcatus*.

Even though during the first 40 days SA provided better pigmentation than RP or MF, final total carotenoid accumulation in the flesh was similar in all the experimental groups ($P > 0.05$). On the other hand, all dietary carotenoid sources provided carotenoid accumulation higher than the BD at the end of the experiment ($P < 0.05$). Total carotenoid content of the abdomen muscle of the shrimps ranged between 20 mg/kg (for MF) and 22 mg/kg (for RP and SA). In other studies, depending on the carotenoid level and application

time, carotenoid content in the entire body of different crustacean species has been reported to range between 10 and 40 mg/kg (6,7,15).

Our results clearly showed that the shrimp were able to successfully utilise the natural carotenoid sources (RP and MF) at a rate similar to SA. Unlike salmonids (17), our results, and those of Miki et al. (13) and Arredondo-Figueroa et al. (14) suggest that penaeid shrimps are capable of readily metabolising plant carotenoids such as zeaxanthin and lutein, which are plentiful in RP, MF, and spirulina. Similar results were also reported for other crustaceans such as crayfish (*Cherax tenuimanus*) fed a diet containing alfalfa meal (15).

In the current study, a yellow pigmentation partially occurred in the muscle of the shrimp fed MF, which was distinctly different from the other groups. This yellow pigmentation might have occurred due to the accumulation in the flesh of the shrimp of xanthophyll type carotenoids in the MF. Similar pigmentation was also observed in rainbow trout when fed a diet of MF meal (19). Further studies are recommended to determine what types of carotenoids from MF, other than astaxanthin, are deposited in the flesh of shrimps.

In conclusion, both natural carotenoid sources (RP and MF meal) successfully promoted pigmentation comparable to SA in *P. semisulcatus*. This is a highly significant development for commercial aquaculture, because it shows that a similar result can be achieved by supplementing diets with RP or MF instead of SA, which is much more expensive. It is therefore recommended that either of these natural carotenoid sources can be incorporated into shrimp diets to obtain desired pigmentation.

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