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RESEARCH ARTICLE

The effects of dietary supplementation synthetic Astaxanthin on body astaxanthin, survival, growth of white leg shrimp (*Litopenaeus vannamei*)

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Abstract

The effect of dietary astaxanthin was evaluated for survival, growth parameters and astaxanthin accumulation of postlarval *Litopenaeus vannamei*. The experimental diets supplemented at 0, 50, 100 and 200 mg astaxanthin kg^{-1} and was performed with 8 day-old postlarval shrimp (mean initial wet weight 5.3±1.6 mg) for 30 days. Shrimp fed with a diet without astaxanthin pigment served as control. Shrimp fed diets containing 50, 100 and 200 mg astaxanthin kg^{-1}, survival and growth was significantly higher than the control group. The pigment accumulation rate was determined by high performance liquid chromatography. The analysis of pigment accumulation showed that the increasing astaxanthin pigment amount in the diets, survival and resistance to salinity stress test increased in postlarvae. Due to nutritional properties of astaxanthin pigment and the positive effect on growth and survival, the feeding postlarval white leg shrimp with the diet containing at least 100 mg astaxanthin/kg is recommended.

INTRODUCTION

The contribution of aquaculture in the supply of fish, crustaceans, molluscs and other aquatic animals is growing, so rising from 3.9 percent in 1979 to 36 percent in 2006 (FAO, 2008). Shrimp production has experienced exponential growth since the early 1980s. In recent years, concerns about the stability of shrimp culture has increased. Therefore, the quality of postlarvae to be stored in the nursery and grow-out ponds is involved in the sustainable production of shrimp culture (Lovens and sorgeloos, 2000).

The main source of synthetic carotenoid pigments used in aquaculture is astaxanthin and canthaxanthin and most important of natural astaxanthin source are red yeast (*Xanthophyllomyces rhodorhous*) and green algae (*Haematococcus Pluvialis*) (Bjerkeng, 2008). Aquatic animals cannot synthesize carotenoids and obtain carotenoids from their diets (Boonyaratpalin et al., 2001;; Meyers, 1994; Wouters et al., 2001). Astaxanthin is main and dominant carotenoid in shrimp (Chien and Shiau, 2005; Darachai et al., 1998; Meyers, 1994).Besides pigmentation properties of carotenoids, increasing attention is being directed toward defining the biological function of astaxanthin in aquatic animals (Meyers and Latscha, 1997). Survival improvement in kuruma prawn, *Marsupenaeus japonicus* (Chien and Jeng, 1992), and tiger prawn, *Penaeus monodon* (Thongrod et al., 1995), by dietary astaxanthin supplementation was reported. Recent studies showed that enhancement of resistance to oxygen depletion stress (Chien et al., 1999), salinity stress (Darachai et al., 1998; Merchie et al., 1998; Chien et al., 2003), thermal stress (Chien et al., 2003), ammonia stress (Pan et al., 2003a), and pathological stress (Pan et al., 2003b) in penaeid postlarvae was associated with an increase in dietary and body astaxanthin. Niu et al., (2009) and Parisenti et al., (2011) studied carotenoid sources as dietary supplements and found that carotenoids pigments can increase the growth parameters. Studies suggest that, astaxanthin pigment may also enhance the survival of aquatic animals. Niu et al. (2009) found that survival of white leg shrimp (*Litopenaeus vannamei*) postlarvae increased through diet supplemented with astaxanthin.This study was thus designed to systematically compare different levels of synthetic astaxanthin in diets, for their effects on body astaxanthin, survival, and growth of white leg shrimp.

2.Material and method

In this study the effect of synthetic astaxanthin dietary (Lucanthin Pink, 10% Astaxanthin, Basf Nutrition, Germany) at various levels (0, 50, 100 and 200 mg astaxanthin kg^{-1}) on survival and resistance to salinity stress test for the white leg shrimp was evaluated. Eight-day-old postlarvae (mean initial wet weight 5.3±1.6 mg) were obtained a local commercial hatchery. The study included four treatments and each treatment was performed with three replications. The experiments were carried out at the Aquaculture Development Center of Kolahi located in port Kolahi, Hormozgan, Iran. Before the experiment, they were acclimated for 3 days to experimental conditions and during this period the postlarvae were fed by the without supplementing astaxanthin diet (control diet) (Niu et al., 2009). The culture performed in twelve 300-litre fibreglass tanks and stocking density at each tank was 400 postlarvae, the experiment duration was 30 days. To obtain the mean initial wet weight, 100 postlarvae were collected randomly in three groups, then the obtained weight of each group was divided by the number of postlarvae. Postlarvae were fed four times per day (0700, 1300, 1800 and 2200 hours) at 12% of body weight. Leftover and faces were siphoned and 35% of the water was exchanged daily. During the experiment period, physicochemical properties of water, included temperature, dissolved oxygen, salinity and pH were 29±1 C, 5.7±0.4 mg/L, 40 ‰ and 8.3±0.04, respectively. At the end of the experiment, postlarvae from each tank were counted to evaluate the survival rate.

2.1.Growth and Survival parameters

After 30 days of experimenting, growth parameters such as Specific growth rate (SGR, % /day), final body wet weight and weight gain (WG) and survival rate according to the following equations:

$SGR (\%/day) = 100 \times (\ln W_e - \ln W_s) / t$

$WG (\%) = ((We - Ws) / Ws) \times 100$

Survival (%) = 100 × (final number of shrimp / initial number of shrimp)

Where W_e are final weight, Ws is initial weight and t is the duration of the experiment (number of days).

2.2.Diet Preparation

All diet has the same ingredients except the level of the astaxanthin pigment (Lucanthin Pink, 10% Astaxanthin, Basf Nutrition, Germany) , including 0 (control diet), 50, 100 and 200 mg astaxanthin kg^{-1}. The experimental diets Composition is shown in table 1. All ingredients were weighed and thoroughly mixed in mixer, then the soybean oil was added and mixed again for 5 min. Deionised water (350 ml kg^{-1}) was added and the mixing for 5 min (Supamattaya et al., 2005). The mixture was then extruded through a 1.5-mm diameter dies in a meat grinder. Then the resulting pellets were exposed to sunlight for 24 hours. Then the dried pellets were broken up and sorted through a series of different-sized (200, 500 µm, 800, 1000 and 1200 µm) metal sieves. The diets were stored at -20 C until the start of the experiment.

2.3.Biochemical analysis

For biochemical analysis some of the experimental diet was randomly taken out from each diet (triplicate samples). The analysis of crude protein, crude lipid, ash and moisture of the diets were determined according to AOAC methods (AOAC, 1990). Crude protein and crude lipid were determined by Kjeldahl method and Soxhlet extraction, respectively. Moisture was determined by oven drying at 105°C. Ash was determined at the residue after muffle furnace ignition at 550°C for 6 h. Analysis of the experimental diets is shown in table 2.

2. 4.Determination of astaxanthin content of whole body postlarvae

After 30 days of experimentation, the samples were sent to the laboratory for determination of astaxanthin. Analysis of astaxanthin content was carried out according to the method described by Chien et al. (2003).

2.5.Statistical analysis

Statistical analysis was performed using Excel and SPSS 17.0 statistical software. Effect

By treatment was carried out using a one-way ANOVA followed by a post-hoc Tukey's multiple comparison test with a significance level of $P < 0.05$.

3.Result

Table 1. Composition of experimental diets (g per kg dry matter)

Ingredient	Diet 0 mg kg^{-1}	Diet 50 mg kg^{-1}	Diet 100 mg kg^{-1}	Diet 200 mg kg^{-1}
Fish meal	520	520	520	520
Soybean meal	130	1310	130	130
Wheat meal	188	187.5	187	186
Corn gluten	50	50	50	50
Soybean oil	30	30	30	30
Vitamin mix	15	15	15	15
Vitamin C	7	7	7	7
Mineral mix	40	40	40	40
Binder	20	20	20	20
Astaxanthin	0	0.5	1	2

Table 2. Chemical analysis of experimental diets

Crude protein (%)	40.89 ± 0.19
Crude lipid (%)	11.63 ± 0.18
Moisture (%)	9.85 ± 0.24
Ash (%)	12.08 ± 0.31

3.1.Growth and survival

Post-larvae that were fed with astaxanthin diets, growth parameters were higher than the control group. After 30 days, specific growth rate (SGR), Final body wet weight and weight gain (WG) of post-larvae fed the control diet, significantly lower than supplementing diets with astaxanthin pigment ($p<0.05$). The growth rate in experimental diets was shown in table 3.

Table 3. Growth parameters of white leg shrimp postlarvae fed diets with various levels of astaxanthin

Treatments	Initial weight (Mean ±SE)*	Final wet weight (Mean ±SE)*	SGR (%) (Mean ±SE)*	WG (%) (Mean ±SE)*
0 mg kg^{-1}	5.3 ± 1.6	482.2 ± 28.9[a]	15.1 ± 0.2[a]	9036±546[a]

50 mg kg^{-1}	5.3 ± 1.6	670 ± 27.4^{b}	16.1 ± 0.1^{b}	$12542±517^{b}$
100 mg kg^{-1}	5.3 ± 1.6	690.1 ± 13.5^{b}	16.2 ± 0.1^{b}	$12921±254^{b}$
200 mg kg^{-1}	5.3 ± 1.6	700.6 ± 12.7^{b}	16.3 ± 0.1^{b}	$13118±239^{b}$

*Mean ±SE Values in any row with different superscript are significantly different (P<0.05).

The survival rate in shrimp fed the experimental diets were shown in table 4. Survival of shrimp was from between 54.7% to 82%. Survival was significantly greater in shrimp fed with astaxanthin supplemented diets than shrimp fed the control diet (p<0.05). Among the supplemented diets with astaxanthin, Survival postlarvae fed diet containing 200 mg kg^{-1} had significantly higher than shrimp fed diet containing 50 mg kg^{-1} (p<0.05).
No significant differences in survival were observed among the supplemented diets with 100 and 200 mg kg^{-1} (p>0.05).

Table 4. The survival rate of white leg shrimp postlarvae fed diets with various levels of astaxanthin

Treatments	Diet 0 mg kg^{-1} (Mean ±SE)*	Diet 50 mg kg^{-1} (Mean ±SE)*	Diet 100 mg kg^{-1} (Mean ±SE)*	Diet 200 mg kg^{-1} (Mean ±SE)*
Survival	$54.7±4.5^{a}$	$76.5±2.3^{b}$	$78.5±1.8^{bc}$	$82±1.5^{c}$

*Mean ±SE Values in any row with different superscript are significantly different (P<0.05).

3.2.Astaxanthin accumulation

After 30 days of feeding, the amount of astaxanthin pigment in the shrimp body was between 17.5 to 23.4 mg kg^{-1} (Table 5). Astaxanthin content in shrimp fed with supplemented diets with 100 and 200 mg kg^{-1} were significantly higher than the control group and diet containing 50 mg kg^{-1} astaxanthin (p<0.05).

Table 5. Astaxanthin content (mg kg^{-1}) in shrimp fed diets with various levels of astaxanthin

Treatments	Diet 0 mg kg^{-1} (Mean ±SE)*	Diet 50 mg kg^{-1} (Mean ±SE)*	Diet 100 mg kg^{-1} (Mean ±SE)*	Diet 200 mg kg^{-1} (Mean ±SE)*
Astaxanthin content	$17.5±0.8^{a}$	$18.1±1.1^{a}$	$22.1±1.2^{b}$	$23.4±1.1^{b}$

*Mean ±SE Values in any row with different superscript are significantly different (P<0.05).

4.Discussion

There are various functions of carotenoid pigments. So the carotenoids positive role in the metabolism of aquatic animals are known (Segner et al., 1989), such as antioxidant activity, pigmentation, source of provitamin A (Miki, 1991; Latscha 1989; Meyers & Latscha 1997). The effect of carotenoid pigments on the growth parameters and survival in crustaceans is corroborated by other studies as well., but, on the other hand, in some studies, carotenoid pigments have no significant impact on growth and survival. Also, the growth performance and survival improve with increasing the astaxanthin content from 0 to 200 mg kg^{-1} . This agrees with the study of Niu et al., (2009). Niu et al., (2009) found that the growth and survival of postlarvae fed with astaxanthin supplemented diets was better than the control group, in which the highest survival was obtained in postlarvae fed the diet containing 200 mg kg^{-1} astaxanthin. Also, Chien and Jeng (1992) reported that survival in kuruma shrimp (*Marsupenaeus japonicus*) fed with astaxanthin diet was higher than other diets, it was found a positive correlation between survival rates and pigment accumulation in shrimp tissue. On the other hand, the study carried out by Boonyaratpalin et al., (2001) showed that astaxanthin diet has no significant effects on the growth and survival. Nègre-Sadargues et al.,(1993) found that the higher survival in shrimp has been observed that the 50 mg kg^{-1} astaxanthin and 50 mg kg^{-1} canthaxanthin were fed and higher growth rate observed in shrimp that receiving 100 mg^{-1} canthaxanthin. In this study, survival and growth rate of postlarvae was enhanced when a higher proportion of astaxanthin was added to the diet. Diets containing carotenoid pigments, contributed better to the survival rate of shrimp as compared with a diet without carotenoid pigments but there was no significant effect found on the growth performance (Chien and Shiau, 2005). Postlarvae growth and survival can be affected by nutrition and culture conditions (Palacios and Racotta., 2007). Flores et al., (2007) also reported that shrimp fed with a diet supplemented with 80 mg kg^{-1} astaxanthin had higher survival and growth than other diets also Molting frequency in shrimp fed diet containing 80 mg kg^{-1} was higher than other diets. The positive effect of astaxanthin due to being involved the physiological function of aquatic animals (Hunter, 2000) and strong antioxidant properties that the better of β-carotene and α-tocopherol (Miki, 1991). It has been suggested that carotenoids have various effects, for example, enhanced stress resistance, stimulated animal immune systems, resistance to white spot disease (WSSV) and improve survival and growth (Supamattaya et al., 2005; Torrissen and Christiansen, 1995; Niu et al., 2009). The present study confirms that the diet containing astaxanthin improves the survival and growth parameters of postlarvae. Merchie et al., (1998) demonstrated that postlarvae fed the astaxanthin diets had the highest survival and growth, so groups that received high contents of astaxanthin, they had higher survival and growth more than other groups. In the present study, no significant difference was observed between groups that receiving supplemented diets with astaxanthin but with the increasing astaxanthin amount in diets, survival and growth also improved. Carotenoids diet eliminated the oxidative damage and inactive the free radicals produced from cellular and various stresses (Halliwell & Gutteridge 1989; Chew 1995; pan et al., 2010). In this experiment, postlarvae fed with diet containing 200 mg kg^{-1} astaxanthin to be highest survival and growth between the other treatments. The polyene system of astaxanthin is one of a perfect properties that it gives the unique molecular structure (Seabra and Pedrosa, 2010). Chien et al., (2003) have suggested the use of astaxanthin could enhance the antioxidant capacity against the physiological stress caused by abiotic changes. Darachai et al., (1998) also found that postlarvae fed with algal astaxanthin diet had higher growth and survival than postlarvae fed other diets. Pigmentation is one of the important functions in aquatic animals (LIÑÁN-Cabello et al., 2002). The study carried out by Chien and Jeng (1992) showed that positive correlation there is the between survival and pigment accumulation in kuruma prawn. Also, the accumulation of carotenoid pigments in the tissue, protect cells against the photo-oxidation (You et al., 2006). This advantage has the positive effects on early development of aquatic animals. In this experiment, demonstrated that the increasing astaxanthin content in diets improved the survival and enhanced the growth parameters. When the total content of carotenoid decline to 20 mg kg^{-1}, black tiger shrimp occurrence the blue colour which caused the lack of carotenoid pigments in the diet (Howell and Matthews, 1991). In this experiment, the astaxanthin levels in postlarvae fed the 100 and 200 mg kg^{-1} was higher than 20 $mgkg^{-1}$. Carotenoid accumulation was evaluated in white leg shrimp fed the green algae (*Haematococcus Pluvialis*), higher content of carotene pigment for example astaxanthin, observed in shrimp fed the diets containing carotenoid sources that higher than control group (Parisenti et al., 2011). Also, in this study, higher levels of astaxanthin body observed in postlarvae fed the diets containing astaxanthin, so higher content of astaxanthin body was in diets containing 200 mg kg^{-1}. In this experiment, positive correlation was observed between astaxanthin body accumulation with survival and growth parameters. So, Pan et al., (2010) found that growth affected body astaxanthin content, also, the study shown that there is a positive correlation between survival rate and body astaxanthin accumulation. Astaxanthin is dominant carotenoid in shrimp, so that 70 to 90 percent of accumulated carotenoids in black tiger prawn is astaxanthin (Menasveta et al., 1993).

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