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Improved maturation of pond-reared, black tiger shrimp (*Penaeus monodon*) using fish oil and astaxanthin feed supplements

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ABSTRACT

Penaeus monodon female (49 g) and male (37 g) shrimp were fed formulated diets supplemented with 3 or 8% fish oil and 100 or 500 mg kg^{-1} astaxanthin, in addition to fresh squid during a 120 day trail using a 2×2×2 factorial design. Four formulated diets were provided with different combinations of high and low concentrations of lipid (fish oil) and astaxanthin. We found that fish oil addition, at the concentrations we used did not significantly ($P>0.05$) affect shrimp growth, but there was significantly greater growth of male shrimp and higher astaxanthin concentration. Female shrimp growth was not significantly different at either astaxanthin concentration. Likewise, there were no significant interactions on growth between fish oil and astaxanthin or fish oil and shrimp sex, but there was significant interaction between astaxanthin and sex of shrimp. Reproductive performance, as measured by number of eggs in gravid females and number of spermatozoa in male shrimp was significantly enhanced by both higher concentration of fish oil and astaxanthin. There was no significant interaction between fish oil and astaxanthin on number of eggs or spermatozoa. Likewise, there was no significant interaction between fish oil and astaxanthin, or fish oil and astaxanthin concentrations in shrimp muscle, hepatopancreas, ovaries or shell tissues. Astaxanthin concentrations in these respective tissues were similar for both levels of dietary fish oil. There were, however, significant interactions between astaxanthin and shrimp sex with these tissues. Greater dietary astaxanthin concentration resulted in significantly greater astaxanthin concentration in female shrimp muscle, hepatopancreas and ovarian tissues, but not in their shells. Female shrimp had significantly greater astaxanthin concentration in hepatopancreas tissue compared with males, but not in muscle or shells. Shrimp fed diets containing high levels of fish oil and astaxanthin had significantly greater 22:6n-3, total n-3 PUFA and total n-3 HUFA concentrations in muscle and ovary, whereas 20:4n-6, 20:5n-3, 22:6n-3, total n-6 PUFA, total n-3 PUFA and total n-3 HUFA concentrations were significantly greater in hepatopancreas of shrimp fed diet containing high level of fish oil. We concluded that dietary supplementation of formulated diets with 8% fish oil (12% total lipid) and at least 280 mg kg^{-1} astaxanthin will significantly improve *Penaeus monodon* maturation and spawning success.

1. Introduction

Ocean-caught *Penaeus monodon* broodstock are known to produce highest quality shrimp larvae (seed) compared with pond-reared broodstock. However, ocean- or wild-caught broodstock are often infected with shrimp viral diseases (Flegel and Alday-Sanz, 1998; Otta et al., 1999) that can cause mass mortality of seed shrimp after a few months of culture in earthen ponds. In addition to these disease problems, over-fishing has resulted in decreased supply of wild-caught broodstock. A solution to these problems is to develop procedures for producing improved pond-reared, captive broodstock in biosecure settings. This solution has not been realized yet in part due to poor reproductive performance of pond-reared broodstock. This poor performance is thought to be related to nutritional deficiencies of diets fed pond-reared shrimp.

Astaxanthin and lipids are known to be important nutrients affecting reproductive performance of shrimp (Middleditch et al., 1979; Millamena, 1989; Bray et al., 1990; Menasveta et al., 1994a; Pangantihon-Kuhlmann et al., 1998). Known or suspected functions of astaxanthin in aquatic animals include; improved provitamin A activity, antioxidant, improved embryonic and larval development, cellular protection from photodynamic damage, enhanced growth and maturation, and formation of in-chain epoxides that act as oxygen reserves under anoxic condition (Torrissen, 1990). Astaxanthin is the most frequent end product of carotenoid metabolism in crustaceans (Katayama et al., 1972a) and is the primary pigment in adult *P. japonicus* (Katayama et al., 1971, 1972b). A variety of factors affect the

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amount and distribution of carotenoids in crustaceans, including embryogenesis, reproductive cycle, molting, background colors, and hormonal control (Goodwin, 1960). Lipids play a major role in crustacean reproduction. Amount and composition of dietary lipids profoundly affect ovarian maturation and reproductive success (Harrison, 1990). Middleditch et al. (1979, 1980) found that penaeid shrimp need polyunsaturated fatty acids (PUFA) for proper development of gonadal tissues.

In our present study we evaluated the effect of different astaxanthin and fish oil formulations on reproductive performance of pond-reared *Penaeus monodon*.

2. Materials and methods

Our experiment was a 2×2×2 factorial design as a completely randomized design. Two lipid levels (fish oil at 3 and 8%), two levels of astaxanthin (100 and 500 mg kg^{-1}), and two shrimp sexes (male and female) were used. Eight replicates were used per nutrient treatment combination. Trial shrimp were sub-adult *P. monodon* of 48–50 g for females and 35–38 g for males. All shrimp were collected at age six months from a commercial, earthen pond. Shrimp were acclimated in trial tanks (temperature 28±1 °C, salinity 35 ‰) for at least 15 days before beginning the experiment. Shrimp were doubly tagged with a plastic numbered tag glued on their carapace, and a second rubber tube tag with the same number around their eyestalks. All shrimp were randomly placed into each treatment combination.

The four experimental diets all contained about 48% crude protein, including: LFLA (low fish oil and low astaxanthin), supplemented with 3% fish oil and 100 mg kg^{-1} astaxanthin; LFHA, supplemented with 3% fish oil and 500 mg kg^{-1} astaxanthin; HFLA, supplemented with 8% fish oil and 100 mg kg^{-1} astaxanthin; and HFHA, supplemented with 8% fish oil and 500 mg kg^{-1} astaxanthin (Table 1). The lipid source was refined fish oil, while synthetic astaxanthin was purchased from Hoffman-La Roche, Switzerland. Vitamins A, C and E were used in all diets at levels of 20 000 IU kg^{-1}, 200 and 100 mg kg^{-1}, respectively. Dietary ingredients were ground into powder and mixed by a twin blade rolling mixer for 30 min. The homogenized mixtures were pelletized using a California Pelleting Machine (CPM), streamed for 5 min, and then dried in a hot air oven at 60 °C for 2 h. Finished pellets were 3 mm dia. and 5 mm long. Diets were placed in dark containers and flushed with nitrogen gas before storage at −20 °C.

Table 1
Feed ingredients of broodstock shrimp diets

Ingredients	Dry weight (g 100 g^{-1} of diet)			
	LFLA	LFHA	HFLA	HFHA
Fish meal	56	56	56	56
Shrimp head meal	10	10	10	10
Wheat flour	16	16	16	16
Refined tuna fish oil	3	3	8	8
Chlorophyll pink[a]	0.125	0.625	0.125	0.625
Cellulose	9.758	9.258	4.758	4.258
Mineral mixture[b]	1	1	1	1
Vitamin mixture[c]	1	1	1	1
Cholesterol[d]	1	1	1	1
Lecithin[e]	1	1	1	1
Binder[f]	1	1	1	1
Vitamin A[g]	0.04	0.04	0.04	0.04
Vitamin C[h]	0.057	0.057	0.057	0.057
Vitamin E[i]	0.02	0.02	0.02	0.02
Total	100	100	100	100

[a] Chlorophyll pink contains 8% active form of astaxanthin, Roche.
[b] Mineral mixture 100 g contains: K_2HPO_4 2.0 g, $Ca_3(PO_4)_2$ 2.720 g, $MgSO_4$ $7H_2O$ 3.041 g, NaH_2PO_4 $2H_2O$ 0.790 g.
[c] Vitamin mixture 10 g contains: ρ-aminobenzoic acid 10.0 mg; biotin 0.40 mg, inositol 400.0 mg; nicotinic acid, 40.0 mg; Ca-pantothenate, 60.0 mg; pyridoxine-HCl, 12.0 mg; riboflavin, 8.0 mg; thiamin-HCl, 4.0 mg; menadione, 4.0 mg; cyanocobalamine, 0.08 mg; calciferol, 1.20 mg; folic acid, 0.80 mg; choline chloride, 120.0 mg.
[d] Ninety five percent cholesterol, laboratory grade, Sigma.
[e] Soy lecithin, feed grade.
[f] Aquabind, Du Pont.
[g] Five hundred thousand IU g^{-1}, feed grade, Roche.
[h] Stay C 35%, Roche.
[i] Fifty percent, feed grade, Roche.

Table 2
Water quality of the broodstock shrimp experiment

Parameters	Range
Salinity (‰)	35±1
Temperature (°C)	28±1
Dissolved oxygen (mg l^{-1})	6.5–7.7
Alkalinity (mg l^{-1})	148–220
pH	7.5–8.5
Ammonia (mg l^{-1})	0–0.5
Nitrite (mg l^{-1})	0–0.3
Nitrate (mg l^{-1})	0–20

The culture system consisted of one large, 7 m dia. circular tank with a 3.2 m dia. circular biofilter at the center of the larger tank. Water depth was 1 m and water was recirculated between the larger tanks and the biofilter using airlift pumps. This system is described more fully by Menasveta (1982). The tank system was covered with shade cloth, reducing ambient light by >95%. Thirty-two rectangular cages were placed in the larger tank, each cage measuring 0.36 m^2 on top surface. Sixty-four shrimp were placed in these cages, with one female and one male per cage.

Test diets were fed twice daily at 1200 and 1800 h. Fresh chopped squid (*Loligo* sp.) was also provided once a day at 10% of shrimp body weight at 0600 h. Diets were fed at 2% of shrimp weight per day. Uneaten diet, fecal matter and particular detritus were removed before the first feeding at 0600 h.

Newly molted, female shrimp were induced to mature by unilateral eyestalk ablation. Ovarian development was determined by flashing a light through the dorsal part of the abdomen every two days (Motoh, 1981). Gravid females at stage IV were sacrificed and analyzed for egg number, and astaxanthin and fatty acid content of muscle, hepatopancreas, ovary and shell. Spermatophores were obtained from male shrimp by electrical stimulation at 2–4 V and 0.3–0.5 A using a method similar to that described by Sandifer et al. (1984). Total spermatozoa numbers were determined at trial completion as described by Leung-Trujillo and Lawrence (1987). Broodstock growth was determined by weight measurement every 30 days. Shrimp survival was not determined since dead shrimp were replaced with shrimp of similar size and sex during the first two months of the trial. Sacrificed, stage IV females were also replaced. At trial completion, remaining shrimp were sacrificed and muscle, hepatopancreas, ovary and shell were analyzed for astaxanthin and fatty acid content using high performance liquid chromatography and gas chromatography as described by Weber (1988) and Christie (1989), respectively.

Diets were analyzed for crude protein, lipid, ash, fiber and moisture as described by AOAC (1995). Ammonia, nitrite and nitrate concentrations

Table 3
Proximate analysis of experimental broodstock diets as fed basis (means±s.d.)

Proximate	Diets			
	LFLA	LFHA	HFLA	HFHA
Crude protein (%)	48.64±1.71	48.96±0.28	47.52±0.20	48.83±0.83
Crude lipid (%)	7.70±0.03	7.78±0.16	11.51±0.13	12.04±0.30
Moisture (%)	13.56±0.11	12.18±0.08	12.95±0.16	12.87±0.23
Ash (%)	12.51±0.10	12.52±0.37	12.20±0.51	12.40±0.06
Fiber (%)	2.83±0.04	2.92±0.31	2.96±0.17	2.61±0.11
NFE[a] (%)	14.76±1.85	15.64±0.16	12.86±0.85	11.25±0.82
Astaxanthin (mg kg^{-1})	45.58±1.71	296.41±2.86	46.16±1.30	264.55±6.00

[a] NFE=Nitrogen free extract.