Aquat. Living Resour. 11 (6) (1998) 403-409 / © Ifremer/Elsevier, Paris

Effect of dietary astaxanthin and vitamin A on the reproductive performance of *Penaeus monodon* broodstock

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Received May 12, 1997; accepted October 19, 1998.

Abstract – The reproductive performance of pond tiger shrimp given astaxanthin (100 mg·kg^{-1}) and/or vitamin A (20 000 IU·kg^{-1}) for 61 d in tanks was assessed in a 2 × 2 factorial experiment in a completely randomized design. Tissue carotenoid (total carotenoid; astaxanthin free form, AF; monoester, AM; diester, AD) was analyzed using high performance liquid chromatography. The gonad index (GI) of shrimps fed diets with astaxanthin and/or vitamin A (3.05 ± 0.11 to 3.30 ± 0.17) were significantly higher than shrimps not fed any supplement (2.24 ± 0.17). A significant interaction between astaxanthin and vitamin A for ovarian development and spawning was detected (GI, 3.18 ± 0.14). The ovarian total carotenoid (10.85 ± 1.52 μg·g^{-1}) and astaxanthin (AF, 7.65 ± 1.43 μg·g^{-1}; AM, 0.63 ± 0.12 μg·g^{-1}) content of shrimps fed a diet with astaxanthin supplement were significantly higher than those without. In shrimps fed diets with and without vitamin A, no differences in ovarian total carotenoid (9.38 ± 0.58 μg·g^{-1}) and AF (8.12 ± 1.55 μg·g^{-1}) content were observed except for AM (0.64 ± 0.15 μg·g^{-1}) content. In contrast, the ovarian AD level was significantly higher (1.14 ± 0.3 μg·g^{-1}) in shrimps fed diets with both supplements compared to other diets. A significant interaction between astaxanthin and vitamin A was detected for AD storage. In the hepatopancreas of shrimps fed diets with or without astaxanthin or vitamin A, no significant differences in AF and AM content was observed. On the other hand, the AD (4.92 ± 1.3 μg·g^{-1}) level in the hepatopancreas of shrimps fed diet with vitamin A was significantly higher than those without. These results suggest the involvement of astaxanthin in *P. monodon* reproduction and its need for inclusion in broodstock diets. Supplementation of vitamin A to broodstock diet of *P. monodon* appears to enhance its ovarian development and spawning. © Ifremer/Elsevier, Paris

Astaxanthin / vitamin A / *Penaeus monodon* / reproductive performance / broodstock

Resumen – El comportamiento reproductivo de *P. monodon*, que provienen de estanque, alimentado con dietas con astaxanthin (100 mg·kg^{-1}) y/o vitamina A (20 000 IU·kg^{-1}) durante 61 días fue valorado en un experimento factorial 2 × 2 en un esquema totalmente aleatorio. Tejido carotenoide (total carotenoide; astaxanthin forma libre, AF; monoester, AM; diester, AD) fue analizado utilizando cromatografía líquida de gran rendimiento. El índice de gónada (GI) de camarón alimentado con dietas con astaxanthin y/o vitamina A (3,05 ± 0,11 – 3,30 ± 0,17) era notablemente mayor que el de aquellos camarones que no habían sido alimentados con ningún suplemento (2,24 ± 0,17). Se detectó una interacción entre astaxanthin y vitamina A en el desarrollo del ovario y la reproducción (GI, 3,18 ± 0,14). El carotenoide ovárico total (10,85 ± 1,52 μg·g^{-1}) y el contenido de astaxanthin (AF, 7,65 ± 1,43 μg·g^{-1}; AM, 0,63 ± 0,12 μg·g^{-1}) del camarón alimentado con dieta con suplemento de astaxanthin eran notablemente mayores que los que no la recibieron. Y en los camarones alimentados con dietas con o sin vitamina A, no se observaron diferencias en su contenido ovárico de carotenoide total (9,38 ± 1,58 μg·g^{-1}) y AF (8,12 ± 1,55 μg·g^{-1}) con excepción de su contenido AM (0,64 ± 0,15 μg·g^{-1}). Por el contrario, el nivel AD ovárico era notablemente mayor (1,14 ± 0,3 μg·g^{-1}) en el camarón alimentado con dieta con ambos suplementos que con las otras dietas. Una interacción notable entre astaxanthin y vitamina A fue detectada por el almacenamiento de AD. Además, no se observaron diferencias notables en el contenido de AF y AM en el hepatopáncreas de camarón alimentado con dietas con o sin astaxanthin o vitamina A. Por otro lado, el nivel AD (4,92 ± 1,3 μg·g^{-1}) en el hepatopáncreas de camarón alimentado con dieta con vitamina A fue notablemente más alto que en aquellos sin ella. Estos resultados parecen sugerir la participación de astaxanthin en la reproducción de *P. monodon* y la necesidad de incluirlo en las dietas para criaderos. El suplemento de vitamina A en las dietas para criaderos de *P. monodon* parece aumentar el desarrollo del ovario y la producción de huevos o reproducción. © Ifremer/Elsevier, París

Astaxanthin / vitamina A / *Penaeus monodon* / comportamiento reproductivo / criadero

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1. INTRODUCTION

The nutrition of pond *Penaeus monodon* Fabricius, 1798, broodstock plays a vital role in ensuring its good gonadal development and spawning, high fecundity and egg hatchability. However, except for lipids and essential fatty acid, most of *P. monodon* broodstock nutritional needs are not well studied. Astaxanthin, the predominant pigment in penaeids (86–98 % of total shrimp carotenoids) [15] and other crustaceans, can not be synthesized de novo. Its accumulation in the ovary and mobilization into eggs of crustaceans have led to speculation as to its possible role during maturation, embryonic and larval development [7, 8, 12]. Early results on the reproductive role of carotenoid in fish and crustacean, however, were not consistent when β-carotene, astaxanthin or canthaxanthin were included in the diet at 20 to 3 000 mg·kg^{-1} [5, 6, 11, 14, 21–23, 25]. These inconsistencies may be attributed to the possible interconversion of astaxanthin, vitamin A and other carotenoids, their levels of inclusion and/or experimental methods. The possible pathway of interconversion is either from astaxanthin to the β-carotene intermediate to vitamin A or vice-versa [2, 18, 19]. Vitamin A's function in reproduction, on the other hand, is not known in penaeids except for its accumulation in the hepatopancreas during maturation and transfer to the oocyte [9].

The specific function of vitamin A and astaxanthin in penaeid reproduction has not been elucidated. The objectives of this study was to determine the effect of dietary astaxanthin and/or vitamin A supplementation and their possible interconversion on ovarian development and spawning, fecundity and egg hatchability of pond *Penaeus monodon* broodstock.

2. MATERIALS AND METHODS

2.1. Experimental diets

Astaxanthin (100 mg·kg^{-1}) and/or vitamin A (20 000 IU·kg^{-1}) were added to four diets of the same basal composition (*table I*). The four broodstock diets were: diet 1, without astaxanthin and vitamin A; diet 2, with astaxanthin only; diet 3, with vitamin A only; and diet 4, with astaxanthin and vitamin A. The homogenized mixtures were pelletized through a Hobart pelletizer (3.0-mm orifice diameter), steamed for 5–10 min, oven dried at 50–60 °C, packed in plastic bags and flushed with nitrogen gas before storage (–4 °C). The synthetic astaxanthin (Carophyll pink 5 %) and vitamin A (ROVIMIX A 500) supplied in the diet were manufactured by Hoffmann-La-Roche, Switzerland. Both additives were dissolved in oil before being added to the final mixture. Proximate analysis of the diets was done at the Centralized Analytical Laboratory of SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines.

2.2. Broodstock management and culture

Four units of 12 m^3, 4-m diameter, circular concrete tanks covered with black canvas were used. Each tank

Table I. Composition and nutrient content of experimental diets.

Ingredients	(g·100 g^{-1})	Proximate composition	(%)
Squid meal	30.0	Crude protein	57–59
Peruvian fishmeal	33.0	Crude fat	14–15
Soybean meal	6.5	Crude fiber	0.8–1.6
Wheat gluten meal	2.0	Nitrogen free extract	15–17
Wheat flour	6.7	Moisture	3–7
Soybean oil	2.0	Ash	10–11
Squid oil	6.0	Total ME (kcal·100 g^{-1})[5]	419–441
Vitamin mix[1]	4.0		
Mineral mix[2]	4.0		
Cholesterol	0.6		
Lecithin	1.0		
Betaine-glycine (1:7)	1.5		
Carrageenan	2.5		
Basfin	0.2		
Carophyll pink[3]	0 or 0.2		
Vitamin A[4]	0 or 0.004		

[1] Vitamin mix (mg·kg^{-1} diet): cholecalciferol, 10; dl-tocopherol, 550; thiamine.HCl, 50; riboflavin, 60; menadione, 60; nicotinic acid, 300; ca-panthothenate, 300; cyanocobalamine, 0.2; folic acid, 15; ascorbic acid, 5 000; pyridoxine.HCl, 100; biotin, 4; choline chloride, 1 000; inositol, 600; para-aminobenzoic acid, 200.

[2] Mineral mix (mg·kg^{-1} diet): $CaHPO_4.2H_2O$, 12; $NaHCO_3$, 1.2; $FeSO_4.7H_2O$, 0.06; $Al(SO_4)_4.12H_2O$, 0.02; $ZnSO_4.7H_2O$, 0.36; $CuSO_4.5H_2O$, 0.06; $MnSO_4.H_2O$, 0.10; KI, 0.01; $CoCl_2.6H_2O$, 0.02; $NaSeO_3$, 0.05; K_2HPO_4, 1.2; $MgSO_4.7H_2O$, 2.4; KCl, 1.6; $AlCl_3.6H_2O$, 0.01.

[3] Carophyll pink (5 % astaxanthin) is supplied at 100 mg astaxanthin per kg diet.

[4] Vitamin A is supplied as ROVIMIX A 500 (500 000 IU·g^{-1}) at 20 000 IU per kg diet.

[5] ME, metabolizable energy [3].

was subdivided by 85-cm high screen net attached to 15-cm high marine plywood at the tank bottom. Sand-filtered seawater flowed through the tanks at 10 $m^3 \cdot d^{-1}$. Total water exchange was done every 14 d after tank and broodstock disinfection using 1 $mL \cdot 10\ L^{-1}$ formaldehyde for 1 h. Water salinity and temperature during culture ranged from 30–33 ‰ and 26–31 °C, respectively.

Eight-month-old *Penaeus monodon* broodstocks (77–111 g females and 70–90 g males) were obtained from brackishwater ponds. Broodstocks were acclimated to seawater before stocking. Thirty males and 25–27 females were stocked in each tank separated by a screen net. To facilitate individual monitoring of ovarian development and moulting, each female was doubly tagged at the eyestalk and carapace with color coded plastic squares. Broodstocks were fed basal or control diet (without astaxanthin or vitamin A supplement) initially for 14 d to reduce traces of astaxanthin and vitamin A deposited in the tissues. From the 15th day and until the 61st day of the experimental run, the female shrimps were fed the experimental diets. Feeding to satiation was done 3 times daily at 08:00, 17:00 and 22:00 hours. Females at intermoult stage were unilaterally ablated by pinching the eyestalk 14 d after the start of the experimental feeding or at the 28th day of the experimental run. Unimpregnated female shrimps were artificially inseminated with 1–2 spermatophores using a bent tipped needle at their post-moult stage or a few hours before spawning. The shrimps were freeze-tranquilized (water temperature lowered by 3–4 °C) before either eyestalk ablation and artificial insemination was performed.

2.3. Reproductive performance

Monitoring of ovarian development (stage I to IV) [20] was done 3 d after ablation, and every other day thereafter, by visual observation of the ovarian outline of the shrimp at its dorsal exoskeleton against the beam of light. Ovarian development and spawning of each shrimp was evaluated using the gonad index (GI) which indicates the highest stage of maturity attained, and the number of spawnings and rematurations per female. Gonad stage values (GSV) of 1 to 4 were assigned to each shrimp corresponding to the highest ovarian stage (stage I to IV) attained. GI was calculated based on the formula described below:

$$GI = GSV_1 \times OC + \tfrac{1}{2} GSV_2 \quad \text{or} \quad GI = GSV_1{}^*$$

where GSV_1 is the highest gonadal stage value attained (1–4), GSV_2 the highest gonadal stage value attained of rematured broodstock unable to complete an ovarian cycle, OC the number of ovarian cycle completed, and $GSV_1{}^*$ for those broodstock which did not complete an ovarian cycle but attained a certain stage of ovarian development.

Broodstock which did not complete an ovarian cycle but matured to a certain stage had a gonad index value equal to the highest GSV attained. Ready-to-spawn females were individually transfered to a 250-L aerated conical-bottom fiberglass tank. After spawning, fecundity or number of eggs per g body weight of female and per cent hatching rate were measured.

2.4. Astaxanthin and carotenoid analysis

Samples of female *P. monodon* hepatopancreas at day 0, 14 and 61, and ovary at day 61 were analyzed for their total carotenoid (TC), astaxanthin monoester (AM) and diester (AD) and astaxanthin free form (AF) using high performance liquid chromatography [26]. Analysis was done by the Hoffman-La-Roche Co. Ltd Laboratory in Basel, Switzerland.

2.5. Statistical design and analysis

A 2 × 2 factorial experiment in a completely randomized design was used. Each of the individually tagged 25–27 females per tank was considered as a replicate. Data on reproductive performance and tissue carotenoid content were analyzed by two-way analysis of variance (ANOVA) and their means compared by contrast (main and simple effects) using the SAS package [17]. When approriate, data which did not show interaction after ANOVA were pooled and their main effects compared. The gonad index, fecundity and egg hatching rate were subjected to square root, log and arc sine transformation, respectively, before analysis, while data on tissue analyses were subjected to log (TC) and square root (AF, AM, AD) transformation. The level of significance used was 95 %.

3. RESULTS

The gonad index of shrimps fed the diet with astaxanthin and/or vitamin A supplements (3.05 ± 0.11 to 3.30 ± 0.17) were significantly higher than those fed the control diet or unsupplemented (2.24 ± 0.17; *table II*). However, there were no significant differences in the gonad index among shrimp fed any of the supplemented diets. Moreover, a significant interaction between astaxanthin and vitamin A supplements was observed during ovarian development and spawning (*table II*). In contrast, fecundity and hatching rate of *P. monodon* broodstock were not affected by the dietary treatments (*table II*). These were possibly due to the high variability in the number of spawned eggs and number of the spawning female.

The highest pooled survival rate of 80 % was observed in female shrimps fed the diet with astaxanthin and lowest (60 %) in those fed with both supplements. Female shrimps receiving diets with vitamin A supplement and the control diet had pooled survival rates of 68 and 78 %, respectively. Heavy mortality occurred 36 d after rearing or 6 d after eyestalk ablation.

In the ovary, a significant interaction of astaxanthin and vitamin A was observed during ovarian asta-

Table II. Reproductive performance of pond *P. monodon* fed diets with or without astaxanthin and vitamin A supplementation for 61 d (values in parentheses are number of shrimps observed for the entire period).

Astaxanthin (100 mg·kg^{-1})	Without	With	Without	With	Two-way ANOVA (*P* value)		
Vitamin A (20 000 IU·kg^{-1})	Without	Without	With	With	Astaxanthin	Vitamin A	Astaxanthin + Vitamin A
Gonad index[1,2]	2.24 ± 0.17[b] (21)[2]	3.3 ± 0.17[a] (23)	3.05 ± 0.11[a] (21)	3.18 ± 0.14[a] (19)	0.0001	0.0082	0.0013
Hatching rate (%)	0[a] (1)	5.7 ± 2.9[a] (5)	5.6 ± 4.1[a] (3)	45.7 ± 45.7[a] (2)	0.2771	0.2596	0.6686
Fecundity (no. of eggs·g^{-1} female)	453[a] (1)	646 ± 165[a] (5)	591 ± 309[a] (3)	500 ± 388[a] (2)	0.9274	0.6577	0.6994

[1] Means (± SEM) with the same superscript are not significantly different ($P > 0.05$) between treatments for the same parameter.
[2] Gonad index (GI) = $GSV_1 \times OC + \frac{1}{2} GSV_2$ or $GI = GSV_1$* (see explanations in Materials and Methods).

Table III. Ovarian carotenoid content (μg·g^{-1}) of pond *P. monodon* broodstock fed diets with or without astaxanthin (100 mg·kg^{-1}) and vitamin A (20 000 IU·kg^{-1}) supplements for 61 d. *n*: Number of shrimps analyzed.

Astaxanthin	Without	With	Without	With	Two-way ANOVA (*P* value)			
Vitamin A	Without (*n* = 21)	Without (*n* = 14)	With (*n* = 12)	With (*n* = 5)	Block[3]	Astaxanthin	Vitamin A	Astaxanthin + Vitamin A
Total carotenoid[1]	6.89 ± 2.03	11.14 ± 1.8	9.1 ± 1.9	10.04 ± 2.84	0.0001	0.0001	0.0845	0.2483
Astaxanthin								
Free form[1]	5.78 ± 1.68	8.28 ± 1.76	9.06 ± 1.95	5.88 ± 2.42	0.0001	0.0337	0.5797	0.0621
Monoester[1]	0.42 ± 0.09	0.44 ± 0.08	0.42 ± 0.12	1.16 ± 0.31	0.4190	0.0125	0.0204	0.0933
Diester[2]	0.46 ± 0.12[a]	0.56 ± 0.08[a]	0.28 ± 0.07[a]	1.14 ± 0.3[b]	0.0563	0.0001	0.2341	0.0044

[1] There was no interaction ($P > 0.05$) between astaxanthin and vitamin A for total carotenoid, astaxanthin free form and astaxanthin monoester; statistical analysis for pooled data is presented in *table IV*.
[2] There was interaction ($P < 0.05$) between astaxanthin and vitamin A for astaxanthin diester. Means (± SEM) in a row with the same superscript are not significantly different ($P > 0.05$).
[3] Ovarian stage was used as block.

Table IV. Pooled data on ovarian carotenoid content of pond *P. monodon* broodstock fed diets with or without astaxanthin and vitamin A supplements for 61 d. *n*: Number of shrimps analyzed.

Carotenoid (μg·g^{-1})	Astaxanthin supplementation (100 mg·kg^{-1})		Vitamin A supplementation (20 000 IU·kg^{-1})	
	With (*n* = 19)	Without (*n* = 33)	With (*n* = 17)	Without (*n* = 35)
Total carotenoid	10.85 ± 1.52[a]	7.69 ± 1.46[b]	9.38 ± 1.54[a]	8.59 ± 1.45[a]
Astaxanthin free form	7.65 ± 1.43[a]	6.97 ± 1.3[b]	8.12 ± 1.55[a]	6.78 ± 1.23[a]
Astaxanthin monoester	0.63 ± 0.12[a]	0.42 ± 0.07[b]	0.64 ± 0.15[a]	0.43 ± 0.06[b]

Means (± SEM) in a row of a factor (astaxanthin or vitamin A) with the same superscripts are not significantly different ($P > 0.05$) in a 2 × 2 factorial experiment in randomized complete block design with ovarian stage as block. Since there was no interaction detected between astaxanthin and vitamin A ($P > 0.05$), the data were pooled.

xanthin diester storage (*table III*): astaxanthin diester level was significantly higher (1.14 ± 0.3 μg·g^{-1}) in shrimps fed both supplements compared to those fed other diets (0.28 ± 0.07 and 0.56 ± 0.08 μg·g^{-1}, respectively; *table III*). Moreover, ovarian total carotenoid (10.85 ± 1.52 μg·g^{-1}), AF (7.65 ± 1.43 μg·g^{-1}) and AM (0.63 ± 0.12 μg·g^{-1}) were significantly higher in shrimps fed the diet with astaxanthin compared to those without (TC, 7.69 ± 1.46 μg·g^{-1}; AF, 6.97 ± 1.3 μg·g^{-1}; AM, 0.42 ± 0.07 μg·g^{-1}; *table IV*). No significant differences were found in total carotenoid (9.38 ± 1.54 μg·g^{-1}) and AF (8.12 ± 1.55 μg·g^{-1}) content of shrimps fed diets with and without vitamin A except for its AM content (0.64 ± 0.15 μg·g^{-1}).

Table V. Carotenoid content of the hepatopancreas of pond *P. monodon* broodstock fed diets with and without astaxanthin (100 mg·kg^{-1}) and vitamin A (20 000 IU·kg^{-1}) supplements for 61 d (values are mean ± SEM). *n*: Number of shrimp analyzed.

Carotenoid (μg·g^{-1})	Before depletion	After depletion	At harvest, day 61			
			Astaxanthin supplementation		Vitamin A supplementation	
	Day 0 (n = 6)	Day 14 (n = 5)	With (n = 18)	Without (n = 27)	With (n = 19)	Without (n = 26)
Total carotenoid	263.93 ± 50.88	79.96 ± 37.49	17.76 ± 9.82^a	9.82 ± 1.72^b	15.26 ± 2.64^a	11.34 ± 1.44^a
Astaxanthin						
Free form	16.18 ± 3.57	4.36 ± 1.56	8.53 ± 1.48^a	7.1 ± 1.8^a	9.85 ± 2.54^a	6.07 ± 1.04^a
Monoester	75.32 ± 8.14	33.44 ± 17.46	4.49 ± 0.81^a	3.19 ± 0.65^a	3.96 ± 0.91^a	3.53 ± 0.6^a
Diester	107 ± 29	31.98 ± 15.47	3.75 ± 0.66^a	3.22 ± 0.93^a	4.92 ± 1.3^a	2.35 ± 0.38^b

Within a factor (astaxanthin and vitamin A), means with the same superscript are not significantly different ($P > 0.05$) in a 2 × 2 factorial experiment in randomized complete block design with ovarian stage as block ($P < 0.05$). Since there was no interaction detected between astaxanthin and vitamin A ($P > 0.05$), data at harvest were pooled.

In the hepatopancreas, total carotenoid and total astaxanthin were initally very high (day 0) at 263.9 ± 50.9 and 198.5 μg·g^{-1} (*table V*), respectively, and reduced by 65–70 % after being fed a carotenoid-free diet (day 14). At harvest (day 61), there were no significant differences in the astaxanthin content (AF, AM) of the hepatopancreas of shrimps fed diets with or without astaxanthin or vitamin A. On the other hand, the astaxanthin diester content of the hepatopancreas of shrimps fed the diet with vitamin A (4.92 ± 1.3 μg·g^{-1}) was significantly higher compared to those without (2.35 ± 0.38 μg·g^{-1}). The total carotenoid of shrimps (17.76 ± 9.82 μg·g^{-1}) fed the diet with astaxanthin was likewise significantly higher than those without (9.82 ± 1.72 μg·g^{-1}). The astaxanthin-free form level of the hepatopancreas of shrimps given diets without astaxanthin or vitamin A increased from 4.36 ± 1.56 (day 14) to 6.07 ± 1.04–7.1 ± 1.8 μg·g^{-1} (day 61).

4. DISCUSSION

The present study was able to show that the addition of 100 mg·kg^{-1} astaxanthin to practical broodstock diet improves ovarian development and spawning of eight-month-old pond *P. monodon* as indicated by the higher GI found in astaxanthin-fed shrimp. Moreover, the significantly high levels of ovarian astaxanthin (9.42 μg·g^{-1}), and the predominance of astaxanthin (> 90 %) and its free form (> 50–76 %) in the tissues examined suggest their association with lipoprotein complex and nutrient accumulation in the oocyte [4, 12, 16, 24]. The low deposition of astaxanthin in the hepatopancreas (17 μg·g^{-1}) despite supplementation with 100 mg·kg^{-1} astaxanthin may suggest this nutrient's immediate transfer to the ovary during gonadal development and spawning. Several workers [7, 10, 12] have similarly found that other penaeids, *P. schmitti*, *P. esculentus*, and the sand crab, *E. analoga*, mobilize their stored astaxanthin from the hepatopancreas to the ovary in the advent of maturation. Altogether, the findings indicate astaxanthin's involvement in the reproduction of pond *P. monodon*. In a similar study, however, contrasting results were obtained on the reproductive performance and tissue astaxanthin content of pond reared *P. monodon* when fed both diets fortified with astaxanthin (100 mg·kg^{-1}) and natural food such as squid, mussel, *Artemia* and annelid [14].

Similarly, vitamin A when supplemented at 20 000 IU·kg^{-1} in the diet was observed in this study to enhance ovarian development and spawning of *P. monodon*. This positive response, however, is possibly due to the high levels of astaxanthin esters found in the ovary and hepatopancreas since vitamin A levels in the tissues were not determined. Relatively, *P. japonicus* given broodstock diets containing 9 000 IU·kg^{-1} vitamin A has been shown to improve its gonadosomatic index by 3 % and has an ovarian vitamin A content of 0.97 μg·g^{-1} [1]. Moreover, the significantly high levels of astaxanthin accumulated in the ovary (AM, 0.64 μg·g^{-1}) and hepatopancreas (AD, 4.92 μg·g^{-1}) of vitamin A-fed shrimps may possibly suggest the occurrence of vitamin A conversion to astaxanthin and its immediate esterification. The combined effect of astaxanthin and vitamin A is indicated in the interaction of astaxanthin and vitamin A on AD storage in the ovary and on ovarian development and spawning. Conversion of vitamin A may be triggered by hormonal changes during eyestalk ablation and the high levels of vitamin A supplement. β-Carotene, as intermediary product in the possible vitamin A-astaxanthin pathway, has been demonstrated to be effectively converted to astaxanthin with eyestalk ablation in *Macrobrachium rosenbergii* [13]. Further studies using radioisotope labelling, however, are needed to clearly demonstrate vitamin A conversion to astaxanthin, astaxanthin metabolism and their reproductive role in *P. monodon*.

The low fecundity and hatching rate of shrimps in this study may partly be due to monosex stocking, poor quality of sperm used during artificial insemination, and long rearing period (28 d) before induction of maturation.

5. CONCLUSION

Astaxanthin, the red carotenoid pigment found among crustaceans improves the ovarian development and spawning of pond *P. monodon*. The high levels of astaxanthin and the predominance of its free form in the ovary and its poor deposition in the hepatopancreas of *P. monodon* fed with astaxanthin further suggests its involvement in shrimp reproduction. The results also suggest an apparent need for the inclusion of astaxanthin in broodstock diets. However, the optimum levels of its inclusion still need to be established.

The enhanced ovarian development and spawning of *P. monodon* fed the diet with vitamin A is possibly due to the high levels of astaxanthin esters in the ovary and hepatopancreas. Possible conversion of vitamin A to astaxanthin and its esters via the β-carotene intermediate is shown by the significant interaction of astaxanthin and vitamin A during AD storage, ovarian development and spawning. Further studies using radioisotope techniques may clearly elucidate vitamin A-astaxanthin conversion, astaxanthin metabolism and their relationship to *P. monodon* reproduction.

Acknowledgements

The authors are grateful for the support of the Southeast Asian Fisheries Development Center, UP Alumni Association and F. Hoffman-La Roche Switzerland and Philippines to this masteral work; and, special thanks to Neila Sumagaysay and Dr Kai-Jens Kühlmann for reading the manuscript.

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