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CAROTENOID-ENRICHED DIET FOR PREMATURATION STAGE OF POND-REARED TIGER SHRIMP, *Penaeus monodon*: Part II. EFFECT ON GONADAL MATURATION AND BIOCHEMICAL PROFILES OF OOCYTES, SPERMATOPHORES AND HEPATOPANCREAS

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ABSTRACT

Two-phase feeding trials were conducted to evaluate the effect of the carotenoid mixture on gonadal development and biochemical properties of several tissues of tiger shrimp. The treatments were diet enriched with (PC) and without carotenoid mixture (PO). Shrimp with an initial body weight of 31.7 ± 1.3 g were allocated among four of 1,000 m² concrete ponds with a density of 1 shrimp/m² and fed tested diets for five months. Selected shrimps from the pond with a minimum weight of 80 g for females and 60 g for males were stocked into four 10-m³ concrete tanks at 15 pairs per tank. Natural mating rate and ovary development were not stimulated by the carotenoid supplement. However, it significantly improved ($P < 0.05$) both ovary maturation and spermatophore formation of tiger shrimp from 76.7 ± 1.4 to $86.7 \pm 0.0\%$ and from 69.9 ± 4.5 to $82.3 \pm 4.0\%$, respectively. Total carotenoid content in meat, oocyte and hepatopancreas of female tiger shrimp significantly ($P < 0.05$) increased by supplementing carotenoid compared to the control diet. The total amino acid content in the spermatophore of shrimp fed the PC diet was significantly higher (73.82%) than in the PO diet (66.09%). The present study revealed the important effect of carotenoid feed during the pre-maturation stage on the reproductive performances of pond-reared tiger shrimp.

KEYWORDS: prematuration; carotenoid; gonadal maturation; pond-reared tiger shrimp

INTRODUCTION

Carotenoids comprise a widespread group of bioactive compounds, mainly present in the plant kingdom (Yesilayer *et al.*, 2020) but also in algae (Guedes *et al.*, 2011) and some microorganisms (Mussagy *et al.*, 2019; Pagels *et al.*, 2021). Chemically, there are two classes of carotenoids which are carotenes and xanthophylls. The second class of xanthophylls besides containing carbon and hydrogen, also contains oxygen in their chemical structure, such as torularhodin, astaxanthin, and canthaxanthin (Colmán *et al.*, 2016).

In addition to their role as pigments (Parisenti *et al.*, 2011; Daly *et al.*, 2013), carotenoids have been reported to have various biological effects on many aquatic animals, such as a source of pro-vitamin A, antioxidant functions as indicated by decreasing the lipid peroxidation (da Silva *et al.*, 2015; Sallam *et al.*, 2017), alleviating oxidative damage (Santos *et al.*, 2012) and inactivating free radical production caused by cellular metabolism (Pan *et al.*, 2010). These functions may contribute to improving the growth and survival of aquatic animals fed carotenoid diets such as black tiger shrimp, *Penaeus monodon* (Niu *et al.*, 2012), Pacific white shrimp, *Litopenaeus vannamei* (Parisenti *et al.*, 2011) and finfish including fry of European seabass, *Dicentrarchus labrax* (Sallam *et al.*, 2017) and yellowtail cichlid, *Pseudotropheus acei* (Guroy *et al.*, 2012).

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The role of carotenoids in reproductive development has been reported in crustaceans (Paibulkichakul *et al.*, 2008) and finfish (Bjerkeng *et al.*, 2000). In crustaceans, dietary pigment deficiency is associated with a decrease of carotenoids in the ovaries of maturing females and in the egg yolk (Lee & Gilchrist, 1972). In addition, Paibulkichakul *et al.* (2008) found that dietary astaxanthin enhanced fecundity and number of spermatozoa in male black tiger shrimp, *P. monodon*. Furthermore, several studies have revealed that a combination of various carotenoids (astaxanthin, β -carotene, zeaxanthin and lutein) improved fecundity and hatching rate in yellowtail, *Seriola quinqueradiata* (Watanabe *et al.*, 1996) compared to a diet containing only β -carotene or paprika ester as a carotenoid source (Vassallo-Agius *et al.*, 2002). Our recent study on rabbitfish (*Siganus guttatus*) showed that combined carotenoid of astaxanthin, canthaxanthin and carotenoid from *Spirulina* used as a supplement in a maturation diet produced higher fecundity and increased the total fatty acid content of the ovaries compared to the control diet (Laining *et al.*, 2015).

Several main issues faced in domesticating tiger shrimp in ponds conducted at Research Institute for Coastal Aquaculture and Fisheries Extension (RICAPE) include the low number of shrimp reaching the maturation stage and the low mating rate occurring in the cavity both in the pond and in maturation tank (Laining *et al.*, 2015). It was hypothesised that supplementation of carotenoid in diet and applied since prematuration stage can improve reproductive performance of pond reared tiger shrimp *P. monodon* broodstocks. The general objective of this present study was to clarify the effects of combined carotenoids fed since the pre-maturation stage on growth, pigmentation and reproductive performances of pond-reared tiger shrimp, *Penaeus monodon*. The results presented herein concern the reproductive development of both male and female shrimp and the biochemical properties of several tissues including hepatopancreas, muscle, ovary and spermatophore.

MATERIALS AND METHODS

Shrimp and carotenoid-enriched diets fed during the prematuration stage

The feeding experiment was conducted at the Shrimp Hatchery Station of the Research Institute for Coastal Aquaculture and Fisheries Extension (RICAPE) located in Barru District, South Sulawesi Province, Indonesia. Cultured *P. monodon* ($n = 5,000$, an initial body weight of 31.7 ± 1.3 g (mean \pm SD), six months of age, were selected and 4,000 shrimps

were randomly stocked into four 1,000 m² sand-substrate concrete ponds to accommodate two treatments with two replicates. The shrimp were stocked at a density of one shrimp/m². The ponds were supplied with sand-filtered seawater and aerated using one paddle wheel for each pond. The shrimp were fed with one of two diets, one (PC) enriched with a 4.93 g carotenoid mixture /kg diet and the second (PO) without carotenoid supplementation. The carotenoids incorporated into the standard diets were a mixture of astaxanthin (carophyll pink) (1.25 g/kg diet) (Paibulkichakul *et al.*, 2008), canthaxanthin (carophyll red) (0.68 g/kg) and *Spirulina* powder (3 g/kg diet) (Laining *et al.*, 2015). The standard diet used for this experiment was a starter diet for tiger shrimp which were modified to be iso-nitrogenous and iso-lipidic (Table 1).

The standard diet used for the present study was a crumbled commercial diet basically for tiger shrimp applied during the early stocking in the pond. Standard diet was firstly grounded before being mixed with carotenoid (PC) by thoroughly mixing including PO diet and each diet was added water approximately 300-350 g/kg of dry ingredient (Laining *et al.*, 2012). The dough was then re-pelleted using a pelletizer (Hiraga, Co. Ltd, Kobe, Japan) with a 3.1 mm die and steamed for three minutes before being dried (Laining *et al.*, 2017). The pellet was oven-dried and then kept at a low temperature of 18°C during the feeding trial for the two stages. Shrimp were fed the two tested diets three times a day at 07.00, 13.00 and 19.00 at a rate of 2-5% of biomass until reaching the maturation stage or 20 weeks culture period.

During feeding time at the prematuration stage, water quality was monitored every day in the morning and the afternoon for pH, salinity, temperature and dissolved oxygen (DO) while alkalinity was measured once a week only in the morning. When alkalinity was below 100 mg/L, lime was applied at the level of 15 ppm to increase its concentration.

Feeding regime and husbandry during the maturation stage

After 20 weeks of culture at the prematuration stage or at 10 months of age, shrimp from each pond were selected based on weight and morphological appearance. A total of 120 healthy shrimp with a minimum weight of 80 g for females and 60 g for males were transferred to five 3 m³ tanks and maintained in a quarantine room for one week. They were then allocated to corresponding dietary treatments in one of four 10-m³ circular concrete tanks with two replicates for each diet. The stocking density was 30

Table 1. Proximate composition (g/kg, dry matter) and total carotenoid content (mg/kg, dry matter) of commercial standard diet and the two test diets fed to tiger shrimp during the prematuration stage. Values are mean \pm SD (n=3)

Nutrient	Standard diet	Test diets	
		PO	PC
Crude protein	455 \pm 10	445 \pm 19	436 \pm 15
Lipid	66 \pm 8	71 \pm 10	77 \pm 1
Ash	117 \pm 5	113 \pm 0	112 \pm 1
Crude fiber	32 \pm 3	40 \pm 9	40 \pm 4
NFE	330 \pm 7	331 \pm 8	335 \pm 11
Total carotenoid	90.66 \pm 0.40	90.29 \pm 0.05	150.46 \pm 0.04

shrimps/tank with a 1:1 female: male ratio. All tanks were supplied with disinfected seawater from a flow-through system at a rate of 10 L/min.

The test diets applied for the gonadal development process were similar to those used during the prematuration stage, i.e. PO and PC diets. However, these two experimental diets were each combined with fresh feed (equal portions of squid and mussel meat) at a ratio of 60:40% (Laining *et al.*, 2014). The shrimp were fed four times a day at 08.00 (pellet), 12.00 (squid), 16.00 (mussel) and 20.00 (squid) at a level of 2.5% of shrimp biomass.

To induce ovarian maturation, female broodstocks were eyestalk-ablated one week after starting feeding the diets. Female broodstock reaching ovarian maturation stage II or III (Tan-Fermin & Pudadera, 1989) were sacrificed and dissected to sample the ovary, hepatopancreas, muscle and shell. Ovary and hepatopancreas tissues were freeze-dried (Eyela FD-1000, Japan) while muscle and shell tissues were oven-dried at 60°C (Memmert, Germany). All dried samples were pulverized and kept at -20°C until analysis.

Evaluation of reproductive performance

At the end of the feeding trial (20 weeks) at the concrete pond during the prematuration stage, natural mating was observed by checking the presence of spermatophores on the thelycum of individual females from each pond. Accordingly, ovarian maturation was checked by observing the ovary development stage using a torch. The assessment of spermatophore formation was not carried out at the prematuration stage but performed during the maturation development stage in controlled tanks.

Parameters used to evaluate female shrimp broodstock during the maturation process were: the number of females that matured before and after ab-

lation (reaching the ovarian development stage II and III), gonadosomatic index (GSI) and hepatosomatic index (HSI). For male broodstock, the recorded parameters were: the number of males producing spermatophores at maturation and the first re-maturation. Unlike the females, the stages of testes and spermatophore development of male tiger shrimp are not visible externally through either the dorsal or ventral exoskeleton. A technique to assess spermatophore formation has been developed by artificially releasing the spermatophore out of the *ampulla terminal* (Sandifer & Lynn, 1980). The males which released their spermatophore through artificial ejaculation were assumed to be mature broodstock (Laining *et al.*, 2016). The artificial insemination applied in the present study was an electrical shock (Diwan & Joseph, 2009) using a transformer set up at 5 mA and 8-12 V following a slight modification (Lante & Laining, 2016). The transformer was connected with an electrode placed near the gonophores at the base of the 5th pereopods and the electric shock was applied for two seconds. The electrical shock stimulates contraction surrounding the terminal ampullae, expelling a single spermatophore from each gonophore. After a month of the feeding trial, the electrical shock was applied twice at seven days intervals to release the spermatophore. If a male released spermatophores at the first electrical shock, it was recorded to be at the maturation stage, and if the same male again released spermatophores at the second electrical shock, it was categorized to be at the first re-maturation (Laining *et al.*, 2016). The percentage of males maturing was calculated based on the number of males releasing spermatophores divided by the number of males treated. Spermatophores obtained through the electrical shock were freeze-dried (Eyela FD-1000, Japan), pulverized and kept at -20°C until analysis.

Biochemical analyses

Proximate analysis of the shrimp diets and muscles was done according to AOAC International (2007). Crude protein was determined according to the micro-Kjeldahl procedure and lipid was extracted using chloroform and methanol. Ash was analyzed using a muffle furnace at 550°C (Barnstead, Thermolyne, CA, USA).

Analysis of total carotenoid was carried-out according to AOAC International (2007). Samples were extracted with acetone and hexane and elucidated in a column before being read spectrophotometrically (Spectrophotometer UV VIS Jasco V-70, Japan). Fatty acid analysis of oocytes was determined using gas chromatography (GC, FID Perkin Elmer Clarus 680, US). Amino acid profiles of oocytes were determined using HPLC (Shimadzu 20A, Tokyo, Japan). For the limited sample of spermatophores, amino acid profiles were performed with ultra-performance liquid chromatography (UPLC, Waters H Class with PDA detector, US).

Statistical analysis

Comparisons of reproductive performance and biochemical properties in several tissues of the tiger shrimp broodstock were analyzed using *t*-tests with SPSS software (version 25; SPSS, Inc., Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Natural mating and ovarian maturation during the prematuration stage

During the 20-week culture period in concrete ponds, there were two spermatophore-carrying females from 195 females fed the PC diet which corresponds to a natural mating of 1.0%. None of the female shrimp from 166 females fed the PO diet carried spermatophore in their thelycum. In addition, there was only one shrimp from the PO dietary group found to be reproductively mature while no mature shrimp were observed in the PC group (Table 2).

Application of the test diet for 20 weeks in ponds did not improve either the natural gonadal maturation of female shrimps or the mating rate of pond-reared tiger shrimp. However, after 6 weeks of gonadal maturation in controlled tanks, the number of ablated females that reached ovarian maturation stages II and III significantly improved in shrimp fed the PC diet compared with the PO diet. Similarly, male shrimp fed the PC diet produced more spermatophores compared to males fed the PO diet even without ablation. These results support other studies reporting the beneficial effect of carotenoids in the gonadal maturation of crustaceans (Paibulkichakul *et al.*, 2008) as well as in finfish (Vasallao-Agius *et al.*, 2002). This experiment also demonstrated that ablation is still necessary to stimulate the ovarian maturation of shrimp in line even when the carotenoid-rich diet was used in the maturation phase.

Water quality during prematuration culture

Water quality data for the 20 weeks' prematuration culture in the concrete ponds are shown in Table 3. There were not many differences in the parameter ranges between morning and afternoon samplings. However, the range of several parameters was quite wide due to seasonal changes over the 20 weeks culture period. The temperature ranged from approximately 27- 34°C, while salinity ranged from 18-41 ppt. DO was slightly lower in the morning than in the afternoon with the highest afternoon reading of 9.2 mg/L, while morning readings reached only 8.7 mg/L. The range of pH was from approximately 7.0 to 8.9 in both the morning and afternoon. Alkalinity varied from 65 to 180 mg CaCO₃/L with the lower alkalinity being observed during the rainy season.

Ovarian maturation and spermatophores formation during the maturation stage

The reproductive performance of female shrimp after the six-week feeding trial at the maturation stage is presented in Table 4. None of the females in the two groups showed ovary development (stage II-IV; Tan-Fermin & Pudadera, 1989) at stocking of the re-

Table 2. Natural mating and female maturation rate of shrimp after 20 weeks fed carotenoid-enriched diets during the prematuration stage in the concrete pond. Data are expressed as means ±SD (n=2). Means in the same column with different superscript letters are significantly different (*t*-test, *P*<0.05)

Test diet	Initial weight (g)	Final weight (range of weight) (g)	Natural matings (%)	Female natural maturation (%)
PO	31.5 ± 5.0	82.9 ± 1.9 ^a (41-144)	0.0 ± 0.0	0.5 ± 0.1
PC	32.0 ± 1.1	86.5 ± 0.1 ^a (45-146)	1.0 ± 0.0	0.0 ± 0.0

Table 3. Water quality (range of values) was measured in the experimental ponds during the 20-weeks feeding trial. Alkalinity was measured only once daily, in the morning

Parameter	Morning	Afternoon
Temperature (°C)	27.2-34.0	26.8-34.0
Salinity (ppt)	17.5-40.1	20.5-41.0
Dissolved oxygen (mg/L)	3.8-8.7	3.9-9.2
pH	7.0-8.9	7.2-8.8
Alkalinity (mg CaCO ₃ /L)	65-180	-

productive trial or before ablation of the females. Thirteen females from 15 ablated females in the PC group reached the ovarian development stage of II or III corresponding to 87%. This percentage was significantly higher (*t*-test, $P < 0.05$) than the 77% of shrimp that matured when fed the PO diet. However, the gonadosomatic index (GSI) and hepatosomatic index (HSI) of the maturing females of the two groups were not significantly different ($P > 0.05$) (Table 4).

The number of males releasing spermatophores at first electrical shock (maturation) was significantly higher in males fed the PC diet ($82.3 \pm 4.0\%$) compared with the PO diet ($69.9 \pm 4.5\%$) (Table 5). However, for the second electrical shock or re-maturation, the percentage of males producing spermatophores was not significantly different ($P > 0.05$) for both groups. Furthermore, no differences were detected in the weight of spermatophores both at maturation and re-maturation.

Carotenoid content in tissues of matured female shrimp

Figure 1 shows the total carotenoid content in the whole body, muscle tissue, and shell of the female shrimp that matured during the maturation development stage in concrete tanks. Total carotenoid content in the whole body and muscle were both significantly higher ($P < 0.05$) for female shrimp fed the PC diet than in these tissues of shrimp fed the PO diet. The concentration of total carotenoid in the muscle of shrimp fed the PC diet was more than double that of shrimp fed the PO diet (110 vs $250 \mu\text{g/g}$). In contrast, the pattern of total carotenoid concentration in shell tissue was lower for shrimp fed the PC diet ($100.7 \mu\text{g/g}$) than for shrimp fed the PO diet ($249 \mu\text{g/g}$). This phenomenon might indicate that shrimp fed with a carotenoid diet had better gonadal development and required a higher amount of carotenoid in the gonad which was mainly transferred from shell to gonad.

Table 4. Reproductive performance of female shrimp fed carotenoid-enriched diets for 20 weeks in pre-maturation stage followed by six weeks of feeding at maturation stage. Data are expressed as means \pm SD ($n=2$). Means in the same column with different letters are significantly different (*t*-test, $P < 0.05$)

Test diet	Female shrimp	Females reaching stages II and III (%)	GSI (%)	HIS (%)
PO	15	76.7 ± 1.4^a	3.8 ± 0.0^a	2.9 ± 0.1^a
PC	15	86.7 ± 0.0^b	3.7 ± 0.4^a	3.0 ± 0.1^a

Table 5. Reproductive performance of male shrimp fed a carotenoid-enriched diet for 20 weeks cultured in pre-maturation stage followed by six weeks of feeding at maturation stage. Data are expressed as means \pm SD ($n=2$). Means on the same column with a different letter are significantly different (*t*-test, $P < 0.05$)

Test diet	Male shrimp	Male shrimp releasing spermatophore at maturation (%)	Weight of spermatophore (g)	Males stock releasing spermatophore at re-maturation (%)	Weight of spermatophore (g)
PO	15	69.9 ± 4.5^a	0.096 ± 0.004^a	58.6 ± 2.1^a	0.087 ± 0.004^a
PC	15	82.3 ± 4.0^b	0.104 ± 0.021^a	52.2 ± 1.1^a	0.100 ± 0.032^a

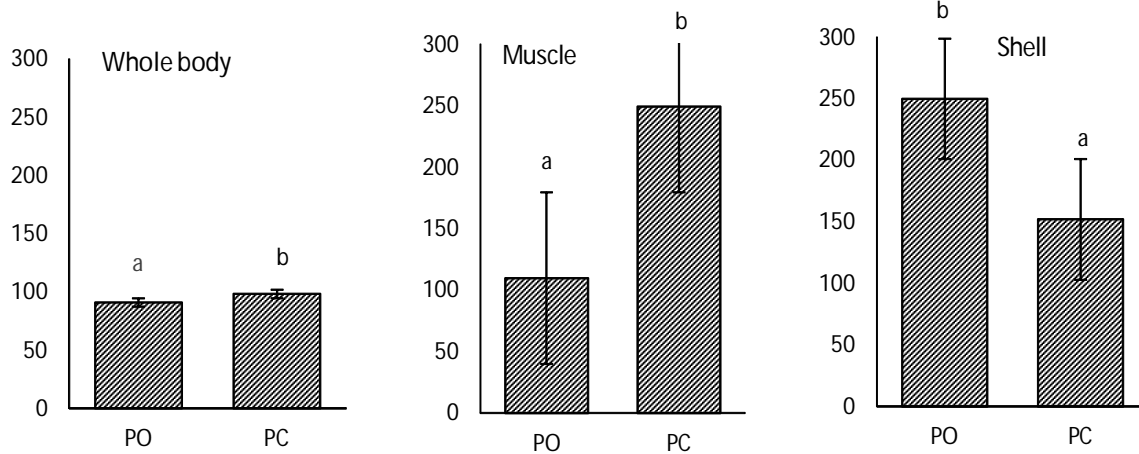


Figure 1. Total carotenoid content ($\mu\text{g/g}$) in the whole body, meat and shell of matured female shrimp fed carotenoid-enriched diets for 20 weeks at the prematuration stage followed by six weeks of feeding at the maturation stage. Data are expressed as means \pm SD ($n=4$). Means with a different letter are significantly different (t-test, $P<0.05$).

Figure 2 shows the total carotenoid content in the ovary and hepatopancreas. Total carotenoid in ovary tissue was significantly higher ($P<0.05$) in shrimp fed the PC diet (558 $\mu\text{g/g}$) than in shrimp fed the PO diet (470 $\mu\text{g/g}$). Similarly, total carotenoid content in the hepatopancreas of shrimp fed the PC diet was also higher (558 $\mu\text{g/g}$) than in shrimp fed the PO diet (465 $\mu\text{g/g}$). The concentration of total carotenoid in these two tissues was about twice that of meat tissue.

Amino acid profile of oocyte and spermatophore

Table 6 presents amino acid content in ovarian tissue of shrimp after being fed the test diets for 20

weeks at the prematuration stage followed by six weeks at the maturation stage. The total amino acid content in the ovary of shrimp fed PC diet averaged 44.47% and did not significantly differ from shrimp fed PO diet (45.88%).

The amino acid profile of spermatophores of shrimp fed the PC diet was significantly higher (73.82%) than those fed the PO diet (66.09%) (Table 7). The concentrations of most of the individual amino acids in the spermatophore were improved after they had been fed with the PC diet. On the other hand, several amino acids of the spermatophore including lysine and aspartic acid were not enhanced by the tested dietary treatment. Moreover, cysteine was not detected in shrimp fed either the PO or PC diets.

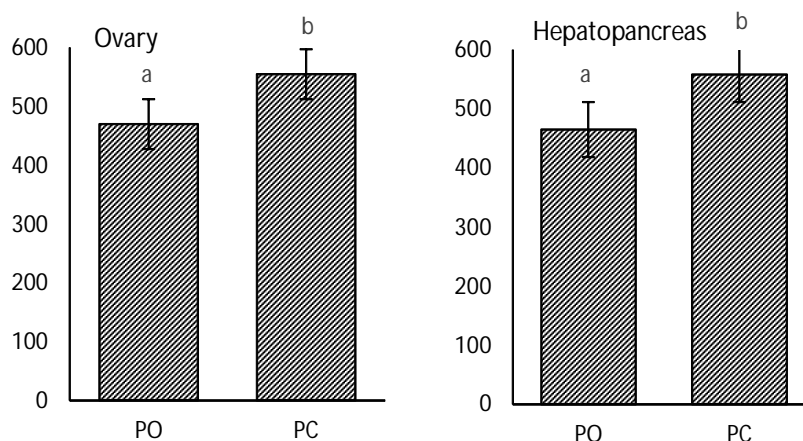


Figure 2. Total carotenoid content ($\mu\text{g/g}$) in ovary and hepatopancreas of matured female shrimp fed carotenoid-enriched diets for 20 weeks at pre-maturation stage followed by six weeks of feeding at maturation stage. Data are expressed as means \pm SD ($n=4$). Means with a different letter are significantly different (t-test, $P<0.05$).

Table 6. Amino acid profile (% dry matter) of the ovary of tiger shrimp fed a carotenoid-enriched diet for 20 weeks at the prematuration stage followed by six weeks of feeding at the maturation stage. Data are expressed as means ± SD (n=2)

Amino acid	Test diets	
	PO	PC
Histidine	1.20 ± 0.11 ^a	1.18 ± 0.07 ^a
Threonine	2.89 ± 0.28 ^a	2.89 ± 0.02 ^a
Isoleucine	2.50 ± 0.21 ^a	2.44 ± 0.06 ^a
Leucine	3.81 ± 0.30 ^a	3.72 ± 0.00 ^a
Phenylalanine	2.36 ± 0.12 ^a	2.32 ± 0.04 ^a
Lysine	3.33 ± 0.31 ^a	3.13 ± 0.09 ^a
Valine	3.01 ± 0.18 ^a	2.97 ± 0.03 ^a
Methionine	0.81 ± 0.11 ^a	1.01 ± 0.23 ^a
Serine	2.47 ± 0.23 ^a	2.46 ± 0.26 ^a
Glycine	2.90 ± 0.23 ^a	2.67 ± 0.08 ^a
Proline	ND	ND
Tyrosine	1.87 ± 0.05 ^a	1.90 ± 0.02 ^a
Arginine	2.85 ± 0.18 ^a	2.81 ± 0.26 ^a
Alanine	3.35 ± 0.33 ^a	3.18 ± 0.11 ^a
Aspartic acid	5.02 ± 0.32 ^a	4.91 ± 0.17 ^a
Glutamic acid	7.54 ± 0.70 ^a	6.92 ± 0.45 ^a
Cysteine	ND	ND
Total	45.88 ± 3.67 ^a	44.47 ± 0.95 ^a

Means on the same line with a different "letter" are significantly different (t-test, "P<0.05)."

ND: not detected

Table 7. Amino acid profile (% dry matter) of spermatophores of shrimp fed a carotenoid-enriched diet for 20 weeks at the prematuration stage followed by six weeks of feeding at the maturation stage. Data are expressed as means ± SD (n=2)

Amino acid	Test diets	
	PO	PC
Histidine	0.5513 ± 0.0187 ^a	1.3365 ± 0.0362 ^b
Threonine	4.4610 ± 0.0006 ^a	5.3633 ± 0.0197 ^b
Isoleucine	3.3140 ± 0.0064 ^a	3.4154 ± 0.0251 ^b
Leucine	5.3760 ± 0.0039 ^a	5.6766 ± 0.0291 ^b
Phenylalanine	1.2532 ± 0.0397 ^a	1.7945 ± 0.0289 ^b
Lysine	6.3206 ± 0.0262 ^b	5.7023 ± 0.0095 ^a
Valine	2.7724 ± 0.0387 ^a	3.3706 ± 0.0157 ^b
Methionine	1.9651 ± 0.0014 ^a	3.1361 ± 0.0346 ^b
Serine	5.0690 ± 0.0208 ^a	5.8857 ± 0.0013 ^b
Glycine	4.2184 ± 0.0549 ^a	5.1757 ± 0.0179 ^b
Proline	5.1890 ± 0.0078 ^a	5.6722 ± 0.0214 ^b
Tyrosine	0.0613 ± 0.0001 ^a	0.1187 ± 0.0003 ^b
Arginine	5.2716 ± 0.0035 ^a	6.8453 ± 0.006 ^b
Alanine	2.4454 ± 0.0211 ^a	2.6056 ± 0.0064 ^b
Aspartic acid	9.0997 ± 0.0697 ^a	8.8076 ± 0.0076 ^a
Glutamic acid	8.7219 ± 0.0184 ^a	8.9154 ± 0.0009 ^b
Cysteine	ND	ND
Total	66.0901 ± 0.0892 ^a	73.8215 ± 0.1087 ^b

Means on the same line with a different letter are significantly different (t-test, P<0.05)

Fatty acid profile in the meat of female broodstock

The total fatty acid (TFA) content of shrimp meat fed the PC diet was significantly higher than for those fed the PO diet ($P < 0.05$) (Table 8). Several fatty acids significantly increased in shrimp fed the PC diet, in particular the HUFAs arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

A high concentration of total carotenoid in oocytes indicated that this micronutrient is importantly

required for ovary maturation as many previous studies reported. The important function of astaxanthin in the yolk sac is probably related to its function to improve antioxidant enzyme activities which further increase the tolerance against environmental stress including salinity, pH, oxygen depletion and nitrite stress (Flores *et al.*, 2007; Diaz *et al.*, 2014; Wang *et al.*, 2018) which may happen during the earlier stage of larval development.

Astaxanthin has a hydroxyl (-OH) and ketone (-CO) functional groups that make its structure polar and

Table 8. Fatty acid profile (g/100g) in the meat of matured female shrimp fed a carotenoid-enriched diet for 20 weeks at the prematuration stage followed by six weeks of feeding at the maturation stage. Data are expressed as means \pm SD ($n=2$)

Fatty acid	Test diet	
	PO	PC
C 8:0	0.0033 \pm 0.0003	ND
C 10:0	0.0066 \pm 0.005	ND
C 12:0	0.0819 \pm 0.0012 ^b	0.0051 \pm 0.0011 ^a
C 14:0	0.0829 \pm 0.0002 ^a	0.0388 \pm 0.0025 ^a
C 15:0	0.0174 \pm 0.0002 ^a	0.029 \pm 0.0009 ^b
C 16:0	1.0316 \pm 0.0075 ^b	0.8113 \pm 0.0006 ^a
C 16:1	0.0616 \pm 0.0013 ^a	0.0800 \pm 0.0007 ^b
C 17:0	0.0453 \pm 0.0010 ^a	0.0836 \pm 0.0022 ^b
C 17:1	ND	0.0123 \pm 0.0103
C 18:0	0.5239 \pm 0.0030 ^a	0.6038 \pm 0.0049 ^b
C 18:1	0.5390 \pm 0.0050 ^a	0.6756 \pm 0.0041 ^b
C 18:2	0.7140 \pm 0.0080 ^a	0.7245 \pm 0.0008 ^a
C 18:3	0.0273 \pm 0.0002 ^a	0.0343 \pm 0.0008 ^b
C 20:0	0.0256 \pm 0.0011 ^a	0.0239 \pm 0.0024 ^a
C 20:1	0.0138 \pm 0.0010 ^a	0.0165 \pm 0.0006 ^a
C 20:2	0.0245 \pm 0.0008 ^a	0.0386 \pm 0.0025 ^a
C 20:3n-3	0.0113 \pm 0.0020 ^a	0.0069 \pm 0.0009 ^a
C 20:3n-6	0.0045 \pm 0.0002 ^a	0.0049 \pm 0.0004 ^a
C 20:4n-6 (ARA)	0.1617 \pm 0.0064 ^a	0.3483 \pm 0.0028 ^b
C 20:5n-3 (EPA)	0.2812 \pm 0.0006 ^a	0.4888 \pm 0.0021 ^b
C 21:0	0.0027 \pm 0.0006 ^a	0.0051 \pm 0.0004 ^a
C 22:0	0.0205 \pm 0.0005 ^a	0.0289 \pm 0.0022 ^a
C 22:1n-9	0.004 \pm 0.0003 ^a	0.0038 \pm 0.0001 ^a
C 22:6n-3 (DHA)	0.2311 \pm 0.0038 ^a	0.4050 \pm 0.0041 ^b
C 23:0	0.0063 \pm 0.0016 ^a	0.0069 \pm 0.0015 ^a
C 24:0	0.0095 \pm 0.0015 ^a	0.0129 \pm 0.0007 ^a
Total	3.9282 \pm 0.0100 ^a	4.4888 \pm 0.0600 ^b

Means in the same row with different superscript letters are significantly different (t-test, $P < 0.05$). ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid. ND: not detected

susceptible to oxidation, thereby imparting antioxidant properties (Hussein *et al.*, 2006). It can react with free radicals to convert them into a more stable product and terminate the reactions. The antioxidant level of astaxanthin is 10 times more than zeaxanthin and lutein and even 100 times higher than α -tocopherol (Miki, 1991).

The significant increase in total amino acid content in the spermatophores of shrimp fed the PC diet shows that dietary carotenoids can improve amino acid metabolism in shrimp gonads. As an antioxidant which is capable of protecting cells from free radicals such as hydroxyl and reactive oxygen, carotenoids could minimize destroyed cell membranes and protein (DellaPenna & Pogson, 2006; Bailey & Grossman, 2008). However, this effect was not observed in female shrimp where the total amino acid content of the ovaries in shrimp fed the PC diet did not significantly differ from those fed the PO diet. This finding may imply that the effect of carotenoid was more pronounced on amino acid metabolism in male shrimp than in females. Higher protein levels (64-74%) in spermatophores might be a possible reason why this organ was more sensitive to a change in protein metabolism caused by dietary carotenoid compared to the ovary. Furthermore, undetected cysteine in both spermatophore and ovary of shrimp fed the two diets might indicate that this amino acid was not required for gonadal development of tiger shrimp. Recent publications on the biochemical organ of matured shrimps in particular spermatophore fed carotenoid diet are scarcely available, and thus difficult to compare with the finding of this present study.

Another biological function of carotenoids is to reduce lipid peroxidation (Santos *et al.* 2012; da Silva *et al.*, 2015) which is necessary to maintain the stability of fatty acids. This was in line with the pattern of total fatty acid found in the meat of matured female shrimp which was significantly enhanced when shrimp consumed the carotenoid diet (PC). Unfortunately, in this study, fatty acids profiles in both ovaries and spermatophores were not analyzed due to insufficient samples.

In this study, the commercial shrimp diet used as the standard diet already contained a total carotenoid of approximately 90 mg/kg diet (Table 1). However, this concentration seemed insufficient to support the reproductive traits of tiger shrimp indicated by the lower rate of gonadal maturation of both female and male shrimps fed control PO diet compared to carotenoid enriched PC diet. On the other hand, the concentration of total carotenoid in the standard diet used could support the growth and survival of the shrimp (Laining *et al.*, 2017). Therefore, it is impor-

tant to incorporate a greater level of carotenoids in the maturation diet to improve the reproductive traits of tiger shrimp as illustrated in this present study.

CONCLUSION

Total carotenoid content in meat, oocyte and hepatopancreas of female tiger shrimp increased by supplementing carotenoid in the diet fed since the prematuration stage. Dietary carotenoid significantly improved ovarian maturation of female shrimp from 76.7% to 86.7% and improved the spermatophore formation of male tiger shrimp from 69.9% to 82.3%. The TAA in spermatophores increased significantly by feeding shrimp with dietary carotenoid, as did TFA in meat, in particular the HUFAs ARA, EPA and DHA.

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