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CAROTENOID-ENRICHED DIET FOR PRE-MATURATION STAGE OF POND-REARED TIGER SHRIMP, *Penaeus monodon* PART I. THE EFFECTS ON GROWTH, PIGMENTATION AND WHOLE BODY NUTRIENT CONTENT

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ABSTRACT

Carotenoids, besides as a natural pigment, may have vital roles in the growth of crustacean. The aim of this study was to clarify the influence of combined carotenoids given since pre-maturation stage on the growth performances, pigmentation and biochemical composition of the whole body of pond-reared tiger shrimp, *Penaeus monodon*. Two experimental diets were supplemented with or without carotenoid mixture consisting of astaxanthin, canthaxanthin and other carotenoids contained in Spirulina. The carotenoid mixture was supplemented in the commercial diet normally used as a starter feed for tiger shrimp, re-pelleted and fed to tiger shrimp with an initial body weight of 31.7±1.3 g. Shrimp were stocked in four 1,000 m^2 concrete ponds with a density of 1 shrimp/m^2 and fed until the shrimp reached maturation stage (broodstock size). Variables observed were growth performances and pigmentation properties during the pre-maturation stage and total carotenoid content in several tissues of the female broodstock after being fed with the diets until maturation stage. After 16 weeks, shrimp fed with carotenoid-enriched diet (PC) diet produced significantly ($P<0.05$) higher biomass than the diet without the enrichment (PO). The color of raw shrimp fed with PC diet was darker with greenish-brown compared to shrimp fed PO diet which was greenish blue. The visual appearances of 3-min steamed shrimp produced the color of red-orange for shrimp fed carotenoid compared to orange-yellow for control PO. The total carotenoid content in the whole body of shrimp fed PC diet were significantly ($P<0.05$) enhanced compared to control PO diet which was 42.8 ±5.8 and 55.8± 5.1 μg/g for PO and PC diet, respectively. Supplemental carotenoid in the pre-maturation diet increased the biomass production from 23.1±1.9 kg to 30.2 ±0.1 kg and enhanced the color of the shrimp which was in line with carotenoid content in the whole body of pond-reared tiger shrimp.

KEYWORDS: **pre-maturation; carotenoid-enriched diet; pigmentation; pond-reared tiger shrimp**

INTRODUCTION

Carotenoids, the main pigments of many aquatic animals, have been associated with freshness and product quality of seafood including shrimp (Higuera-Ciapara *et al*., 2006; Niu, *et al*., 2011; Parisenti *et al*., 2011). The colors of cooked and raw shrimp are important quality criteria in world markets and may be associated with premium pricing (Hunter, 2001). Besides as pigments, carotenoids also have several biological functions including as sources of pro-vitamin A (DellaPenna & Pogson, 2006; Krinsky & Johnson, 2005), cellular protection from photo-dynamic damage (Nakano *et al*., 1999), as a biological antioxidant (Miki *et al*., 1994, da Silva *et al*., 2015), and enhancement of growth and reproductive potential (Bjerkeng *et al*., 2000; Choubert *et al*., 1998; Pangantihon-Kuhlmann *et al*., 1998). Some evidences suggest that these pigments may perform vital roles in the growth of crustaceans (Niu *et al*., 2011; Supamattaya *et al*., 2005) and fish (Vasallao-Agius *et al*., 2001; Watanabe *et al*., 1991).

Specifically, astaxanthin is the predominant carotenoid found in penaeid shrimp and other crustaceans (86-98% of total carotenoid) attributing the characteristic color to these crustaceans (Armenta & Guerrero-Legarreta, 2009), which in turn increase consumer's acceptance to the products (Parisenti *et al*., 2011). Astaxanthin has a stronger antioxidant property than other carotenoids, such as β-carotene,

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lutein, and zeaxanthin (Naguib, 2000). The presence of astaxanthin protects crustaceans from the lipid peroxidation (da Silva *et al*., 2015) such as oxidation of polyunsaturated fatty acids and cholesterol (Palozza *et al*., 2008).

Microalgae have been widely acknowledged to contain various carotenoids such as *Haematococcus pluvialis* (Parisenti *et al*., 2011), *Botryococcus brauni* (Rao *et al*., 2006) and *Dunaliella salina* (Boonyaratpalin *et al*., 2001). A recent study reported that *Tetraselmis* sp DS3 contains > 5% β-carotene and 0.48% lutein of biomass and also has the same biological functions as other carotenoid sources (Tsai *et al*., 2016). In this present study, *Spirulina* sp. was used as one of the carotenoid sources which are widely available as a commercial product.

The development of broodstock diet for pond-reared tiger shrimp should be programmed not only for maturation stage (Paibulkichakul *et al*., 2008), but also for growth and pre-maturation stages. Specific nutrients requirement during pre-maturation stage such as carotenoid is important to be determined as very little information is available for this stage. Therefore, this present study was carried out to clarify the influence of combined carotenoids fed since pre-maturation stage on the growth, pigmentation and biochemical composition of the whole body of pond-reared tiger shrimp, *Penaeus monodon.*

MATERIALS AND METHODS

Experimental Diets

Two experimental diets were enriched either with or without carotenoid mixture into a standard diet and designated as PC and PO diets, respectively. A standard diet is a crumbled commercial diet normally used as a starter feed for tiger shrimp during initial stocking in the ponds. Three types of carotenoid were used for the enrichment including astaxanthin at a level of 0.125% (Paibulkichakul *et al*., 2008), canthaxanthin (0.068%) and carotenoids in *Spirulina* powder (0.300%) following the previous experiment dealing with rabbitfish (Laining *et al*., 2015). The carotenoids were supplemented to a standard diet by thoroughly mixing them with the commercial diet and added with water approximately 300-350 g/kg of dry ingredient (Laining *et al*., 2012; 2017). 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. The moisture content of the dried pellet was below 12%. The test diets were kept in low temperature at 18°C during the feeding trial for the two stages.

Proximate composition of the two diets is presented in Table 1 in which both diets were iso-nitrogenous containing approximately 44% crude protein and 7% lipid. The *Spirulina* powder used in the enrichment contained relatively high total carotenoid content which was 1,464 ± 6.15 mg/kg. The total carotenoid content of the commercial standard diet and diet without carotenoid enrichment (PO) were similar at approximately 90 mg/kg while PC diet contained higher level at 150.5 mg/kg (Table 2).

Growth Trial at Pre-maturation Stage in Concrete Ponds

Five thousand tiger shrimps with an average initial body weight of 31.7 ± 1.3 g with a weight range of 30-33 g were transferred from the Brackishwater Aquaculture Development Centre in Takalar to the Installation of Tiger Shrimp Hatchery of Research Institute for Coastal Aquaculture (RICA) in Barru Regency, South Sulawesi, Indonesia. The age of the shrimp was approximately six months old at the stocking time. The shrimp were selected and stocked in four 1,000 m^2 concrete ponds with a density of one shrimp/m^2. Shrimp were fed with 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 maturation stage or five months culture period. Water quality was monitored every day in the morning and in 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, then lime was applied at the level of 15 ppm to increase its concentration.

The growth of tiger shrimp was evaluated by measuring the weight every month for five months. The variables observed during the pre-maturation phase were growth performances and organoleptic evaluation for pigmentation. At the end of the feeding trial, organoleptic evaluation was carried out by comparing the color between the two groups for both raw and steamed shrimp. Ten raw shrimps from the two groups were steamed for 3-minutes and evaluated color differences.

Samples Collection and Biochemical Analysis

At the end of the feeding trial, fifteen shrimps from each group were used for biochemical analysis. The whole body shrimp were dried using an oven at 60°C (Memmert, Germany), pulverized and kept in -20°C until subsequent analysis.

Proximate analysis of the diets and the shrimp were done according to AOAC International (2005). Crude protein was determined according to the mi-

Table 1. Proximate composition (%, dry basis ± SD of triplicates) of tested diets fed to tiger shrimp during pre-maturation stage

Diets	Crude protein	Lipid	Crude fiber	Ash	NFE*
PO	44.5 ± 1.9	7.1 ± 1.0	4.0 ± 0.9	11.3±0.0	33.1
PC	43.6 ± 1.5	7.7 ± 0.1	4.0 ± 0.4	11.2±0.1	33.5

Note: * Nitrogen free extract

Table 2. Total carotenoid content (mg/kg ± SD of duplicates)in *Spirulina* powder, commercial standard diet and the two tested diets

	Spirulina powder	Standard diet	Tested diets	
			PO	PC
Total carotenoid	1,464.48 ± 6.15	90.66 ± 0.40	90.29 ± 0.05	150.46 ± 0.04

cro-Kjeldahl procedure. Lipid was extracted using chloroform and methanol. Ash was analyzed using muffle furnace at 550°C (Barnstead, Thermolyne, CA, USA). The analysis of total carotenoid was carried-out according to AOAC (2005). The samples were extracted with acetone and hexane and elucidated in the column prior reading the carotenoid content spectrophotometrically (Spectrophotometer UV VIS Jasco V-70, Japan).

Calculation and Statistical Analysis

The biomass of the shrimp (g) was calculated by multiplying the number of shrimp survived at the end of the feeding trial with the average final body weight.

Data on the growth traits and biochemical component in several tissues were statistically analyzed using Independent *t*-test with SPSS software (version 21; SPSS, Inc., Chicago, Illinois, USA). Differences were considered significant at $P<0.05$. Pigmentation of raw and cooked shrimps was descriptively presented.

RESULTS AND DISCUSSION

Growth, Survival and Biomass Production of Shrimp

The pattern of shrimp growth fed with the two diets during pre-maturation stage is illustrated by Figure 1. The shrimp still grew linearly even at the age of 11 months indicating that the culture condition was at the optimum level. According to Rothlisberg (1998), penaeid shrimp in nature grow faster from first to ninth months post-hatching and then reach the stagnant phase. The weight gain of 170% obtained in the present study was over five times higher than the weight gain of shrimp reared for 16 months which was only 30% as found in our previous study (Laining *et al*., 2014). The low weight gain found in our previous study indicated that at 16 months of age, the growth of tiger shrimp reached the stagnant phase. This stagnant phase could be an indicator of the maturation stage of the shrimp or the phase where the shrimp has been categorized as a broodstock.

Table 3 presents the survival rate, weight gain and biomass of tiger shrimp after 16 weeks culture during the pre-maturation stage. The survival rate of shrimp fed with PO diet was 33.8% lower than PC diet (36.9%) but both were not significantly different ($P> 0.05$). Weight gains of shrimp fed the two diets were also not significantly different. However, shrimp fed with PC diet produced significantly ($P<0.05$) higher biomass than PO diet which was 23 kg and 30 kg, respectively. The significant higher biomass found in PC diet indicated that to some extent, the dietary carotenoid had a beneficial effect on biomass production of the tiger shrimp. This was in line with a previous study dealing with black tiger shrimp where the growth and survival were positively affected by the addition of carotenoids (Niu *et al*., 2012). A similar effect was also reported on Kuruma prawn (*Penaeus japonicus*) fed with β-carotene from *Dunaliella salina* (Supamattayaa *et al*., 2005). In addition, supplementation of microalgae *Haematococcuspluvialis* as a dietary carotenoid source presented higher survival rate and weight gain of juvenile vaname shrimp (*Litopenaeus vannamei*) (Parisenti *et al*., 2011). Even though these previous studies did not provide suffi-

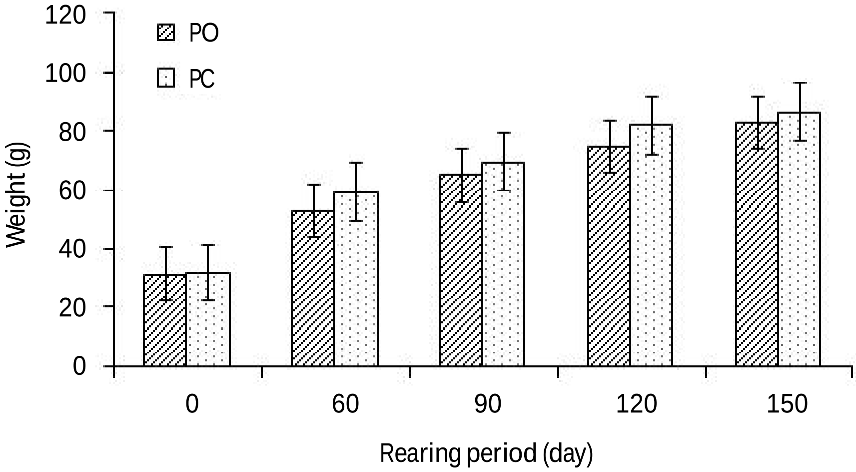

Figure 1. The pattern of weight increment of tiger shrimp during pre-maturation stage reared in the concrete ponds. PO: diet without carotenoid supplement, PC: diet with supplemental carotenoid.

cient data on biomass, the significant higher growth and survival detected in those studies implied a significantly higher biomass which was similar to that of found in the present study.

Data are expressed as means (n = 2) ± SD. Means on the same column with different letter are significantly different (*t*-test, $P < 0.05$). PO: diet without carotenoid supplement, PC: diet with supplemental carotenoid

The positive effects of carotenoid on the growth, survival and feed utilization have been also reported on fish species such as fry of European seabass (Sallam *et al*., 2017). Furthermore, the specific growth rate of yellowtail cichlid significantly improved even when *Spirulina* meal included solely as the dietary carotenoid source (Guroy *et al*., 2012). The improvement in the performance parameters for the growth of shrimp fed with diets supplemented with the carotenoid mixture in the present study may be due to the role of carotenoid as an antioxidant (Pan *et al*., 2010) and its involvement in intermediary metabolism (Talebi *et al*., 2013). In the present study, antioxidant status of the shrimp fed dietary treatments was not determined. However, previous studies have found that carotenoids decreased the levels of TBARs (thiobarbituric acid-reactive substances), indicating lipid peroxidation (da Silva *et al*., 2015; Palozza *et al*., 2008; Sallam *et al*., 2017), alleviating oxidative damage (Santos *et al*., 2012) and limiting free radical molecule production caused by cellular metabolism and other various stressors (Pan *et al*., 2010).

Pigmentation of Tiger Shrimp

Based on the organoleptic evaluation, the color of the shrimp fed with PC diet tended to be

Table 3. Survival rate, weight gain and biomass of tiger shrimp after 20 weeks fed with carotenoid-enriched diet at pre-maturation stage

Test diet	Survival rate (%)	Initial weight (g)	Final weight (Range) (g)	WG (%)	Biomass (kg)
PO	33.8 ± 3.8[a]	31.5 ± 5.0	82.9 ± 1.9 (41-144)	163.2 ± 5.9[a]	23.1 ± 1.9[a]
PC	36.9 ± 2.5[a]	32.0 ± 1.1	86.5 ± 0.1 (45-146)	170.3 ± 3.5[a]	30.2 ± 0.1[b]

Data are expressed as means (n = 2) ± SD. Means on the same column with different letter are significantly different (*t*-test, $P < 0.05$). PO: diet without carotenoid supplement, PC: diet with supplemental carotenoid dark with greenish brown (Figure 2-2) compared to shrimp fed without carotenoid which was greenish blue (Figure 2-1). Moreover, Figure 2-4 shows the visual appearances of 3-min steamed shrimp which produced the color of red-orange for shrimp fed carotenoid diet compared to orange-yellow for control PO (Figure 2-3).

Pigmentation occurs in the tiger shrimp fed with the diet enriched with carotenoid in this present study supported the previous findings demonstrating the role of carotenoid in pigmentation of aquatic animal including crustacean (Hatlen *et al*., 1998; Bowen *et al*., 2002; Armenta & Guerrero-Lagarreta, 2009). The green or brown coloration of raw tiger shrimp and the orange-red coloration of cooked tiger shrimp is due to the presence of naturally occurring pigments, carotenoid, in which astaxanthin is the principal pigment deposited (Springate & Nickell, 2000). Wild tiger shrimp performing a dark green or brown appearance of raw shrimp and a dark orange-red appearance of cooked shrimp may contain 40-60 ppm of total astaxanthin (Latscha, 1989).

Other penaeid species, *L. vannamei* fed with the dietary carotenoid also showed higher red-like color than the control group. The color difference was in line with the higher carotenoid content in both muscle and exoskeleton of the *L. vannamei* shrimp fed with the microalgae compared to control. Similar effects of carotenoid extracted from *H. pluvialis* on muscle and skin pigmentation were also reported in rainbow trout (Bowen *et al*., 2002) and on red porgy (*Pagrus pagrus*)when fed with esterified astaxanthin from *H. pluvalis* (Tejera *et al*., 2007).

Basically, dark-colored shrimps of wild catch and shrimp grown under extensive and semi-intensive conditions are mainly caused by the algal food chain which contains astaxanthin and other carotenoids. In

(2-1) Pigmentation of raw tiger shrimp without carotenoid (PO)

(2-2) Pigmentation of raw tiger shrimp fed carotenoid (PC)

(2-3) Pigmentation of 3-min steamed tiger shrimp without carotenoid (PO)

(2-4) Pigmentation of 3-min steamed tiger shrimp with carotenoid (PC)

Figure 2. Colour appearances of raw and steamed tiger shrimps after 20 weeks fed with carotenoid-enriched diets during the pre-maturation stage. PO: diet without carotenoid supplement, PC: diet with supplemental carotenoid.

contrast, intensively grown shrimp has been reported to often have pale color since less natural feed is available which resulted in light green, blue or off-white raw shrimp and pale yellow or yellow-orange when cooked (Hunter, 2001). The color of cooked shrimp fed with PO diet containing un-supplemented carotenoid was not pale yellow as indicated by Figure 2-3 indicating that the carotenoid contained in the commercial diet used as the basal diet for this current study was probably sufficient in forming color pigmentation in the shrimp. In addition, the shrimp may also consume sufficient carotenoid from algal food chain under semi-intensive culture system used in this present study. Therefore, when shrimp is cultured under the intensive condition where natural feed may be insufficient to provide the carotenoid, the diet needs to be supplemented with carotenoid to fulfill its requirement.

Proximate Composition and Total Carotenoid Content in the Whole Body of Female Broodstock

Table 4 presents crude protein, lipid and total carotenoid in the whole body shrimp at the end of the pre-maturation stage in concrete ponds. Crude protein and lipid content of shrimp fed with the two diets were relatively similar, but total carotenoid content in shrimp fed with PC diet was significantly higher ($P < 0.05$) than that of fed with control diet (55.8 vs42.8 μg/g). These total carotenoid values were in line with the pigmentation appearance of the shrimp in the two groups as shown in Figure 2.

Table 4. Crude protein, lipid and total carotenoid content in the whole body of tiger shrimp after 20 weeks fed carotenoid enriched-diet during pre-maturation stage

Test diets	Crude protein (% dry matter)	Lipid (% dry matter)	Total carotenoid (μg/g)
PO	68.9 ± 1.7[a]	5.7 ± 1.3[a]	42.8 ± 5.8[a]
PC	69.8 ± 1.2[a]	5.8 ± 0.9[a]	55.8 ± 5.1[b]

Data are expressed as means (n = 2) ± SD. Means on the same column with different letter are significantly different (*t*-test, $P < 0.05$). PO: diet without carotenoid supplement, PC: diet with supplemental carotenoid

A similar finding was found on seabass where no clear trend was observed regarding the effect of different carotenoid sources on the whole body composition. However, the total body carotenoid content was significantly higher in fish fed with carotenoid diet than fish fed with control diet (Sallam *et al.*, 2017).

In the present study, *Spirulina* sp. was used as one of the carotenoid sources which were suspected to function synergetically with the synthesized astaxanthin and canthaxanthin in enhancing several biological performances in particular biomass production and pigmentation for the pre-maturation stage of tiger shrimp.

Water Quality During The Culture

The ranges of water quality parameters during the feeding trial for 20 weeks are presented in Table 5. Generally, there were not many differences in the parameter ranges between the morning and the afternoon measurements. Temperature ranged from approximately 27- 34°C, while salinity ranged from around 18-41 ppt. DO was higher in the afternoon with the highest concentration was up to 9.2 mg/L than in the morning (8.7 mg/L). The range of pH was also similar between the morning and afternoon measurements from approximately 7.0 to 8.9. In case of alkalinity, its concentration varied from 65 to 180 mg $CaCO_3$/L in which the lower alkalinity was detected during the rainy season. The application of dolomite at the level of 15 mg/L was able to increase the alkalinity over 100 mg/L.

Table 5. Range of water quality measured in the experimental ponds during 20 weeks feeding trial

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	-

Note: * Measured only in the morning

CONCLUSION

Combined carotenoid of astaxanthin, canthaxanthin and *Spirulina* meal in the pre-maturation diet at the supplemental level of 0.493% improved the biomass of tiger shrimp from 23.1 ± 1.9 kg to 30.2 ± 0.1 kg. Dietary carotenoid involved in the pigmentation of premature tiger shrimp and improved the total carotenoid content in the whole body from 42.8 ± 5.8 to 55.8 ± 5.1 μg/g.

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