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Research

Effects of Dietary Oil Palm Carotenes on Colour Intensity and Astaxanthin Content in Giant Freshwater Prawn (*Macrobrachium rosenbergii*)

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ABSTRACT

Prawns have the metabolic ability to convert dietary carotenoids, such as beta-carotene, into astaxanthin, which increases the colour intensity and boosts immunity, thereby improving prawn growth performance. Currently, prawn feed manufacturers are using expensive synthetic astaxanthin as a feed supplement, which leads to higher feed costs. Oil palm carotene can be used in prawn feed production to replace the commercial synthetic astaxanthin in the market. A study was conducted to evaluate the effects of dietary oil palm carotene on the colour intensity, total carotenoids, and astaxanthin content of giant freshwater prawns (*Macrobrachium rosenbergii*). Five different treatments that are isonitrogenous and isocaloric were used in this study, including T1 (commercial feed); T2 (control, without oil palm carotene); T3 (3% crude palm oil (CPO) inclusion); T4 (3% palm pressed fibre oil (PPFO) inclusion); and T5 (3% commercial oil palm-mixed carotene inclusion). Prawns were fed to satiation five times daily during the 10-week feeding trial. At the end of the feeding trial, prawn samples were collected for colour intensity, total carotenoids, and astaxanthin content analysis. The T4 and T5 prawn samples showed better colour intensity in terms of lightness in cooked samples as well as yellowness and redness for both raw and cooked samples. Prawns fed with diets consisting of oil palm carotenes (T3, T4 & T5) contained higher total carotenoids content (more than 40 ppm) and astaxanthin content (ranging from 14.65 to 16.96 ppm) than the commercial (T1) and control (T2) groups. These results indicate that supplementation of oil palm carotenes in the giant freshwater prawn can potentially replace expensive commercial synthetic astaxanthin that is practically used by aquaculture feed producers.

Key words: Astaxanthin, body colour, carotenoids, giant freshwater prawn, oil palm carotene

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INTRODUCTION

Macrobrachium rosenbergii, a giant freshwater prawn, is one of the commercial aquaculture species being given high attention by the Malaysian Department of Fisheries (Anon, 2010) as food and food products for export and consumption (Rubia & Annie, 2016). This species has become the most important cultured freshwater prawn species as a decent way to make money due to its superior cultivable attributes such as fast growth rate, large size, and tolerance to salinity fluctuations (New & Valenti, 2000). Demand for this species is increasing, especially throughout Asia, for its good taste (Whangchai *et al*., 2007) and ability to be integrated with other agricultural farms such as rice or fish productions (New, 2005; Alam *et al*., 2022).

In aquaculture farming, feed costs are the most expensive operating expense, accounting for 60–70% of total production costs (Khan *et al*., 2018; Prodhan & Khan, 2018). Hence, it is essential to look for feed ingredients that can improve the growth rate and survival of the cultured species to reduce the production cost. Carotenoids are a family of more than 600 naturally occurring lipid-soluble pigments that are generated by algae (including phytoplankton), higher plants, as well as photosynthetic bacteria (Meyers & Latscha, 1997; Maoka, 2020; Sonia *et al*., 2021). Due to their beneficial characteristics, carotenoid pigments are used as key nutritional additives in several fish and crustaceans' diets for example, as a precursor of vitamin A and to protect organism cells from oxidative damage caused by active oxygen species (Misawa, 2010).

Crude palm oil (CPO) is one of the most highly produced plant oils in the world (Kenari *et al*., 2011; Ng, 2002; Ng & Gibon, 2011) and has several beneficial characteristics conducive to the aquaculture industry, including being relatively cheap and containing a high carotenoid content. Generally, Malaysian CPO contains 500 to 700 ppm mixed-carotene (Lau, 2016), of which 37% is alpha-carotene and about 50% is beta-carotene (Yap *et al*., 1991). In recent years, the dark red compounds of alpha and beta-carotene have become popular natural food colouring agents in the food and beverage sector. Besides CPO, palm-pressed fibre oil (PPFO) is the residual oil that is extracted from palm-press fibres and contains 19% alpha-carotene and 31% beta-carotene (Akanda *et al*., 2012). The commercial oil palm-mixed carotene is a natural oil suspension of mixed-carotene complexes that is dark brownish, viscous, and derived from virgin crude palm oil. This product is specifically formulated for use as an additive in animal feed formulations to give the maximum health benefits to the animals. It is mainly used as a source of pro-vitamin A and as an antioxidant.

Prawns can metabolically transform dietary carotenoids, such as beta-carotene, into astaxanthin, which intensifies colour and boosts immunity, hence improving prawn growth performance (Zhao *et al*., 2022). Furthermore, carotenoids can improve the colour and appearance of prawns (Kim *et al*., 2013), which contain a wide spectrum of vitamin E isomers that prevent lipid peroxidation (Wang *et al*., 2006). Currently, prawn feed producers are using pricey synthetic astaxanthin as a feed supplement, which increases the cost of the feed. Oil palm carotene can be used in prawn feed production to replace the commercial synthetic astaxanthin in the market. A study was conducted to evaluate the effects of dietary oil palm carotene on the colour intensity of total carotenoids, and astaxanthin content in giant freshwater prawns (*Macrobrachium rosenbergii*).

MATERIALS AND METHODS

Materials

Giant freshwater prawns for the experiment were obtained from a hatchery in Johor. Commercial feed (Cargill, Malaysia) and all raw materials for feed production, such as fish meal (origin: Malaysia) and soybean meal (origin: Argentina), were purchased from local suppliers. CPO was collected from a palm oil mill in Pahang, palm pressed fibre oil (PPFO) was obtained from Lekir Palm Oil Mill, Perak while the commercial oil palm-mixed carotene was supplied by a local company in Perak.

Methods

Feeding trial

A feeding trial was conducted at the Aquaculture Complex, Feed Research Group (FRG), MPOB Keratong Research Station, Pahang. The experimental system consisted of 15 rectangular glass aquaria (48 (L) × 13 (W) × 20 (H) inches) with a capacity of 250 L water each, complete with continuous aeration and supplied with fresh water from reservoirs. Five treatment groups of prawns (*Macrobrachium rosenbergii*), with almost equal in length (25 mm) and weight (3.00 g), were stocked in triplicate in the aquaria at a density of 10 prawns per aquarium. The treatment groups that are isonitrogenous and isocaloric were used in this study, including T1 (commercial feed); T2 (control, without oil palm carotene); T3 (3% CPO inclusion); T4 (3% PPFO inclusion); and T5 (3% commercial oil palm-mixed carotene inclusion) (Table 1). Prawns were fed to satiation five times daily for a 10-week feeding trial. At the end of the feeding trial, the prawns were subjected to 24 hr of fasting before final sampling. The prawn samples were collected using three random samples from each aquarium before being euthanized and kept in a freezer (-20°C) for further analysis.

Colour intensity

The analysis of prawn colour intensity was carried out according to the method by Parisenti *et al*. (2011a), using three random samples from each aquarium. The colour of the prawn from each treatment was determined on raw and cooked samples. The cooked samples were prepared by cooking for 1 min in 200 mL water at 100 °C and being immediately placed in iced water thereafter for another minute. The samples were tested right after collection using a Chroma Meter CR-400 (Konica Minolta, Japan) colourimeter that was calibrated using a white reference. Measurements were taken from three parts along the prawn body (close to the head; middle; close the tail) and performed in the colorimetric space L* (lightness), a* (redness), and b* (yellowness), at 25 ± 1 °C. To confirm the colouration of the shrimp as it is marketed on the market, colour measurements were made on raw and cooked samples using the prawn's exoskeleton.

Table 1. Giant freshwater prawn (*Macrobrachium rosenbergii*) dietary treatments supplemented with different types of oil palm carotene

Raw material (%)	Treatment			
	T2	T3	T4	T5
	Control	CPO	PPFO	Commercial oil palm-mixed carotene
Wheat flour	31.49	31.49	31.49	31.49
Fishmeal 60%	31.40	31.40	31.40	31.40
Soybean meal 48%	27.66	27.66	27.66	27.66
Fish hydrolysate	2.43	2.43	2.43	2.43
Fish oil	4.00	1.00	1.00	1.00
CPO	-	3.00	-	-
PPFO	-	-	3.00	-
Commercial oil palm-mixed carotene	-	-	-	3.00
Feed additives*	3.03	3.03	3.03	3.03

Note: T1 (Commercial feed) was purchased from a local supplier; CPO – crude palm oil; PPFO – palm pressed fibre oil

* Includes dicalcium phosphate, antioxidant, vitamin and mineral premixes

Total carotenoids and astaxanthin content

The total carotenoid content of the prawn sample was determined according to Tolasa *et al*. (2005) with modifications. The whole body of the prawn sample was ground and blended before being extracted using acetone and collected in funnel separation. After that, hexane (Sigma-Aldrich®, Germany), water, and sodium chloride (NaCl) (Systerm®, Malaysia) were added to separate water-soluble compounds. The funnels were kept in the dark for 20 min and shaken once. Hexane extract was filtered and dehydrated with anhydrous sodium sulphate (Systerm®, Malaysia), and then poured into a volumetric flask. Total carotenoid concentration was spectrophotometrically determined by using a Hewlett-Packard, HP8452A (Stockport Cheshire, UK) spectrophotometer at 470 nm and calculated according to the developed standard curve.

Quantitative analysis of the astaxanthin content in the whole body of the prawn was conducted using Agilent 1260 Infinity (Germany) high-performance liquid chromatography (HPLC) according to Hu *et al*. (2019) with slight modifications. The HPLC separation was performed on a ZORBAX Eclipse Plus C18 (4.6 mm × 100 mm) (particle size 3.5 µm, Agilent, Germany) column. Astaxanthin content was determined at a wavelength of 474 nm. The mobile phase, at a flow rate of 0.6 mL/min, was acetonitrile/methanol/dichloromethane (80/15/5, v/v/v). The injection volume was 50 µL. Astaxanthin content was calculated using peak area according to astaxanthin standards of known concentration.

Statistical analysis

Data obtained for all parameters were analysed using a one-way analysis of variance (ANOVA) of SAS Statistical Package 9.1 (SAS Inst. 2003). The Duncan multiple range test was used to further compare means at $P<0.05$ for significant differences.

RESULTS AND DISCUSSION

Colour intensity

Colour differences were observed manually (Figure 1) and confirmed by instrumental colour measurement. The colour of raw prawn was darker, much greener and blueish than in cooked samples. After cooking, the prawns from each treatment presented a more intense orange colour. Parisenti *et al*. (2011a) revealed that the blue/green hue seen is caused by the buildup of crustacyanin, a protein-astaxanthin complex that turns orange upon complex dissociation. Heat (cooking) or the presence of substances like acetone may induce this dissociation (Muriana *et al*., 1993; Velu *et al*., 2003; Darachai *et al*., 2019). The bathochromic effect (a change in the wavelength absorbed by compounds) is another cause for this colour shift brought on by the complex's formation and dissociation. However, green, blue or purple colouration was observed from the absorbance at 632 nm when astaxanthin and protein were combined. Meanwhile, free astaxanthin absorbs light at wavelengths between 470 and 472 nm (yellow, orange & red) (Weesie *et al*., 1995; Cianci *et al*., 2002).

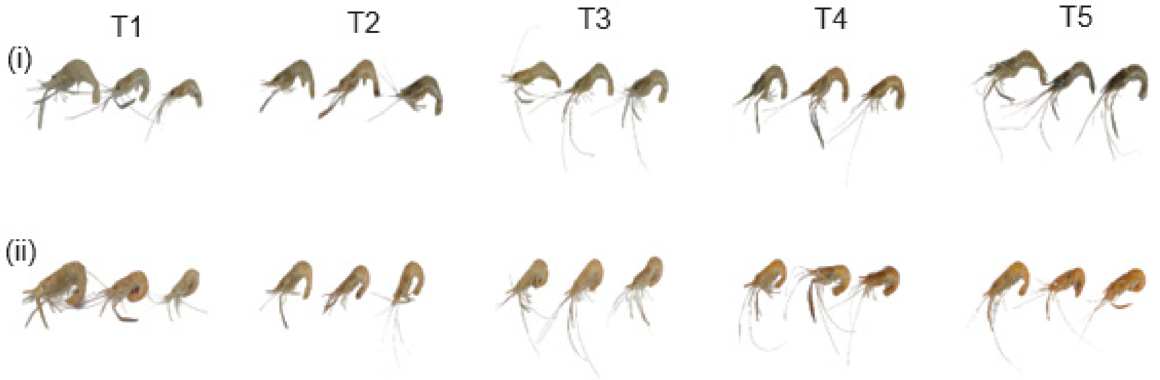

Fig. 1. Colour analysis of (i) raw and (ii) cooked giant freshwater prawn (*Macrobrachium rosenbergii*) samples fed with different oil palm carotenes.
Note: T1 – Commercial; T2 – Control; T3 – CPO; T4 – PPFO; T5 – commercial oil palm-mixed carotene

Results of instrumental colour analysis of raw and cooked prawn samples are shown in Table 2. There were no significant differences (*P*>0.05) in L* values across all diet groups in the raw prawn samples. Meanwhile, raw prawn samples from T4 had a significantly higher value of a* (*P*<0.05) compared to raw prawn samples from other treatment groups, but it was not significantly different (*P*>0.05) when compared to that of T5. After cooking, prawns obtained greater values of L*, a*, and b* than raw samples. Cooked prawn samples of T1, T2 and T3 showed significantly higher (*P*<0.05) L* values than T4 and T5. However, cooked prawn samples of T4 and T5 showed much greater (*P*<0.05) values of a* than the cooked prawn samples from the other treatment groups. Meanwhile, cooked prawn samples of T4 had a significantly higher value of b* (*P*<0.05) compared to cooked prawn samples from other treatment groups but were not significantly different (*P*>0.05) from T5.

Table 2. Colour analysis of raw and cooked giant freshwater prawn (*Macrobrachium rosenbergii*) samples fed with different oil palm carotenes

Parameter	Raw prawn samples				
	T1	T2	T3	T4	T5
L*	52.99±5.69	56.80±7.07	51.80±2.43	52.71±7.12	51.15±11.04
a*	-3.28±0.81[b]	-2.55±0.93[b]	-2.78±1.20[b]	-0.62±1.44[a]	-1.96±1.02[a]
b*	6.32± 2.76[b]	6.97±2.91[b]	6.51±2.14[b]	10.08±3.92[a]	7.04±3.43[b]
Parameter	**Cooked prawn samples**				
	T1	T2	T3	T4	T5
L*	64.24±5.26[a]	64.34±5.37[a]	65.41±5.79[a]	61.01±6.75[b]	61.53±3.84[b]
a*	1.94±3.60[b]	0.99±2.10[b]	2.64±3.58[b]	9.75±3.46[a]	7.75±3.61[a]
b*	17.11±4.28[b]	15.22±1.94[b]	19.94±4.89[b]	32.68±5.79[a]	23.28±3.31[ab]

Note: T1 – Commercial; T2 – Control; T3 – CPO; T4 – PPFO; T5 – commercial oil palm-mixed carotene. L* - lightness; a* - redness; b* - yellowness. Means in the same row with different superscripts were statistically different (*P*<0.05)

In the present study, prawns fed with PPFO have the strongest red-orange colour than other treatment groups, indicating a positive effect of its carotenoid content on prawns' colour. The colour parameters (L*, a*, & b*) are one of the important indices to evaluate the colouration of crustaceans (Smith *et al*., 1992). Moreover, Supamattaya *et al*. (2005) also mentioned that the content of astaxanthin in tissues that have been converted by prawns from dietary carotenoids in PPFO directly affects the colourations of prawns (Zhao *et al*., 2022). A study by Su *et al*. (2018) and Stachowiak & Szulc (2021) showed that body colouration in crustaceans is influenced by the distinct pigments found in the primary layer of their carapace and the subepidermal chromatophores. The most important of them are carotenoids, and astaxanthin is the carotenoid that is found most frequently in commercially important crustaceans.

In general, according to consumer preference based on subjective analysis, orange colour after cooking is preferable for prawns (Tume *et al*. 2009). Parisenti *et al*. (2011b) reported that consumers prefer lighter raw shrimp (light grey & grey) and brightly orange cooked shrimp (orange & intense orange), which indicates that adding pigments either natural pigment such as PPFO and CPO or synthetic pigment like astaxanthin to prawn feed should be followed to improve acceptance by the customer.

These findings suggest a challenge for the prawn industry, as a consequence of these findings because the lightest raw shrimps do not retain their bright orange colour after cooking.

Total carotenoids and astaxanthin content

Significant differences have been observed in total carotenoids and astaxanthin content between prawns in all treatment groups (P<0.05) (Figure 2). The total carotenoid content of the prawns fed with T3, T4, and T5 was significantly higher (P<0.05) than that of those fed with commercial (T1) and control (T2) feeds. However, prawns fed with T4 exhibited the numerically highest total carotenoid concentration compared to CPO and commercial oil palm-mixed carotene. There was a significant difference (P<0.05) in the level of astaxanthin in the whole body of prawns fed with oil palm carotenes as compared to the commercial and control groups. The lowest level of astaxanthin was 9.96 ppm in T2, while the highest was in T5 with 16.96 ppm, but it was not significantly different (P>0.05) with T3 and T4.

Even though the commercial oil palm-mixed carotene contains a higher amount of carotene than other oil palm carotenes, T5 showed a lower amount of total carotenoids than PPFO, while interestingly exhibiting the highest astaxanthin level in the prawn among other tested samples. This finding suggested the high efficiency of bioconversion of beta- and alpha-carotenes into astaxanthin in the prawn. Similar findings were reported by Weaver *et al*. (2018) and Fawzy *et al*. (2022), where dietary carotene supplementation can provide an alternative to astaxanthin in crustaceans and marine copepods. They also found that the higher the carotene supplementation, the higher the astaxanthin content, as one of the main roles of astaxanthin in crustaceans, is to improve immunity, thereby enhancing growth performance. The low amount of carotenoids detected in T5 was also due to the bioconversion of beta- and alpha-carotenes in commercial oil palm-mixed carotene into pro-vitamin A in the prawn body, which exerts its health benefits. Beta- and alpha-carotenes are good sources of pro-vitamin A, which will be converted into vitamin A according to what the body needs (Grune *et al*., 2010).

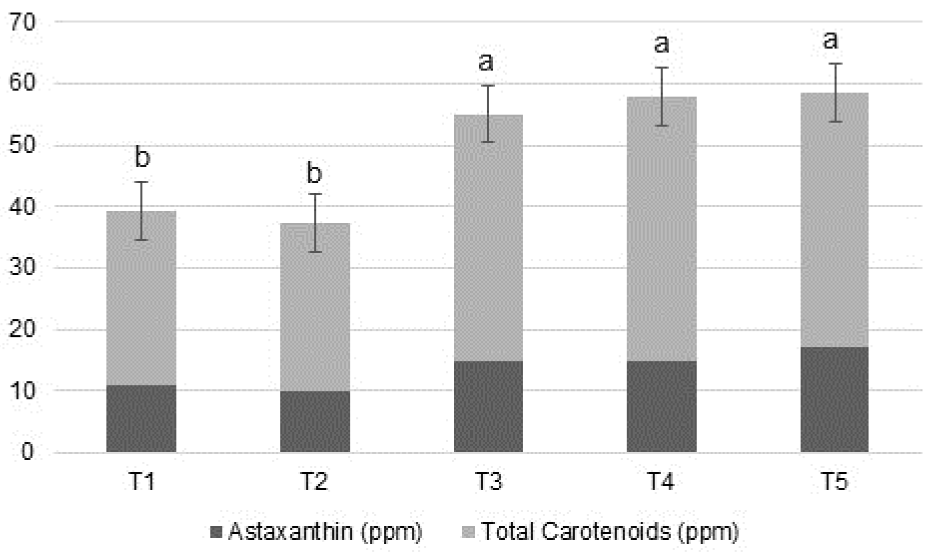

Fig. 2. Total carotenoids and astaxanthin content (± standard deviation of the mean) of whole-body freshwater giant prawn (*Macrobrachium rosenbergii*) supplemented with oil palm carotenes for 10 weeks.

Note: T1 – Commercial; T2 – Control; T3 – CPO; T4 – PPFO; T5 – commercial oil palm-mixed carotene. [ab] Means with different superscripts were statistically different (P<0.05)

CONCLUSION

The supplementation of oil palm carotenes in the giant freshwater prawn diet resulted in better colour intensity with the prawn samples fed with PPFO and commercial oil palm-mixed carotene showed better lightness in cooked samples as well as yellowness and redness for both raw and cooked samples. Furthermore, prawns fed with formulations containing oil palm carotenes (CPO, PPFO & commercial oil palm-mixed carotene) contained higher total carotenoids and astaxanthin content than the commercial and control groups. This finding could lead to new applications of oil palm carotenes as natural additives for giant freshwater prawns to replace synthetic astaxanthin on the market.

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ETHICAL STATEMENT

Ethical approval is not applicable as prawn is an invertebrate species, which is excluded from the policy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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