

## The Use of Synthetic and Natural Carotenoid in Diet for Color Enhancement on Red Cherry Shrimp *Neocaridina heteropoda*

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### ABSTRACT

Color enhancement of red cherry shrimp, *Neocaridina heteropoda*, was studied in 2 experiments in order to evaluate the feasibility of including different sources of carotenoid in shrimp diet. In the first experiment, 3 concentrations (0, 80 and 160 mg.kg<sup>-1</sup> of synthetic carotenoid diet were tested. Shrimp were fed twice a day. After 8 weeks, shrimp fed with 160 mg.kg<sup>-1</sup> showed more significant coloration improvement than those fed with lower carotenoid diets. This shows that the color of red cherry shrimp could be enhanced by incorporating carotenoid into their diet. The second experiment aimed to reduce cost by using a cheap available source of natural carotenoid, i.e. marigold petal, at rates of 0, 50, 100 and 200 mg.kg<sup>-1</sup> diet. Results showed that the reddish color of shrimp fed with the natural carotenoid source at 200 mg.kg<sup>-1</sup> were most enhanced compared with the other groups, including obtaining the highest survival rate (99%). Carotenoid content analysis from whole shrimp revealed that increasing dietary carotenoid concentration resulted in a significant increase in total carotenoid concentration. It is concluded that natural color enhancer diets for red cherry shrimp can be prepared using carotenoid from marigold petals at 200 mg.kg<sup>-1</sup>.

**Keywords:** red cherry shrimp, carotenoid, marigold

### INTRODUCTION

Red cherry shrimp, *Neocaridina heteropoda*, is an ornamental dwarf shrimp with vibrant red color. The trade value of this shrimp is based on the visual appearance of body color. The main red pigment material of shrimp is the carotenoid pigment. It provides the tissue with red orange pigmentation (Yamada *et al.*, 1990). The

occurrence of pigments in shrimp is mainly due to the absorption of carotenoids from their diet. Varieties of carotenoid pigments- both synthetic and natural products - are used for color enhancement. Synthetic carotenoids are expensive, therefore, natural carotenoids are also used in aquaculture feed formulation. So far, no report on red cherry shrimp has been made. The aim of this study was to evaluate the optimum

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concentration of synthetic and natural carotenoids for color enhancement of red cherry shrimp.

## MATERIALS AND METHODS

### Feed preparation

Two experiments were conducted in this study. In both trials, a control group diet without carotenoid was used. Feed preparation used powder commercial fish diet (Nutrena 8000, manufactured by Cargill Siam Ltd.) with the following contents: 40% protein and 12% lipid. In trial 1, synthetic carotenoid from Carophyll Pink (DSM) was used at the rate of 0, 80 and 160 mg.kg<sup>-1</sup> feed. In trial 2, natural carotenoid from marigold petal (*Tagetes erecta*) was supplied in diet at 0, 50, 100 and 200 mg.kg<sup>-1</sup> feed. The total carotenoid concentration of marigold flower was determined to be 3331.4 mg.kg<sup>-1</sup> by homogenized marigold petal in 95% ethanol (1 mg per 10 ml, three times), centrifuged at 10,000 × g for 10 mins. The clear supernatant was read at 473 nm on the spectrophotometer using an extinction coefficient of 2500 (Britton *et al.*, 1981) and was calculated for result in assigned concentration before adding in the trial diet. The powder diet and carotenoid source were mixed thoroughly and processed into 5 mm diameter pellet. The pellets were oven dried at 60°C and kept at -4 °C, in the dark to prevent degradation until required.

### Feeding trial

One-month-old red cherry shrimp from the hatchery of King Mongkut's Institute

of Technology Ladkrabang, Thailand, were used in both experiments and acclimated to experimental conditions for 7 days. At the beginning of the experiment, shrimp were weighed and average initial weight was 10.35±0.16 mg. Four replicates with 20 shrimps each in 25x20x12 cm<sup>3</sup> PE box were fed with experimental diets at 3% of body weight twice a day for 8 weeks. Water was exchanged about 50% every other day. Survival rate, final weight and carotenoid from whole shrimp were checked at the end of the trial.

### Total carotenoid from shrimp body analysis

The carotenoid content of shrimp was extracted by the modified method of Ingle de la Mora *et al.* (2006). Whole shrimp were homogenized and total carotenoid concentration was determined spectrophotometrically in petroleum-ether-acetone-water solution (15:75:10) 1:19 (w/v) incorporated with BHT 0.01%. Total carotenoid concentration was estimated with an  $E_{(1\%,1\text{cm})} = 1910$  at 473 nm.

### Statistical analysis

All data were subjected to analysis with Duncan's new multiple ranges test (Duncan, 1955).

## RESULTS AND DISCUSSION

### Experiment 1

Weight gain, survival rate and total carotenoid from body content of shrimp fed with diet supplemented with carotenoid were

significantly higher ( $P<0.05$ ) than those of the control group (Table 1). The study showed that as dietary carotenoid increased to  $160 \text{ mg.kg}^{-1}$ , carotenoid concentration in the whole shrimp body also increased to  $88.37\pm6.58 \text{ mg.kg}^{-1}$  shrimp (Figure 1). The weight gain and survival rate in shrimp fed with supplementary carotenoid diet were higher than those in the control group. Latscha (1991) reported that carotenoid could improve pigmentation and growth

development through metabolites as precursors of retinoids passed to gonad development and maturation. Additionally, carotenoids played an important role as antioxidant, providing pro-vitamin A and having a function in reproduction (Stahl and Sies, 2005). Meanwhile, dietary level of carotenoid was not likely to reach the saturation point. For that reason, the next trial on natural carotenoid adapted the concentration of  $200 \text{ mg.kg}^{-1}$  diet.

Table 1. Final weight gain, survival rate and total carotenoid contents of red cherry shrimp after 8 weeks of feeding with diets having various concentrations of synthetic carotenoid.

Carotenoid concentration ( $\text{mg kg}^{-1}$ diet)	Weight gain ( $\text{mg.ind}^{-1}$ )	Survival rate (%)	Carotenoid content ( $\text{mg kg}^{-1}$ shrimp)
0	$22.70\pm0.49^b$	$22.70\pm0.49^b$	0
80	$22.88\pm0.35^b$	$22.88\pm0.35^b$	80
160	$24.68\pm0.50^a$	$24.68\pm0.50^a$	160

Note: Means in the same column with different superscripts are significantly different ( $P<0.05$ )

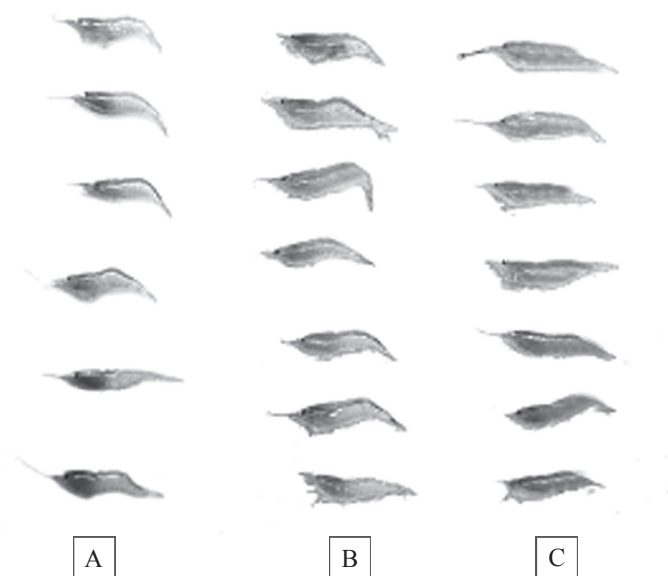


Figure 1. Photograph of red cherry shrimp after 8 weeks of feeding by synthetic carotenoid from Carophyll pink at various concentrations: 0 (A),  $80 \text{ mg.kg}^{-1}$  diet (B) and  $160 \text{ mg.kg}^{-1}$  diet (C).

## Experiment 2

Marigold rich in carotenoid is an interesting dietary alternative to provide skin coloration in shrimp (Figure 2). Diets with natural source of carotenoid showed similar results as in Experiment 1 (Table 2). Red cherry shrimp from this study could obtain natural carotenoid up to 200 mg.kg<sup>-1</sup> diet, leading to an increase in carotenoid levels in the body to a maximum of 204.91±12.3 mg.kg<sup>-1</sup> shrimp. Weight gain and survival rate,

which were higher than that of the control, are related to carotenoid concentration because carotenoid is deposited in the shrimp body in free form and then associated with protein as carotenoprotein. This elevates serum complement and lysozyme activity and growth (Supamattaya *et al.*, 2005). Carotenoid functions as an intracellular oxygen reserve (Chien and Jeng, 1992). Thus, supplementation of natural carotenoid from marigold is a better alternative source in red cherry shrimp culture.

Table 2. Final weight gain, survival rate and total carotenoid content of red cherry shrimp after 8 weeks feeding with varying concentrations of carotenoid from marigold in the diet.

Carotenoid concentration (mg kg <sup>-1</sup> diet)	Weight gain (mg ind <sup>-1</sup> )	Survival rate (%)	Carotenoid content (mg kg <sup>-1</sup> shrimp)
0	23.50±0.89 <sup>c</sup>	78.50±3.75 <sup>c</sup>	46.25±7.46 <sup>d</sup>
50	27.20±1.98 <sup>b</sup>	85.00±2.04 <sup>b</sup>	67.96±3.12 <sup>c</sup>
100	27.78±3.06 <sup>b</sup>	90.00±2.04 <sup>b</sup>	93.80±4.42 <sup>b</sup>
200	34.80±1.16 <sup>a</sup>	99.75±0.25 <sup>a</sup>	204.91±12.3 <sup>a</sup>

Note: Means in the same column with different superscripts are significantly different (P<0.05)

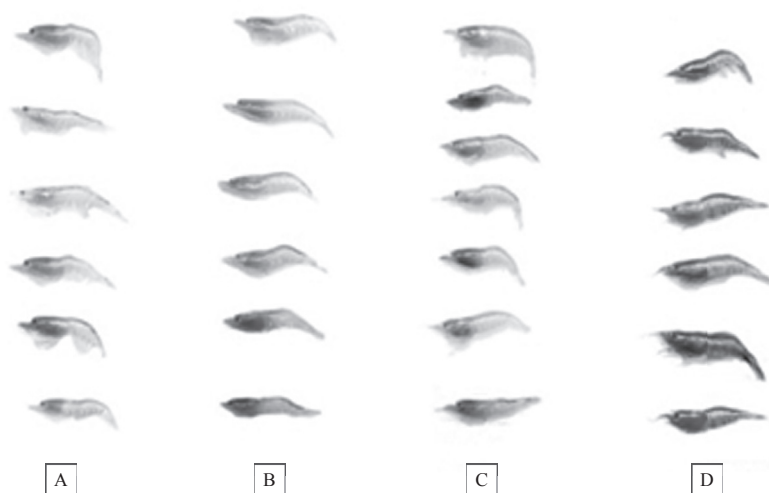


Figure 2. Red cherry shrimp after 8 weeks of feeding with natural carotenoid from marigold petal at various concentrations: 0 (A), 50 mgkg<sup>-1</sup> (B), 100 mgkg<sup>-1</sup> (C) and 200 mgkg<sup>-1</sup> (D) diet.

## CONCLUSION

The study showed that natural carotenoid was effective in enhancing the color of red cherry shrimp at a lower concentration than synthetic carotenoid. The results suggest that natural color enhancer diets could be prepared using 200 mg.kg<sup>-1</sup> diet of carotenoid from marigold petal.

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