CHAPTOR VII

EFFECT OF ALGRO NATURAL® ON GROWTH PERFORMANCE, HEALTH CONDITION, IMMUNE RESPONSE AND DISEASE RESISTANCE IN BLACK TIGER SHRIMP

Introduction

In shrimp culture industries, color is one of the major factors, which determines the market price of black tiger shrimp in the international market. Supplementations of carotenoids pigment into diets have been demonstrated to yield higher pigments in shrimp body. Moreover, carotenoid pigments have positive effect on the immunological and stress response (Latscha, 1991; Estermann, 1994). However, synthetic astaxanthin is still expensive for use in aquaculture. Several attempt have been made to find alternative sources of astaxanthin and other carotenoid such as yeast *Phaffia* sp.(Sanderson and Jolly, 1994), many species of algal (Liao, *et al*., 1993 ; Sommer *et al*., 1991 ; Boonyaratpalin *et al*., 2000). In black tiger shrimp (*Penaeus monodon*), the color is provided mainly by astaxanthin as the free or esterified form (Tanaka *et al.*, 1978). Black tiger shrimp are unable to biosynthesis carotenoid *de novo* but can convert β-carotene, and synthetic canthaxanthin in feed to deposit in the body as astaxanthin (Boonyaratpalin *et al*., 2001). Therefore price and efficiency are used for determining the sources of pigment use in shrimp feed. Moreover, carotenoid was known to enhance immune function and disease resistance in higher animal (Bendich and Shapiro, 1986; Jyonouchi *et al.*, 1993; Thompson *et al*., 1995)., but little information is available for aquatic animal especially in shrimp. In this study, different levels of carotenoid extract from Dunaliella (Algro Natural®) was used in shrimp feed and effect of this substance at different levels on growth performance, health condition, immune

function and disease resistance in black tiger shrimp were evaluated.

Materials and Methods

Test animal

Trial 1 : Small shrimp

Ten thousand PL-15 were grown on commercial shrimp feed in two 5 ton tanks for one and a half month until weight attained 0.5-1 g, then graded to the same size and stocked in 50x100x50 cm aquaria with a density of 15 shrimp per aquarium.

Trial 2 : Juvenile shrimp

One thousand, healthy shrimp with a weight range of 10-12 g were purchased from a shrimp farm where no severe disease outbreak has been recorded. Hundred and twenty shrimps were stocked in each 10-ton concrete tank equipped with aeration. Each concrete tank was covered with black plastic blanket to control the temperature in the pond (29-30 ^{0}C). Five tanks were used in this study. Shrimp were acclimated and were fed with commercial shrimp feed 5 time daily until satiation for one week before start feeding with test diet. Sediment from the pond bottom was clean water exchange was done daily with 10% changing rate and water salinity was kept constantly at 15 ppt throughout the experiment.

Test diet

Feed formulation

Five isonitrogenous test diets were formulated to contain 40 % protein and 8 % fat. All diet have the same composition except the level of Algro Natural® added to achieve 0, 125, 200 and 300 ppm β-carotene in diet 1,2,3 and 4 respectively 0.9% NaCl was added to diet 5 for another control group of Algro Natural® (Algro Natural® powder contained 0.9% NaCl). (Table 7-1) Comparison of the test diets for trial 1 and 2 are the same except the pellet size.

Feed processing

Ingredients were grinned; weight according to each formulation in Table 1, mixed in bowl mixer for 5 min then oil was added and mixed well again. After that 350 ml of freshwater per kilogram of feed was added and mixed for well another 5 min. Then passed through the meat grinder, the spaghetti like feed was broken into pellet and air dry in a air flow oven at 60 ^{0}C for 4-6 hr or until the moisture was less than 10 %. The dry pellets were kept in plastic bags in the refrigerator until used.

Growth performance analysis

For the experiment in small shrimp, the stocking rate was 15 shrimp per aquarium (0.5x1.0x0.5m). Shrimp was fed to satiation 5 times daily for 8 weeks. Data for growth performance was recorded such as growth, weight gain, survival, FCR and color changes. Data recorded was determined every two weeks for the 8 weeks experimental periods.

Immune function analysis

After 6 weeks culture period of juvenile shrimp in trial 2, body color of test animal was examined by using color

fan. When the color change was detected, immune function and disease resistant were performed in the test group of juvenile shrimp as follows

Total hemocyte count : Blood from each shrimp was collected by using a 1 ml syringe with 25 G needle from the base of a walking leg. After withdrawal, the hemocytes was counted, the blood was diluted with 0.15% trypan blue solution, hemocyte was counted using a hemacytometer and calculated as number of blood cell (total hemocytes) per cubic millimeter.

Phenoloxidase activity : Twenty shrimps from each experimental group were collected on week 6 for PO activities determination. PO activity from hemocytes was measured by the following methods : Blood was collected from each shrimp using a 1 ml plastic syringe. L-cycteine was used as an anticoagulant (50 mg/ml). After the blood was withdrawn, the hemocyte was washed three time with Lobster hemolymph medium (LHM). Hemocyte lysate was prepared in a cacodylate buffer pH 7.4 by using sonicator (Vibra Cell^{TM} sonicator and material, USA) at 30 amplitude for 20 second. PO activity from hemocyte lysate was measured by modified method of Smith and Soderhall (1983). L-3,4-dihydroxyphenylalanine (L-DOPA) was used as a substrate and enzyme activity was measured at 630 nm. Protein content in hemocyte lysate was measured by using Lowry's method (Lowry *et al.*, 1951). PO activity was defined by the increase of OD per minute per mg protein.

Bacterial clearance ability : At the end of the 6 weeks feeding period, 20 shrimp from each experimental group were tested for their ability to remove bacteria. The ability of each shrimp to remove bacterial cell from the blood circulation system was measured by the modified method of Martin *et al.* (1993). A bacterial suspension was prepared from 24 h cultured of *Vibrio harveyi*. A bacterial suspension of 0.1 ml (2.7×10^7 cell/ml) was injected into the tail muscle of each shrimp and was

kept in aquaria equipped with seawater and aeration for three hours. Blood was collected from each shrimp without an anticoagulant and 30 µl of whole blood (without dilution) was dropped on TCBS agar plus 1.5% NaCl. A two-fold dilution of whole blood was made using sterile 2.5% NaCl solution. The number of bacteria was counted on the TCBS as above. The number of bacterial cells in the hemolymph was reported as cfu/ml.

Stress tolerance

At the end of experiment, in trial 1, 10 shrimp from each treatment were transferred to duplicates of stress test chambers (100 l glass aquaria). Low dissolved oxygen conditions were maintained by stop the water flow through system and aeration, using plastic sheet overlying on the water surface in each stress test chamber for 10 hr / day. Dissolved oxygen were linearity decreased to below 1 ppm within 10 hr. Mortality of the shrimp in each group was recorded for 9-10 days.

Disease resistance

Virus stock preparation

For preparation of viral stock, normal and healthy shrimp weighing about 12-15 g was injected with whie spot syndrome virus (WSSV) and kept in a 250 l tank until they shown the symptoms of red disease with white spots. Viral stock using for injection were prepared by withdrawn the hemolymph from moribund shrimps and diluted to 1:2 with PBS, stored at -80^0 C until used. The diluted hemolymph was further diluted at 1:10,000, 1:50,000, 1:100,000 with sterile 1.5% NaCl and 20 shrimps were injected with 0.1 ml of each viral suspension.

Mortality was recorded for 10-15 days and LD_{50} was determined by using probit analysis.

Challenge procedure

At termination of the experiment in small shrimp, the shrimp from each treatment were transferred to duplicates of 150 l glass aquaria. Each shrimp were injected with 0.1 ml of viral suspension at the concentration of LD_{50}. The mortality was recorded every day for the periods of 14 days.

Carotenoids analysis

After 6 weeks periods of feeding trial in juvenile shrimp (trial 2), 10 shrimp from each treatment were sampled and analyed for total carotenoids by the method described by Sommer *et al*. (1991). Thin layer chromatography (TLC) from the method described by Yamada *et al*. (1990) was used for the analysis of free astaxanthin, astaxanthin mono-ester and astaxanthin di-ester.

Table 7-1 Compositions of test diet.

Ingredients	**g/kg**				
	T1	**T2**	**T3**	**T4**	**T5**
Fish meal	280	280	280	280	280
Shrimp head meal	100	100	100	100	100
Squid meal	50	50	50	50	50
Wheat gluten	60	60	60	60	60

Soybean meal	100	100	100	100	100
Wheat flour	200	200	200	200	200
Rice flour	101	97.45	91	86	92
Fish oil	20	20	20	20	20
Lecithin	20	20	20	20	20
Cholesterol	5	5	5	5	5
Vitamin - mix	3.3	3.3	3.3	3.3	3.3
Choline	3	3	3	3	3
Vitamin E	1.5	1.5	1.5	1.5	1.5
Vitamin C	1	1	1	1	1
Mineral	40	40	40	40	40
BHT	0.2	0.2	0.2	0.2	0.2
Zeolite	15	15	15	15	15
Algro Natural® (2%)	0	6.25	10	15	0
Salt	0	0	0	0	9

* Vitamin mixture (mg/ kg diet): thiamine 22.5; riboflavin 20; nicotinic acid 36.7; Ca pantothenate 24; inositol 98; biotin 0.5; folic acid 1.68; vitamin B_{12} 0.005; menadione 13.28; vitamin A 1150 IU; vitamin D_3 230 IU; BHT 1; PABA 20

** Mineral mixture (g/kg diet) KH_2PO_4 1; $CaHPO_4$ 1; NaH_2PO_4 1.5; KCL 0.5

Results

Trial 1 (small shrimp)

Growth performance

During 8 weeks of feeding period, there was not significantly difference in average weight of the shrimp among each treatment (Table 7-2). Weight gain, survival rates and FCR of the shrimp in each treatment were shown in table 7-3. Weight gain of shrimp fed diet supplemented with 125-300 ppm β-carotene were significantly higher than the shrimp fed control diet (T1) or diet contained 0.9 % NaCl (T5) ($P<0.05$). As well as, the survival rates, which were lowest in the group fed control diet (T1) and the diet supplemented with 0.9 % NaCl (T5). However, it was not significantly difference in FCR among each treatment ($P>0.05$).

Body color

The body color of each treatment can be distinguished by naked eye in week 3 after feeding with test diet. Shrimp in treatment 1 and 5 looks pale blue while dark blue or dark brown were observed in T2-T4. The color in T4 is more intense than the others. After 8 weeks of feeding trial color score of boiled shrimp in T3 and T4 were highest and the lowest group were found in T1 and T5 as described in Table 7-4.

Blood parameters and disease resistance

There was not significantly difference ($P>0.05$) in total hemocyte count and phenoloxidase activity among each treatment as shown in table 7-5. The survival rate after challenged with WSSV ($1:10^6$) were shown in figure 7-1. After 15 days of challenged period survival of shrimp fed with 300 ppm β-carotene were significantly higher than the others ($P <0.05$).

Table 7-2 Average weigh (g) of small shrimp fed with experimental diet for 8 weeks.

	Week 0	Week 2	Week 4	Week 6	Week 8
T1 control	1.22 ± 0.01 ^{ns}	2.17 ± 0.18 ^{ns}	3.25 ± 0.26 ^{ns}	4.25 ± 0.48 ^{ns}	5.35 ± 0.59 ^{ns}
T2 125 ppm β-carotene	1.22 ± 0.01 ^{ns}	2.32 ± 0.14 ^{ns}	3.56 ± 0.39 ^{ns}	4.70 ± 0.35 ^{ns}	5.76 ± 0.38 ^{ns}
T3 200 ppm β-carotene	1.21 ± 0.01 ^{ns}	2.16 ± 0.10 ^{ns}	3.41 ± 0.33 ^{ns}	4.49 ± 0.36 ^{ns}	5.46 ± 0.18 ^{ns}
T4 300 ppm β-carotene	1.22 ± 0.01 ^{ns}	2.29 ± 0.18 ^{ns}	3.49 ± 0.39 ^{ns}	4.85 ± 0.63 ^{ns}	6.00 ± 0.73 ^{ns}
T5 0.9 % NaCl	1.22 ± 0.01 ^{ns}	2.23 ± 0.17 ^{ns}	3.38 ± 0.19 ^{ns}	4.33 ± 0.50 ^{ns}	5.35 ± 0.61 ^{ns}

Mean ± SD from 6 replications
ns = non significant

Table 7-3 Growth performance of small shrimp after feeding with experimental diet for 8 weeks.

	Weight gain (%)	Survival rate (%)	FCR
T1 control	323.32 ± 41.89 $_{a}$	88.89 ± 3.44 $_{b}$	1.98 ± 0.19 $_{ns}$
T2 125 ppm β-carotene	372.85 ± 29.52 $_{b}$	100.00 ± 0.00 ^{c}	1.68 ± 0.14 $_{ns}$
T3 200 ppm β-carotene	351.81 ± 16.41 $_{ab}$	100.00 ± 0.00 ^{c}	1.80 ± 0.09 $_{ns}$
T4 300 ppm β-carotene	392.40 ± 58.98 $_{b}$	100.00 ± 0.00 ^{c}	1.72 ± 0.23 $_{ns}$
T5 0.9 % NaCl	323.95 ± 44.46 $_{a}$	86.67 ± 0.00 $_{a}$	1.85 ± 0.28 $_{ns}$

Means not sharing a common superscript are significantly different (P<0.05)
ns = non significant

Table 7-4 Color score and visual apparent of the shrimp in each treatment after boiling for 5 min.

	Color score	Visual apparent
T1 control	< 20	Light- orange
T2 125 ppm β-carotene	26	Orange- red
T3 200 ppm β-carotene	27	Orange- red
T4 300 ppm β-carotene	27	Orange- red
T5 0.9 % NaCl	< 20	Light- orange

Stress tolerance

During 9 days of stress period, the survival of shrimp fed diet containing 300 ppm β-carotene (T4) was the highest (P>0.05) as shown in figure 7-3. Moreover, the survival rate of all groups fed β-carotene supplemented diet were significantly higher than control groups (P<0.05).

Trial 2 (juvenile shrimp)

Blood parameters

At termination of experiment on week 6, total hemocyte were significantly higher in control and the shrimp fed diet containing 0.9 % NaCl (P<0.05), Total hemocyte counts were negatively correlated to the increasing of β-carotene in test

diet (r = -0.97). Phenoloxidase activity was not significantly difference among treatment (Table 7-6).

Body colors and carotenoids analysis

The difference in body color of shrimp in each treatment were first observed on week 4, the shrimp fed pigment supplemented diet exhibited more dark-brown color when compared with control and the group fed with 0.9 % NaCl (Fig. 7-3). At the end of feeding trial, color intensity of boiled shrimp in T 3 and T 4 were higher than T 2, while the light-yellow color were observed in control and the group fed with 0.9 % NaCl (Fig. 7-4). Color score of boiled shrimp fed each experimental diet were shown in table 7-7. The carotenoid content of the shrimp are summarized in table 7-8. Total carotenoid were highest in the shrimp fed with 125-300 ppm β-carotene. However, free astaxanthin was highest in the shrimp fed 300 ppm β-carotene.

Table 7-5 Blood parameters of small shrimp fed experimental diet for 8 weeks

	Total hemocytes count (x 10^4 cell/mm^3)	PO activity (U/min/mg protein)
T1 control	6.83 $\pm$ 1.85 ^{ns}	396.51 $\pm$ 224.73 ^{ns}
T2 125 ppm β-carotene	7.22 $\pm$ 2.63 ^{ns}	421.19 $\pm$ 163.38 ^{ns}
T3 200 ppm β-carotene	7.30 $\pm$ 2.30 ^{ns}	334.57 $\pm$ 167.96 ^{ns}
T4 300 ppm β-carotene	7.64 $\pm$ 2.88 ^{ns}	474.80 $\pm$ 162.82 ^{ns}
T5 0.9 % NaCl	8.05 $\pm$ 2.51 ^{ns}	452.72 $\pm$ 323.31 ^{ns}

Mean $\pm$ SD from 15 shrimp
ns = non significant

Table 7-6 Total hemocyte counts and phenoloxidase activity of juvenile shrimp fed experimental diet for 6 weeks

	Total hemocyte count ($x10^7$ cell/ mm^3)	Phenoloxidase activity (U/min/mg protein)
T1 control	7.04 ± 2.70 ^{bc}	553.42 ± 228.44 ^{ns}
T2 125 ppm β-carotene	5.87 ± 2.95 ^{abc}	547.67 ± 171.14 ^{ns}
T3 200 ppm β-carotene	5.45 ± 1.97 ^{ab}	537.56 ± 175.79 ^{ns}
T4 300 ppm β-carotene	4.91 ± 2.17 ^{a}	595.53 ± 184.45 ^{ns}
T5 0.9 % NaCl	7.91 ± 2.00 ^{c}	605.91 ± 129.42 ^{ns}

Mean ± SD from 20 shrimp
Means within columns not sharing the same superscript are significantly different ($P<0.05$)
ns = non significant

Table 7-7 Salmo fan score and visual apparent of the juvenile shrimp fed experimental diet after boiling for 3 min.

	Salmo fan score	Visual apparent
T1 control	20 -21	Light -orange
T2 125 ppm β-carotene	26-27	Orange -red
T3 200 ppm β-carotene	27-28	Orange -red
T4 300 ppm β-carotene	27-28	Orange -red
T5 0.9 % NaCl	20-21	Light -orange

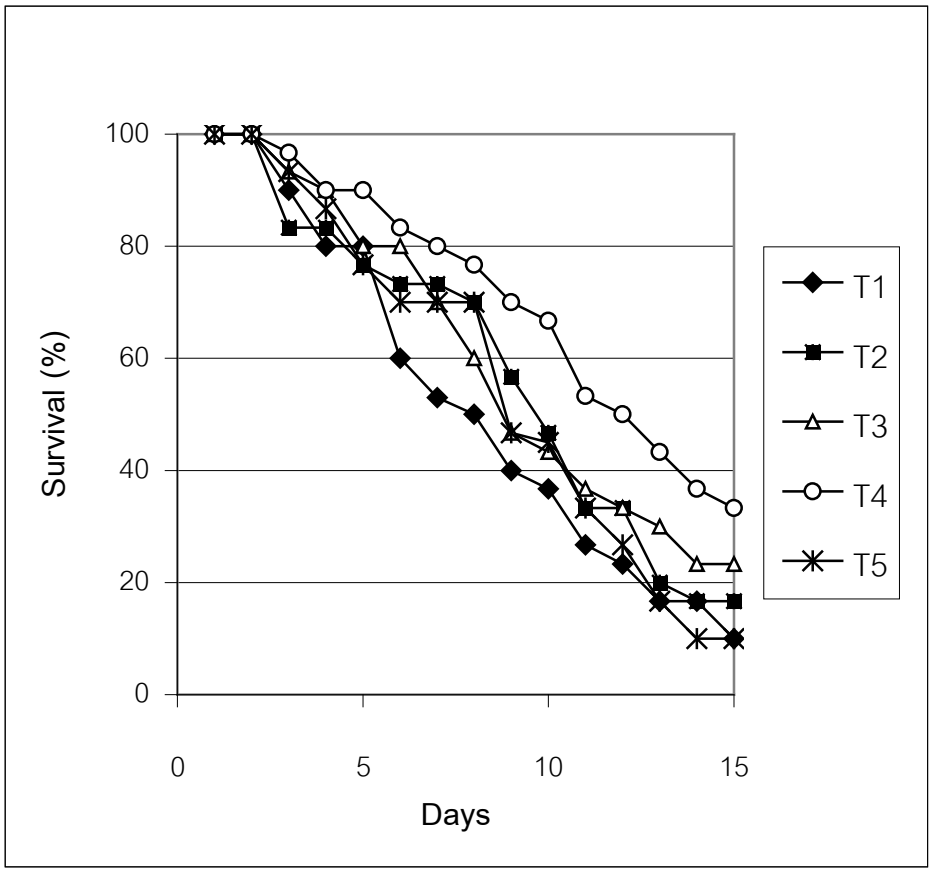
100
80
60
40
20
0
Survival (%)
0
5
10
15
Days
T1
T2
T3
T4
T5

Figure 7-1 Survival rates (%) of the shrimp in each treatment after challenged with $1:10^6$ WSSV for 15 days. (T 1=control, T2 to T5 = 125, 200, 300 ppm β-carotene, and 0.9 % NaCl)

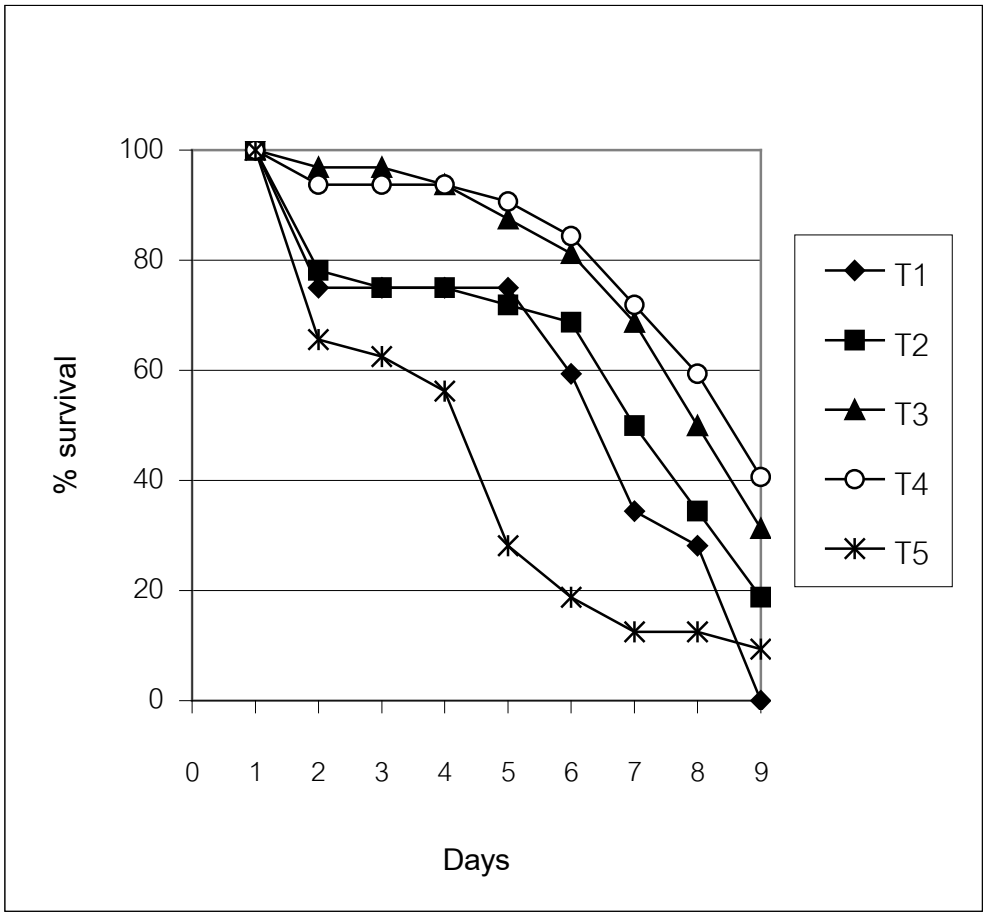

Figure 7-2 Survival rates (%) of the shrimp in each treatment during 9-days of stress period. (T 1=control, T2 to T5 = 125, 200, 300 ppm β-carotene, and 0.9 % NaCl)

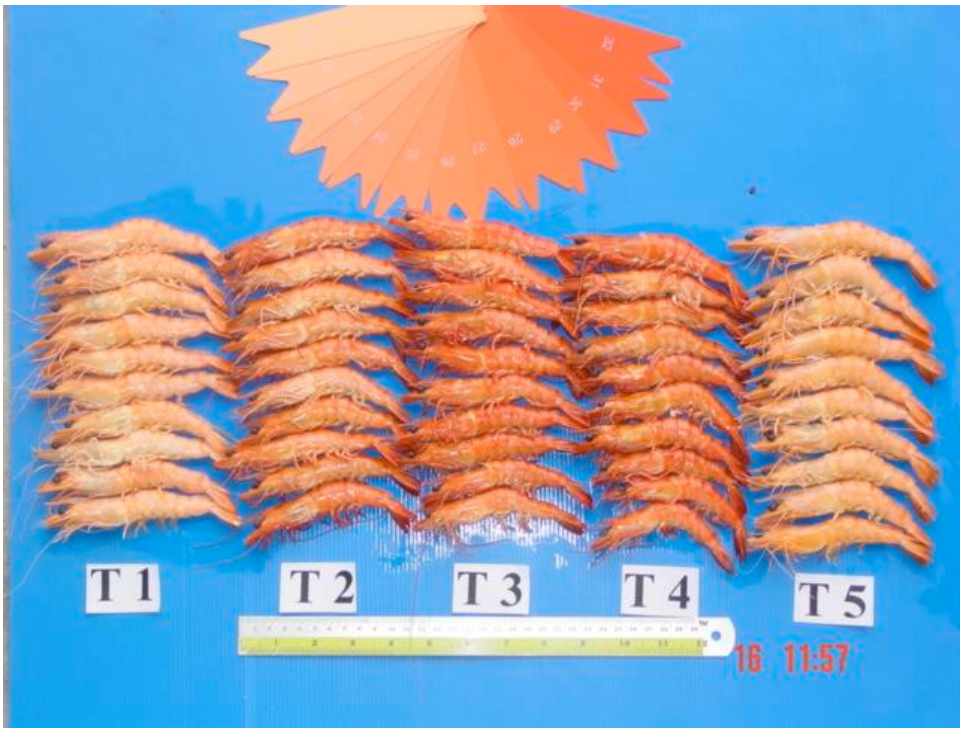

Figure 7-3 Body color of black tiger shrimp fed each test diet for 8 weeks. (Upper = before boiling, Lower = after boiling for 3 mins)

Table 7-8 Total carotenoid and astaxanthin content in juvenile shrimp fed experimental diet for 6 weeks

	Total carotenoid (ppm)	Di ester astaxanthin (ppm)	Mono ester astaxanthin (ppm)	Free astaxanthin (ppm)
T1 control	8.29 ± 0.91 a	1.86 ± 0.23 a	0.12 ± 0.16 a	1.29 ± 0.35 a
T2 125 ppm β-carotene	19.43 ± 2.98 b	2.65 ± 0.18 a	1.69 ± 0.23 bc	3.42 ± 0.33 a
T3 200 ppm β-carotene	20.88 ± 2.34 b	6.94 ± 0.86 b	2.94 ± 0.88 c	3.27 ± 1.54 a

T4 300 ppm β-carotene	24.52 ± 1.22 ^{b}	6.23 ± 1.45 ^{b}	2.71 ± 0.45 ^{c}	9.93 ± 1.51 ^{b}
T5 0.9 % NaCl	11.63 ± 1.79 ^{a}	1.77 ± 0.17 ^{a}	0.54 ± 0.33 ^{ab}	2.01 ± 0.22 ^{a}

Means not sharing a common superscript are significantly different ($P<0.05$)

Bacterial clearance ability

At 3 hour post injection of *Vibrio harveyi* suspension, bacterial cells count in hemolymph from shrimp fed each experimental diet were ranging from 2.15- 3.90 x 10^4 CFU/ml (Table 7-9). However, all bacterial cells count in hemolymp were not significantly difference among treatment ($p> 0.05$).

Table 7-9 Total bacteria count in hemolymph after injected with suspension of *V. harveyi* for 3 hr.

	Total bacteria count (x10^4 CFU/ml)
T1 control	2.15 ± 1.62 ^{ns}
T2 125 ppm β-carotene	2.88 ± 2.81 ^{ns}
T3 200 ppm β-carotene	3.44 ± 2.58 ^{ns}
T4 300 ppm β-carotene	3.90 ± 2.52 ^{ns}

T5 0.9 % NaCl	2.55 $\pm$ 1.48 ^{ns}

Mean $\pm$ SD from 20 shrimp
ns = non significant

Discussion

It is considered that penaeid shrimp, cannot biosynthesize carotenoids from mevalonic acid, but can alter dietary carotenoids by oxidation and deposit them in their tissues. Tanaka *et al*. (1976) reported on the metabolism of carotenoids in kuruma shrimp and suggested that some of dietary carotenoid pigments such as astaxanthin, β-carotene, isocryptoxanthin, echinenone, canthaxanthin, phoenicoxanthin, zeaxanthin and 4-ketozeaxanthin were converted into astaxanthin in shrimp body. Especially, astaxanthin was the most effective substance for pigmentation in shrimp when compared with β-carotene and canthaxanthin. (Yamada *et al.*, 1990). However, Liao *et al*. (1993) studies on the pigmentation of black tiger shrimp by feeding the shrimp with diets containing different carotenoid sources *eg*. β-carotene, spirulina, *Phaffia* yeast and krill oil. A marked increase of carotenoid content in the carapace was observed in the group fed with spirulina supplemented diets and suggested that zeaxanthin, which is major carotenoids in spirulina, has been rapidly converted to astaxanthin. Moreover, 125 ppm of synthetic β-carotene or 125-175 ppm β-carotene extracted from *Dunaliella salina* (Betatene) have been reported as a pigment sources in black tiger shrimp and demonstrated that black tiger shrimp has the metabolic ability to converted β-carotene into astaxanthin (Boonyaratpalin *et al.*, 2001). Such finding was correlated with our results, 200-300 ppm of β-carotene from *Dunaliella salina* (Algro Natural®) shown its high efficiency for pigmentation in black tiger shrimp. From previously reports, dietary carotenoids were converted into astaxanthin and deposited in shrimp body in free form by the association with protein and exists as carotenoprotein and

eaterified forms which are predominatly as mono ester and di ester of long chain fatty acids (Foss *et al.*, 1987 ; Yamada *et al.*, 1990). However, the incorporation of astaxanthin esters in test diets showed less effective than free form as reported by Liao *et al.* (1993) Moreover, Babosa *et al.* (1999) reported that astaxanthin in rainbow trout serum were increased with the increasing of dietary lipids levels.

No evidence shown the positive effect of dietary carotenoid on growth and feed efficiency in black tiger shrimp as in agreement with Liao *et al.* (1993) ; Chein and Jeng (1992) and Boonyaratpalin *et al.* (2001). However, the antioxidant activity of carotenoid pigments are involved in many physiological system in aquatic animal (Estermann, 1994 ; Hunter, 2000). Amar *et al.* (2001) studies on the effect of carotenoids and vitamin A, C and E supplemented diet on bio-defence mechanisms in rainbow trout and found that serum complement activity phagocytic activity and red blood cells in both β-carotene and astaxanthin feeding group. Moreover, the vitamin-containing astaxanthin and β-carotene groups also exhibited better nonspecific cytotoxicity for the peripheral blood lymphocytes. Thus, among the carotenoids studied, β-carotene and astaxanthin elevated humoral factors such as serum complement and lysozyme activity, as well as cellular factors such as phagocytosis and nonspecific cytotoxicity. However, the concentration of β-carotene used in our study did not effect on the immune response and disease resistance to white spot syndrome virus (WSSV). As well as Thompson *et al.* (1995) who reported that the immunomodulatory effects of vitamin A and / or astaxanthin in rainbow trout were small, only serum antiprotease activity was increased by dietary vitamin A (18 ppm) or astaxanthin (100 ppm).

Astaxanthin may serve as an intracellular oxygen supply for shrimp, allowing survival under the hypoxic conditions in pond bottom (Chien and Jeng, 1992). Chien *et al.* (1999) reported that black tiger shrimp fed diet containing 360

ppm synthetic astaxanthin had better survival when exposed to oxygen depletion stress. This finding was agreed with our study, and concluded that deposited β-carotene from Algro Natural® may constitute an intracellular reserve of oxygen in black tiger shrimp. From our results, the increasing of dietary β-carotene reduced the total hemocytes of juvenile black tiger shrimp, while the phonoloxidase activity was not difference from others, such phenomenon was not found in small shrimp. It is hypothesized that the large shrimp has difference in physiological function from smaller shrimp. β-carotene may acts as antioxidant in hemolyph of juvenile shrimp and reduces the hemocytes production. Similar result have been reported by Nakano *et al.* (1995) who found that the activity of serum glutamic-oxaloacetic transaminase (GOT) were lower in rainbow trout fed a diet containing red yeast (Phaffia yeast) or synthetic astaxanthin when compared with those of fish fed control diet. Moreover, the decline in HSI (hepatosomatic indices) has demonstrated that dietary astaxanthin have the potential to improve function of the liver. As well as, Rehulka (2000) who reported that rainbow trout fed diet containing astaxanthin have the significantly lower levels of red blood cells count, hematocrit, hemoglobin, triacylglycerol and Ca^{2+} in the plasma. This finding show that dietary carotenoids influenced the blood components.

Several reports have been demonstrated on the positive effect of microbial products for improve the pigmentation in many aquatic animals (Gentles and Haard, 1991 ; Sommer *et al.*, 1991 ; Sanderson and Jolly, 1994). However, Tangeras and Slinde (1994) suggested that the thick cell wall in *Phaffia rhodozyma* restricted pigment availability, because lack of enzyme able to degrade the cell wall components in digestive tract of salmonid fish. As well as the application of algae, *Haematocuccus pluvialis* in rainbow trout, Sommer *et al*. (1991) reported that the astaxanthin level in fish body was higher in fish fed cell wall disrupted algal cells when compared with intact cells supplemented diet. Moreover, a high supplementation of dried algal cells caused retardation of growth in black tiger

shrimp (Liao *et al.*, 1993) and striped jack (Watanabe *et al.*, 1990). Thus, supplementation of concentrated carotenoids, which extracted from microbial cells are more practical than the incorporation of whole cells in feed stuff.

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