CHAPTER III

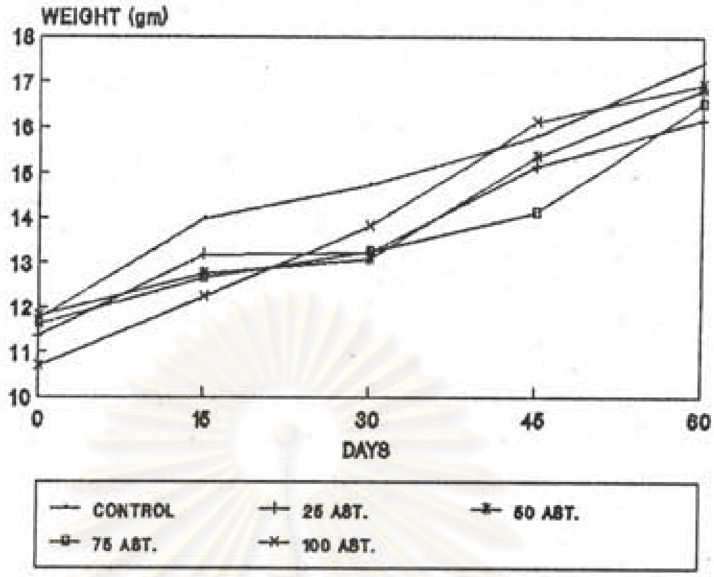
RESULTS

Experiment I : Effect of astaxanthin and canthaxanthin on coloration of juvenile prawns

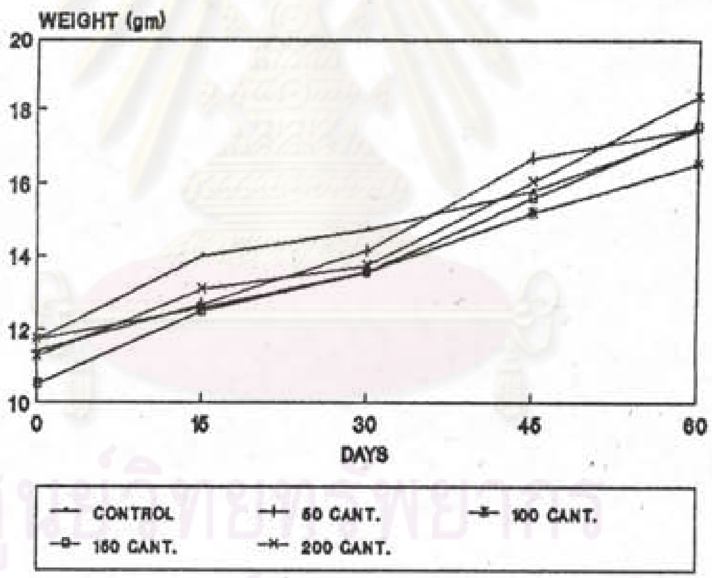
Growth

The growth in term of weight increase of juvenile *P. monodon* fed with the diets containing different concentrations of astaxanthin and the diets containing different concentrations of canthaxanthin were shown in Figure 3. The growth rate of the control group was significantly different from the group of prawns fed diets containing astaxanthin and canthaxanthin. Nevertheless, There was no significant difference within group the prawn fed with either astaxanthin or canthaxanthin. The result of the analysis of covariance is shown in Appendix 1.

It should be noted that data obtained from the control group were taken merely from the first replication. All prawns in the second replication accidentally died at week 3 after a failure of air supply.



A



B

Figure 3 Growth of *P. monodon* fed with the diets containing different concentrations of astaxanthin and the diets containing different concentrations of canthaxanthin.
A = Astaxanthin B = Canthaxanthin

Survival, FCR, and Individual Moulting Rate

Survival, FCR and individual moulting rate of the juvenile *P. monodon* fed with diets containing different concentrations of astaxanthin and canthaxanthin were shown in Table 5. Statistical analysis showed no significant difference among the treatment groups in each of these three parameters.

The analysis suggested that survival, FCR and moulting rates of these treatment groups were somewhat the same, though the average value showed that the treatment with 100 ppm astaxanthin gave the best FCR (2.53).

Visual Observation on Prawn Colouration

The result of the visual observation on the prawn coloration after two months of feeding the experimental diets was shown in Table 6. At week 2 the change in colouration of the prawn appendages could be noticeable in the groups of prawns fed diets containing 100 ppm astaxanthin and 200 ppm canthaxanthin. During the two months feeding trial, the colour hues of the prawns fed with the diets augmented with the two kinds of carotenoids gradually

Table 5 Average food convertion ratio (FCR), average percent survival and average individual moulting rate of juvenile *Penaeus monodon* fed with diets containing different concentrations of astaxanthin and canthaxanthin.

	Control	25 ppm Ast.	50 ppm Ast.	75 ppm Ast.	100 ppm Ast.	50 ppm Cant.	100 ppm Cant.	150 ppm Cant.	200 ppm Cant.
FCR	4.83	3.72	3.25	2.89	2.53	3.48	3.85	2.89	2.86
% Survival	90.00	85.00	85.40	85.80	73.40	78.50	86.70	86.70	86.60
Ave. Individual molting rate per week	0.46	0.48	0.48	0.51	0.58	0.45	0.48	0.53	0.55

Table 6 Ranking order based on visual assessment of the prawn's pigmentation after 2 months of feeding the diets containing different concentration of astaxanthin and canthaxanthin.

Treatments	Raw prawns		Boiled prawns	
	Ranking Order	Colour	Ranking Order	Colour
control	1	blue	1	yellowish orange
25 ppm. Ast	2	light violet	2	redish orange
25 ppm. Ast	2	light violet	2	redish orange
50 ppm. Ast	2	light violet	2	redish orange
50 ppm. Ast	3	dark violet	3	red orange
75 ppm. Ast	3	dark violet	3	red orange
75 ppm. Ast	3	dark violet	3	red orange
100 ppm. Ast	4	grayish brown	4	bright red orange
100 ppm. Ast	4	grayish brown	4	bright red orange
50 ppm Canth	1	blue	2	redish orange
50 ppm Canth	2	light violet	2	redish orange
100 ppm Canth	2	light violet	2	redish orange
100 ppm Canth	3	dark violet	3	red orange
150 ppm Canth	3	dark violet	3	red orange
50 ppm Canth	3	dark violet	3	red orange
00 ppm Canth	3	dark violet	3	red orange
00 ppm Canth	4	grayish brown	4	bright red orange

changed from blue to light violet, dark violet, and grayish brown. The degree of pigmentation was intensified with the increasing concentration of astaxanthin and canthaxanthin in the diets. The changing colour pattern of the astaxanthin groups and the canthaxanthin groups were considered to be comparable. The group of prawns fed a control diet did not show any change in colouration. They maintained the blue colour throughout the whole experimental period.

The colour hues of the boiled prawns varied from yellowish orange to bright red orange depending on the original fresh colours. The blue prawns when boiled usually turned yellowish orange while the darker prawns turned more red. Further study found that the pigment deposited in prawns of this study could maintain their colour efficacy for a long period of time. The grayish brown prawns, after two months of storage, could turn bright red color when boiled.

Carotenoids in the Prawns

The results of the analyses of total carotenoids (TCA), total astaxanthin (TAC) ,free astaxanthin (FAC), and total astaxanthin ester (TAEC) concentrations in the whole prawn during the two months of feeding trial were shown in Tables 7-8.

Table 7 Total carotenoid (TCA), total astaxanthin (TAC), free astaxanthin (FAC) and total astaxanthin ester (TACE) contents of the whole prawn (*Penaeus monodon*) fed with various levels of astaxanthin.

Treatment	TCA in mg/kg			TAC in mg/kg			FAC in mg/kg			TACE in mg/kg		
	Start	1 month	2 months	Start	1 Month	2 Months	Start	1 Month	2 Months	Start	1 Month	2 Months
Control 1	22.70 ±6.18	15.37 ±3.30	12.00 ±3.58	19.52 ±6.39	12.10 ±2.55	9.99 ±3.12	9.30 ±2.78	8.60 ±1.20	6.82 ±1.36	10.22 ±4.19	3.20 ±0.92	3.17 ±1.76
Contorl 2	21.03 ±3.53	-	-	18.57 ±2.48	-	-	10.57 ±1.92	-	-	8.00 ±1.6	-	-
25 ppm Ast.	21.87 ±4.88	22.22 ±1.60	26.73 ±2.49	19.04 ±4.65	21.03 ±2.93	23.71 ±2.79	9.93 ±2.37	12.53 ±0.73	15.69 ±1.30	9.11 ±3.25	5.37 ±1.21	8.02 ±1.81
50 ppm Ast.	21.87 ±4.88	24.22 ±2.80	26.95 ±3.47	19.04 ±4.65	22.47 ±2.94	23.55 ±3.89	9.93 ±2.37	13.25 ±1.80	14.80 ±2.49	9.11 ±3.25	5.67 ±0.84	8.75 ±2.21
75 ppm Ast.	21.87 ±4.88	24.82 ±3.20	33.00 ±8.26	19.04 ±4.65	23.38 ±4.15	28.10 ±8.22	9.93 ±2.37	11.73 ±2.12	17.30 ±3.56	9.11 ±3.25	11.65 ±3.15	10.80 ±6.43
100 ppm Ast.	21.87 ±4.88	30.10 ±6.79	36.57 ±5.24	19.04 ±4.65	29.58 ±5.97	33.92 ±3.07	9.37 ±2.37	14.92 ±4.09	18.73 ±2.28	9.11 ±3.25	14.67 ±2.64	15.18 ±2.51

Table 8 Total carotenoid (TCA), total astaxanthin (TAC), free astaxanthin (FAC) and total astaxanthin ester (TACE), contents of the whole prawn (*Penaeus monodon*) fed with various levels of canthaxanthin.

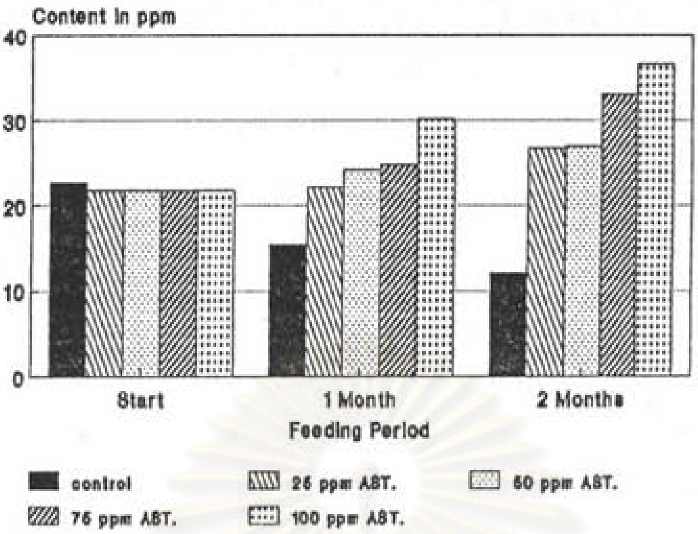
Treatment	TCA in mg/kg			TAC in mg/kg			FAC in mg/kg			TACE in mg/kg		
	Start	1 month	2 months	Start	1 Month	2 Months	Start	1 Month	2 Months	Start	1 Month	2 Months
Control 1	22.70 ±6.18	15.37 ±3.30	12.00 ±3.58	19.52 ±6.39	12.10 ±2.55	9.99 ±3.12	9.30 ±2.78	8.60 ±1.20	6.82 ±1.36	10.22 ±4.19	3.20 ±0.92	3.17 ±1.76
Contorl 2	21.03 ±3.53	-	-	18.57 ±2.48	-	-	10.57 ±1.92	-	-	8.00 ±1.6	-	-
50 ppm Cantha.	21.87 ±4.88	19.23 ±2.22	18.77 ±3.72	19.04 ±4.65	17.07 ±1.41	16.10 ±2.26	9.93 ±2.37	9.43 ±3.01	10.10 ±1.59	9.11 ±3.25	7.63 ±2.35	6.00 ±1.01
100 ppm Cantha.	21.87 ±4.88	21.67 ±2.55	30.90 ±7.11	19.40 ±4.65	19.65 ±3.24	27.47 ±7.52	9.93 ±2.37	11.62 ±1.14	14.97 ±2.49	9.11 ±3.25	8.03 ±3.03	12.50 ±5.70
150 ppm Cantha.	21.87 ±4.88	22.42 ±4.12	25.12 ±4.97	19.04 ±4.65	20.98 ±4.88	20.70 ±5.28	9.93 ±2.37	11.90 ±2.14	13.17 ±2.93	9.11 ±3.25	9.08 ±3.07	7.53 ±3.24
200 ppm Cantha.	21.87 ±4.88	29.13 ±4.17	37.88 ±7.06	19.04 ±4.65	27.67 ±4.14	33.71 ±5.61	9.37 ±2.37	14.47 ±1.81	14.36 ±4.56	9.11 ±3.25	13.20 ±5.45	18.85 ±3.28

The general examination of data revealed that total carotenoids (TCA) concentrations in the group of prawns fed a control diet decreased with time while those in the group of prawns the diets containing astaxanthin increased with time. The rate of increase was highest in the group fed 100 ppm astaxanthin, followed by the group fed 75 ppm. The groups fed 50 ppm and 25 ppm astaxanthin showed a slight increase (Figure 4).

The groups of prawns fed diets containing canthaxanthin showed a similar pattern but with a slight a difference at the lower concentration. The rate of increase was highest in the group fed 200 ppm canthaxanthin. The groups fed 100 ppm and 150 ppm canthaxanthin showed an intermediate increase while the group fed 50 ppm canthaxanthin only slight increase (Figure 5).

The covariance analysis confirmed the significant difference among the carotenoid increasing rates of these treatment groups (Appendix 2).

In every treatment group approximately 90 % of total carotenoid was astaxanthin. The total astaxanthin (TAC) also showed a similar deposition pattern as the total carotenoids. TAC was comprised of two forms, i.e. free astaxanthin (FAC) and astaxanthin esters (TAEC). According to the data



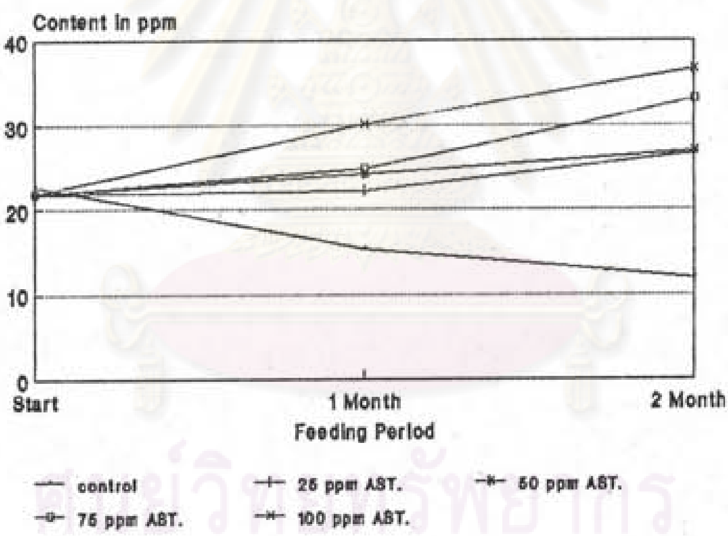
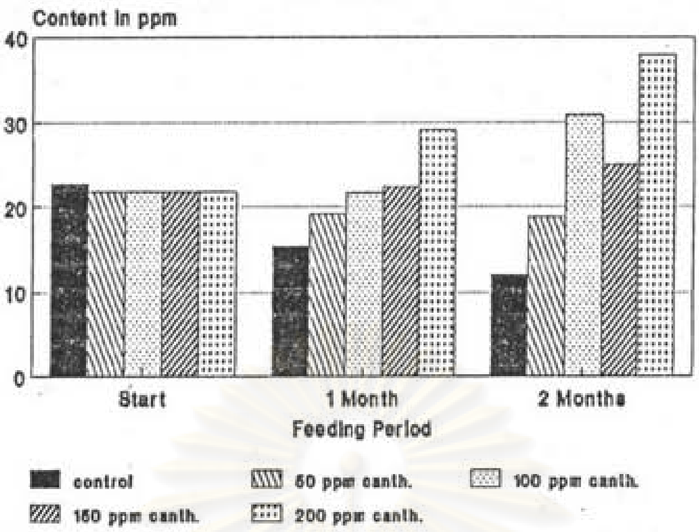


Figure 4 Total carotenoid contents in the whole prawn (*Penaeus monodon*) fed with various levels of astaxanthin.



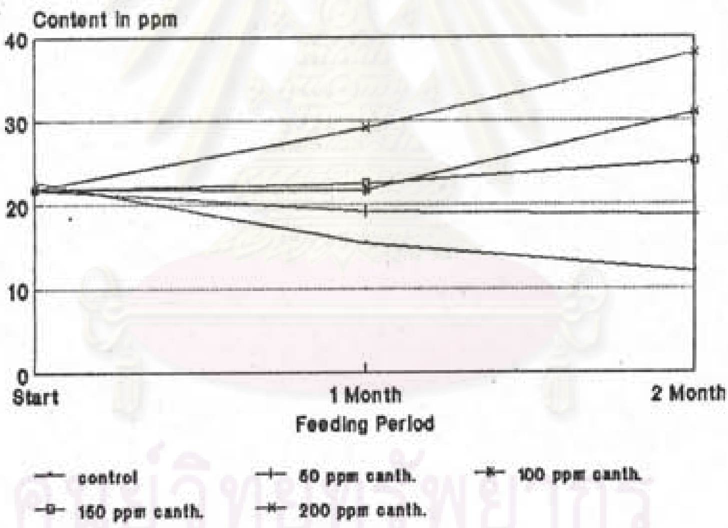


Figure 5 Total carotenoid contents of whole prawn (*Penaeus monodon*) fed with various levels of canthaxanthin.

shown in Tables 7 and 8, the two forms of astaxanthin constituted a similar proportion, approximately 50 % each. Nevertheless the rate of deposition of those two forms not the same. The deposition rate of FAC was seemed to be faster and higher than TACE.

Carotenoid Content in the Feed Pellets

During the experimental period, the nine diets were sampled at start, 1 month, and 2 months for the analysis of actual content of carotenoids in the feed pellets. The results revealed that astaxanthin at the four concentrations were close to the nominal values and the concentrations did not decrease with time. In contrast, canthaxanthin at the four concentrations were a little lower than the nominal values and the concentrations decreased with time (Figure 6).

Efficiency of Astaxanthin and Canthaxanthin

A comparison of pigment deposition efficiency between astaxanthin and canthaxanthin was made by calculation. The result revealed that the realistic ratio between astaxanthin and canthaxanthin efficiency should be 1:1.85 instead of 1:2 Table 9.

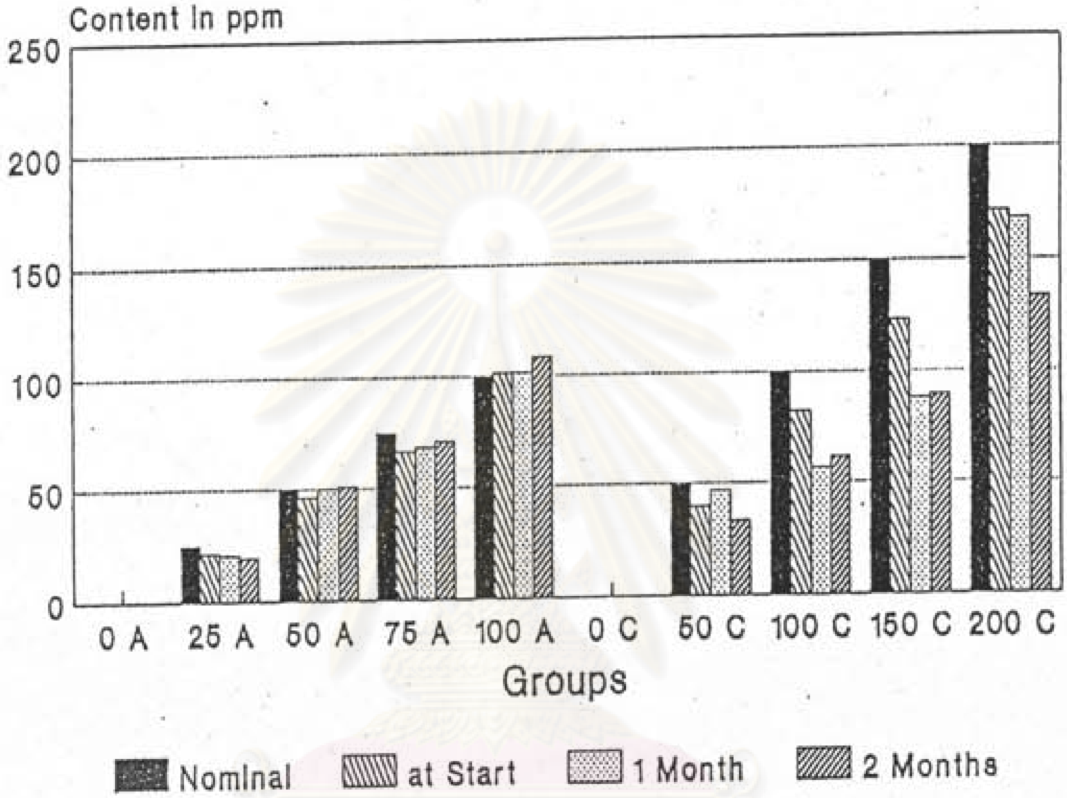


Figure 6 Carotenoid contents in feed pellets at start, 1 month and 2 months.

Table 9 Comparison of astaxanthin and cantaxanthin treatment efficiency. Calculation based on total carotenoid content (TCA) in the prawns and in the feeds.1

Treatment	Prawn content relation TCA	Theoretical Ast:Can.Eff. ratio	Analysed feed content ratio	Realistic Ast:Can.Eff. ratio
25 ppm Ast.:50 ppm Canth.	1.42	1 : 2.84	1.98	1 : 2.81
50 ppm Ast.:100 ppm Canth.	0.87	1 : 1.74	1.35	1 : 1.18
75 ppm Ast.:150 ppm Canth.	1.31	1 : 2.62	1.47	1 : 1.39
100 ppm Ast.:200 ppm Canth.	0.96	1 : 1.92	1.52	1 : 1.47
Total	4.65	4 : 9.12	6.92	4 : 7.39
Average	1.14	1 : 2.28	1.58	1 : 1.85

Water Quality

Water quality during the feeding trial is shown in Table 10. These water quality parameters were in the optimal ranges for the nursery of giant tiger prawns.

Table 10 Water quality of the recirculating seawater system used for the feeding trial in Experiment 1.

Tank	D.O. (ppm)	pH	Temperature (°C)	Salinity (ppt)	NH_4-N (mg/l)	NO_2-N (mg/l)	NO_3-N (mg/l)
Bio-filter	7.0 - 4.0	7.2 - 7.9	28.9 - 30.1	20.0 - 23.5	0.0 - 1.0	0.0 - 1.1	0.0 - 2.0
Experimental tanks	6.9 - 7.1	7.0 - 7.7	28.9 - 31.5	20.0 - 24.2	0.0 - 0.05	0.0 - 1.0	0.0 - 1.0

Experiment II : Effects of astaxanthin on ovarian maturity and spawning of giant tiger prawns

Ovarian Maturity

The results on ovarian maturity of giant tiger prawns fed with non-astaxanthin added diet and 100 ppm astaxanthin added diet are shown in Table 11. The percentage of gravid female (stage IV ovarian maturity) of these two treatments suggested that the prawns fed with astaxanthin added diet became gravid more frequently than another group fed with non-astaxanthin added diet. The eyestalk ablated prawns could become gravid significantly higher than the no-ablated groups. A non-eyestalk ablated prawn in the group fed astaxanthin added diet could become gravid (stage IV), while none in the group fed non-astaxanthin diet became gravid.

The elapsed time between the eye-stalk ablation and the first gravid (stage IV) of these treatment groups did not differ significantly (P>0.05). Furthermore, the elapsed times for the other consecutive gravids were shorter than first gravid.

Spawning

The results on the spawning of the two treatment groups are shown in Table 12. Similar to the ovarian

Table 11 Ovarian maturity of giant tiger prawns fed with two maturation diets (Non-astaxanthin added diet and astaxanthin added diet) during the 80 days experiment.

Maturation diets	Eye-stalk condition	Stage IV ovarian maturity		Elapsed time (days) B		
		No.of gravid females	Percent gravid female	Average	Minimum	Maximum
Non-astaxanthin added diet	Ablated female A					
	1	15	48.0	14.3[a]	5	38
	2	4	12.9	3.5[b]	3	5
	3	1	3.2	2	2	2
	Non ablated female					
	1	0	0	0	0	0
Astaxanthin added diet	Ablated female					
	1	16	51.2	24.6[a]	7	43
	2	8	25.6	12.2[b]	2	23
	3	3	9.6	8	3	9
	4	1	3.2	15	15	15
	Non ablated female					
	1	1	2.5	28	28	28
	2	1	2.5	8	8	8
	3	1	2.5	12	12	12
	4	1	2.5	16	16	16

A. Numbers are the consecutive gravids (stage IV).

B. Time in days after the eye stalk ablation and the consecutive gravids.

Significant differences ($P<0.05$) of the average elapsed time are indicated by different letters i.e. a and b within the column.

maturity results, eye-stalk ablated prawns produced more spawners than the non-ablated prawns. Besides the prawns fed astaxanthin diet produced more spawning successes than the prawns fed non-astaxanthin added diet.

The elapsed time between the eye-stalk ablation and the first spawning of these two treatment groups did not differ significantly (P>0.05). Furthermore, the elapsed time for the other consecutive spawnings were shorter than those of the first spawning.

<u>Egg Quantity and Quality</u>

Results on egg quantity and quality are summarized in Table 13. It should be noted that although the experimental design was a factorial one, the statistical analysis for factorial design could not be applied because one of the factorial treatment (non-eyestalk ablated with non-astaxanthin diet) did not spawn any egg. Because of this the analysis of variance was applied to analyse the data of the rest these factorial treatments.

The general observation on the results shown in Table 13 revealed that the highest egg production was obtained in the treatments feeding astaxanthin added diets. The eyestalk ablated prawns produced higher egg production than the non-eyestalk ablated prawns.

Table 12 Spawning success of giant tiger prawns fed with two maturation diets (Non astaxanthin added diet and astaxanthin added diet) during the 80 days experiment.

maturation diets	Eye-stalk condition	Spawning		Elapsed time (days) B		
		No.of spawners	Percent	Average	Minimum	Maximum
Non-astaxanthin added diet	Ablated female A					
	1	11	35.2	16.3[a]	6	40
	2	4	12.8	7.0[b]	3	14
	3	1	3.2	3.0	3	3
	Non ablated female					
	1	0	0	0	0	0
Astaxanthin added diet	Ablated female					
	1	15	48.0	25.2[a]	8	45
	2	8	25.6	13.2[b]	4	24
	3	3	9.6	6.3	4	10
	4	1	3.2	16.0	16	16
	Non ablated female					
	1	1	2.5	30.0	30	30
	2	1	2.5	10.0	10	10

A. Numbers are the consecutive spawnings.

B. Time in days after the eye stalk ablation and the consecutive spawning.

Significant differences ($P<0.05$) of the average elapsed time are indicated by different letters i.e. a and b within the column.

Table 13. Egg quantity and quality of the giant tiger prawns fed with the two types of maturation diet.

Egg quantity and quality	Non-astaxanthin added diet		Astaxanthin added diet	
	Non ablated female	Ablated female	Non ablated female	Ablated female
Total egg production	-	4,345,000	250,000	6,725,000
Ave. no. of eggs per female	-	140,161^{a}	8,065^{b}	216,936^{a}
Ave. no. of eggs per spawner	-	395,000	250,000	448,333
Eggs dimeter (mm)	-	0.27+0.0069^{a}	0.28+0.0097^{b}	0.28+0.0078^{b}
Ave. % fertility	-	41.5 (0-81.0)	71.2 -	40.1 (0-77.8)
Ave. % hatching rate	-	25.8 (0-64.0)	35.2 -	20.0 (0-45.1)
Ave. % metamorphosis from egg to protozoea stage	-	6.57 (0-16.7)	16.15 -	10.8 (0-38.8)

Note : Significant differences (P<0.01) are indicated by different letters, i.e. a and b.

The average number of eggs per female of the eyestalk ablated groups were significantly higher than the non-eyestalk ablated group ($P<0.05$). Besides the eggs per females of the group fed astaxanthin added diet did not differ significantly from the group fed non-astaxanthin added diet.

The average number of eggs spawned per spawner of the rest three groups of prawns did not differ significantly ($P<0.05$). The number of eggs per spawner averaged at 419,259 eggs. The amount of eggs spawned in the consecutive spawnings did not differ significantly ($P>0.05$).

The diameter of eggs produced from astaxanthin treatments was significantly higher ($P<0.05$) than the non-astaxanthin treatments.

The eggs quality in terms of eggs fertility (fertilized eggs), hatching rate and metamorphosis rate (from egg to protozoea stage) varied greatly. The average percent fertility of total eggs spawned of the three treatment groups did not differ significantly ($P>0.05$). Nevertheless, egg quality of this study was seemed to be better than the previous studies. The consequence mat be due to the improved maturation diets.

Moulting

The moulting pattern of female prawns in the four treatment groups are shown in Table 14. The first moulting after eyestalk ablation took slightly shorter time than the following consecutive moultings. Non ablated female took longer time to moult than the ablated ones.

Mortality

The analysis of covariance revealed that the mortality rate of prawns in the groups fed astaxanthin added diet, regardless the eye-stalk manipulation, was significantly lower than the groups fed non-astaxanthin added diet (Figure 7). The mortality rate of females was slightly higher than that of the males (Figure 8). It was also found that astaxanthin added diet could reduce the mortality rate in the eyestalk ablated females (Figure 9).

Carotenoids in Hepatopancreas and Ovaries.

The results of the analyses of the total carotenoids and total astaxanthin and its duivatives in hepatopancreas and ovaries of giant tiger prawns fed the two maturation diets are shown in Table 15.

Table 14 Average elapsed time between consecutive moltings of giant tiger prawns.

Maturation diet	Eye-stalk condition	Sequential moltings				
		1	2	3	4	5
Astaxanthin added diet	Non-eyestalk ablated	9.2 + 6.75	17.6 + 7.16	17.9 + 5.44	18.7 + 7.95	-
	eyestalk ablated	9.5 + 9.50	13.1 + 6.46	15.8 + 7.72	13.1 + 4.10	16.2 + 4.06
Non-astaxanthin added diet	Non-eyestalk ablated	17.1 + 9.42	26.8 + 8.66	22.0 + 5.24	-	-
	eyestalk ablated	9.2 + 5.37	19.6 + 9.36	15.1 + 8.13	3.0 + 0.00	-

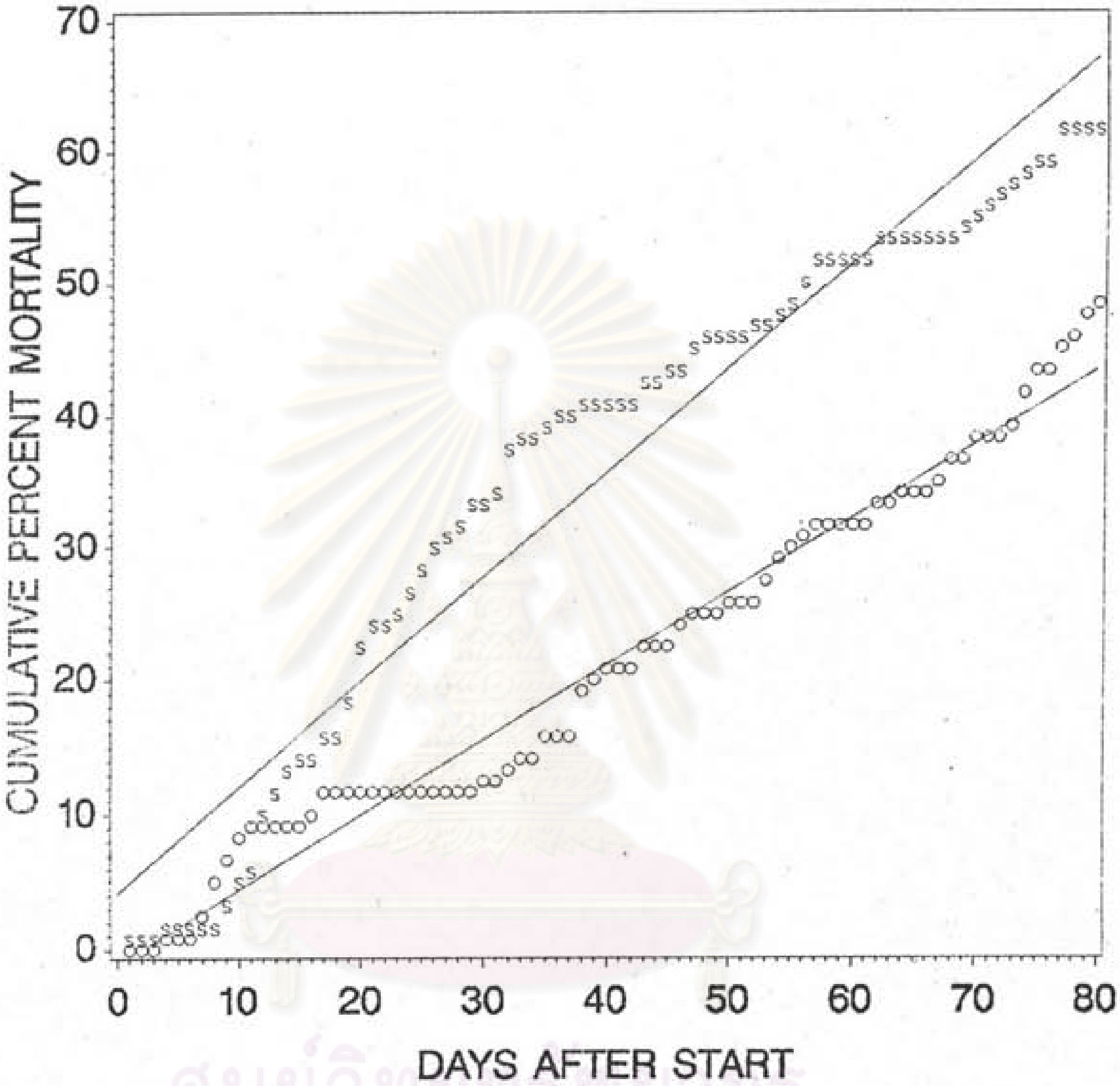


Figure 7 Cumulative percent mortality of giant tiger prawns fed the two maturation diets.

S = Non-astaxanthin added diet.

O = Astaxanthin added diet.

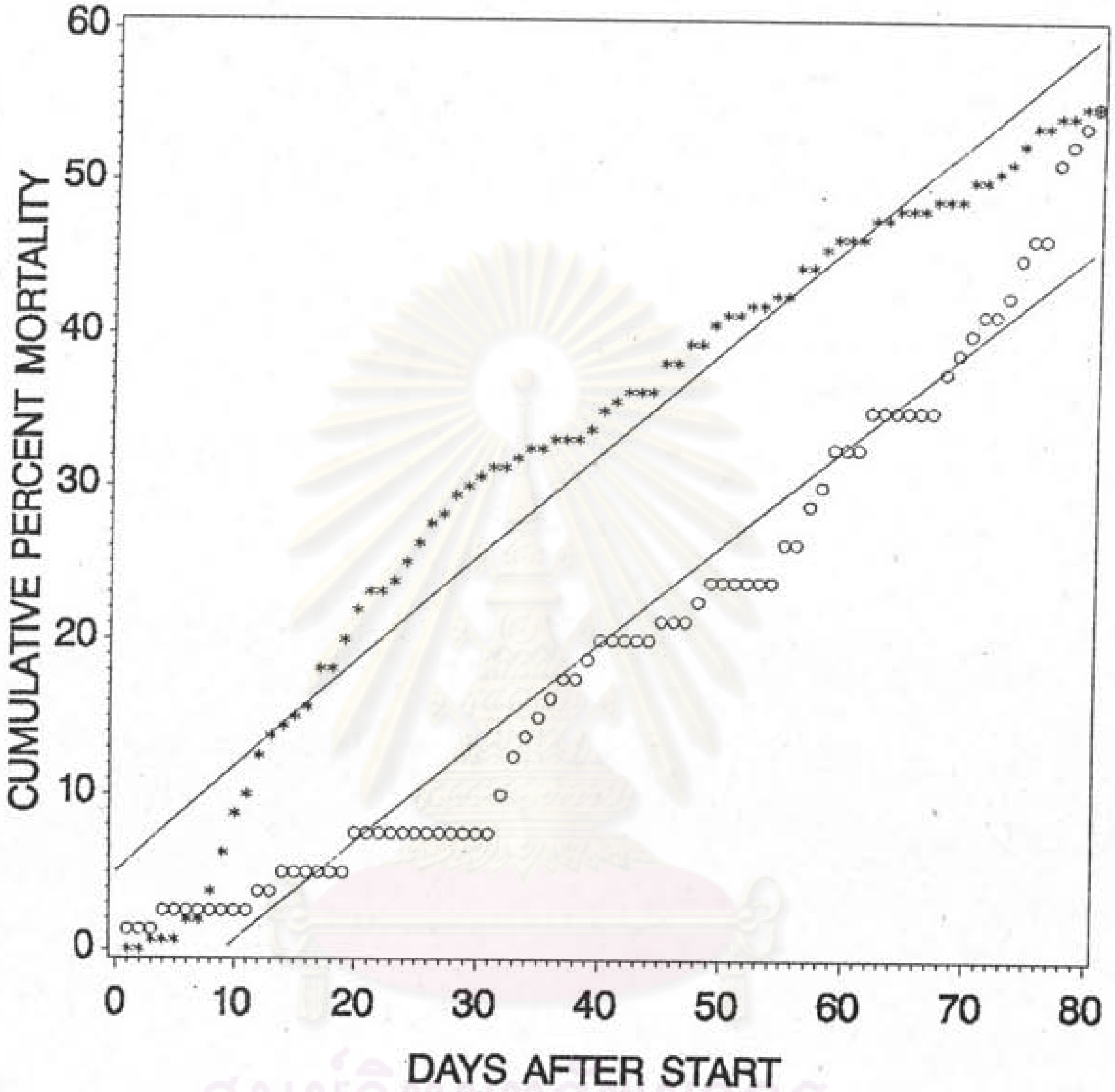


Figure 8 Cumulative percent mortality of female and male giant tiger prawns.

$*$ = Female

0 = Male

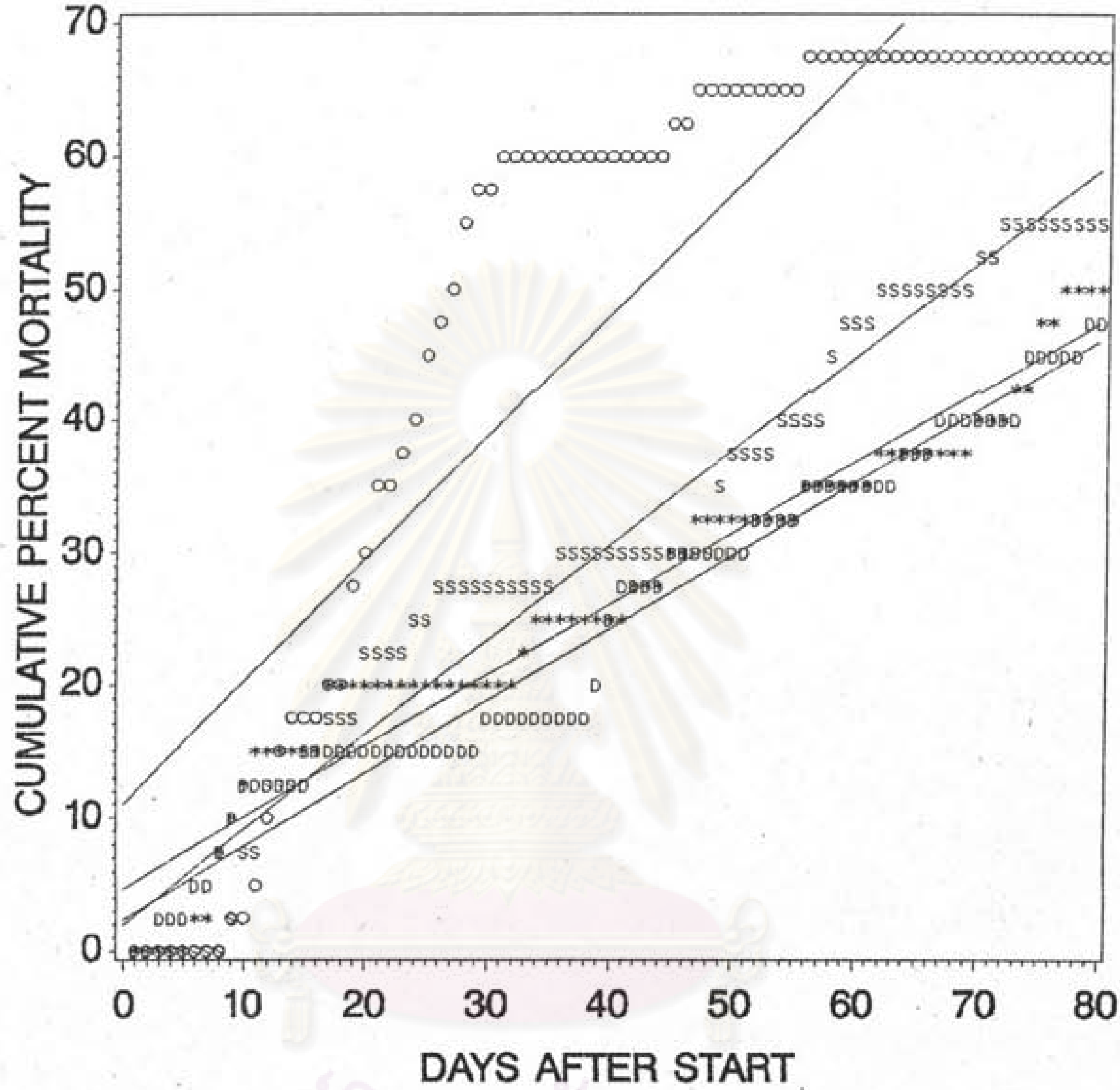


Figure 9 Cumulative percent mortality of female giant tiger prawns fed with the two maturation diets and with eye-stalk manipulation.

O = Ablated females fed non-astaxanthin added diet.

S = Non ablated females fed non-astaxanthin added diet.

* = Ablated females fed astaxanthin added diet.

D = Non ablated females fed astaxanthin added diet.

Table 15 Total carotenoids (TCA), total astaxanthin (TAC), free astaxanthin (FAC) and astaxanthin esters (TACE) in hepatopancreas and ovaries of *P. monodon* fed the two maturation diets.

Treatments	Day after ablation	Stage of ovaries	Hepatopancreas				Ovaries			
			TCA mg/kg	TAC mg/kg	FAC mg/kg	TACE mg/kg	TCA mg/kg	TAC mg/kg	FAC mg/kg	TACE mg/kg
Initial	0	1	0	0.77	0.49	0.30	0	1.01	0.70	0.40
(before	0	1	0	0.40	0.33	0.10	0	3.24	2.70	0.60
feeding)	0	1	72.4	45.40	4.51	40.90	0	2.82	1.10	1.70
	0	1	0	3.14	2.40	0.74	0	2.44	1.10	1.30
Average			18.10	12.43	1.93	10.51	0.00	2.38	1.40	1.00
Non astaxan-	8	2				65.80	5.30	2.10	1.90	1.20
thin added	12	2				114.10	0.00	1.50	0.50	1.00
diet	9	2				38.50	13.50	10.80	9.70	1.10
	12	3	6.60	5.44	1.06	4.40	12.60	9.53	8.60	1.00
	17	3	134.60	118.10	6.21	111.90	20.40	16.67	1.20	15.50
	22	3	356.60	302.92	12.83	28.60	22.60	10.58	4.20	6.40
	16	4	13.90	8.44	1.13	7.30	45.40	31.71	31.60	0.10
	12	4	19.30	16.38	1.17	15.20	34.30	27.01	26.80	0.20
	18	4	39.20	32.70	4.12	28.58	54.40	44.13	39.47	0.60
Average 3-4			95.03	80.66	4.42	32.66	31.62	23.27	18.65	3.97
Astaxanthin	72	1	14.8	11.9	1.5	10.4	0	0.9	0.3	0.6
added diet	9	2	37	26.3	6.6	19.7	9.2	6.6	5.5	1.1
	8	2	27.2	18.4	2.24	16.2	11.3	7.48	7.4	0.1
	18	2	369.5	331.1	10.3	320.8	0	1.89	0	1.6
	20	3	446	364.96	29.5	335.5	31.8	3.84	23.6	0.3
	17	3	97.7	75.13	14.7	60.4	31.9	4.36	24.1	0.3
	21	3	525.8	436.65	22.4	414.2	83.1	8.74	0	52.3
	14	4	173.5	150.96	9.1	141.9	16.6	9.39	8	1.4
	20	4	745.5	620.77	30.4	590	41	31.4	31.0	0.4
	9	4	78.8	60.86	9.7	51.2	7.5	4.15	1.2	2.98
Average 3-4			344.55	284.89	19.30	265.53	35.32	25.31	14.65	9.61

At the initial period (before feeding the two maturation diets) the level of total carotenoids (TCA) and total astaxanthin (TAC) in hepatopancreas were quite low. However, when the two maturation diets commenced and extended the level of TCA and TAC in the hepatopancreas showed the increase (Figure 10). The group fed with astaxanthin added diet shown remarkably higher TCA and TAC than the control group (non-astaxanthin added diet). This circumstance suggested that astaxanthin passing to the body of the prawn would be deposited first in hepatopancreas before distributing to other organs in the later stage.

The concentrations of total carotenoids as well as the total astaxanthin in ovaries were relatively lower than those in hepatopancreas. The concentrations of TCA and TAC significantly elevated in the ovaries after feeding the two diets (Figure 11). Nevertheless the ovary samples collected during the feeding were in the developing stages (stage 2-4). Hence, the increase of carotenoids could be affected by both nutritional and physiological factors. The carotenoid contents of the two treatment groups not show a significant difference like in the hepatopancreas.

As regards the different forms of astaxanthin, it was found that a large portion (>98 %) of astaxanthin in

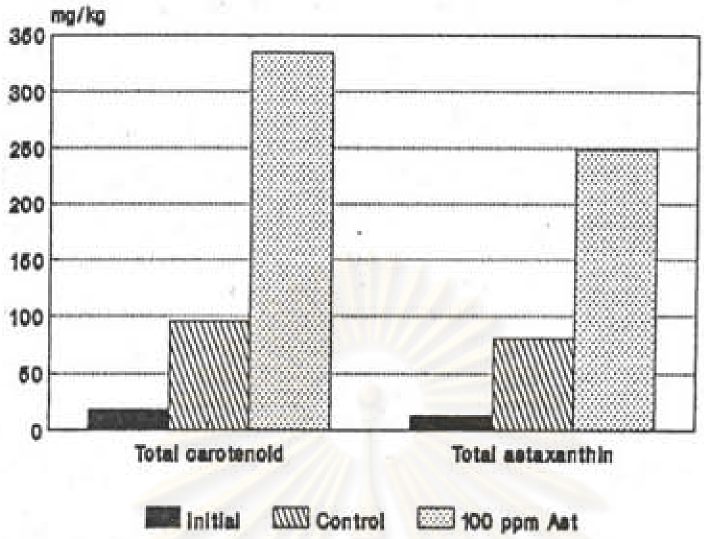


Figure 10 Total carotenoids and total astaxanthin in hepatopancreas at initial, and after feeding the non-astaxanthin added diet and the astaxanthin added diet.

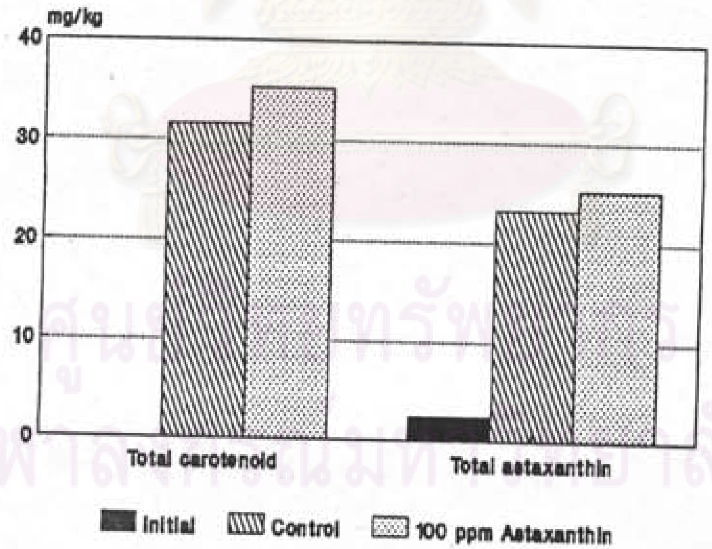


Figure 11 Total carotenoids and total astaxanthin in ovaries at initial, and after feeding the non-astaxanthin added diet and the astaxanthin added diet.

hapatopancreas was the esterified forms. Besides the group fed astaxanthin added diet showed significantly higher astaxanthin esters than the control group (Figure 12). In contrast, higher portion of astaxanthin in the ovaries was the free astaxanthin (Figure 13). This circumstance suggested that the ovaries might gave much lower saturation point of carotenoids than the hepatopancreas and carotenoids in ovaries was not directly levels in the hepatopancreas.

Water Quality

Water quality of the rearing tanks in this study are summarized in Table 16. These water quality parameters were seemed to be in the optimal ranges throughout the whole period of the experiment

Nutritional Values of the Diets

Nutritional values of the diets used in this study were shown in Table 17. Crude protein (51.78 %) and crude fat (11.78%) of maturation diet were relatively higher than the commercial diet. Whereas crude fiber, crude ash and moisture content of maturation diet were slightly lower when compared with the commercial diet.

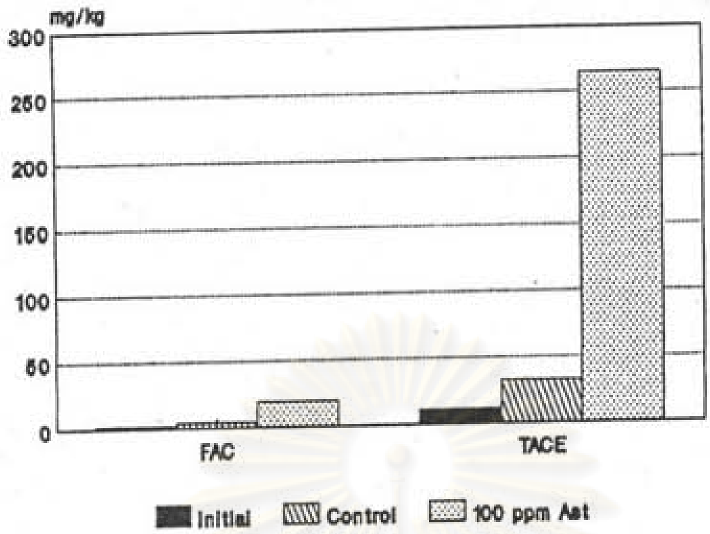


Figure 12 Free astaxanthin (FAC) and total astaxanthin esters (TACE) in hepatopancreaus at initial, and after feeding the non-astaxanthin added diet and the astaxanthin added diet.

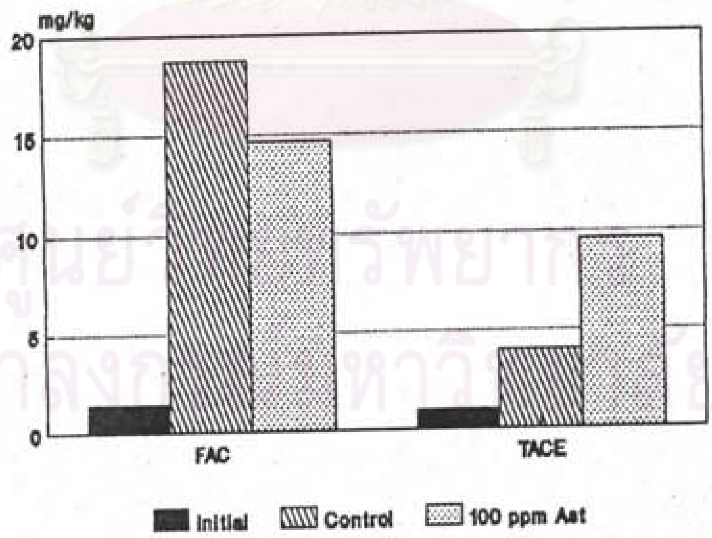


Figure 13 Free astaxanthin (FAC) and total astaxanthin esters (TACE) in ovaries at initial, after feeding the non-astaxanthin added diet and the astaxanthin added diet.

Table 16 Water quality of the two treatment groups.

Treatments	D.O. (ppm)	pH	Temperature (°C)	Salinity (ppt)	NH_4-N (mg/l)	NO_2-N (mg/l)	NO_3-N (mg/l)
Non-astaxanthin added diet	6.16 ± 0.88 (4.4 - 8.4)	8.37 ±0.48 (7.0 - 9.0)	28.15 ± 1.36 (26.2 - 30.0)	31.18 ± 1.98 (28 - 34)	0.03 ± 0.02 (0.0 - 0.7)	0.346±0.566 (0.01-1.5)	0.0028 ± 0.0037 (0 - 0.009)
Astaxanthin added diet	6.08 ± 0.8 (4.1 -7.2)	8.36 ± 0.4 (7.5 - 8.9)	28.15 ± 1.54 (24.9 - 30.0)	31.21 ± 1.86 (27 -34)	0.112 ± 0.13 (0 - 1.48)	0.29 ± 0.51 (0 - 7.5)	1.39 ± 2.66 (0.0004-0.0011)

Table 17 Proximate analysis of artificial diets used in Experiment 1 (coloration) and Experiment 2 (Maturation diets).

Diet types	Moisture content	Crude protein	Crude fat	Crude fiber	Ash
Experiment 1					
Coloration diets	7.61 ± 0.28	41.50 ± 0.50	11.41 ± 0.00	3.48 ± 0.04	16.91 ± 0.07
Experiment 2					
Maturation diets					
Trial 1	6.42 ± 0.86	51.78 ± 0.58	11.87 ± 0.00	3.29 ± 0.02	17.25 ± 0.45
Trial 2	6.41 ± 0.83	50.98 ± 0.49	11.89 ± 0.50	3.30 ± 0.01	17.24 ± 0.39
Commercial diet	9.71 ± 0.52	42.45 ± 0.17	5.12 ± 0.30	3.52 ± 0.39	18.24 ± 0.37