CHAPTER IV

RESULTS AND DISCUSSION

Proximate analysis of experimental diets

There were no significant differences of crude protein, crude fat, crude fiber, moisture and ash in all diets (Table 10). All experimental diets contained 46.9-48.3% of crude protein, 7.6-10.7% of crude lipid, 3.8-4.6% of ash, 3.8-5.3% of moisture and 10.9-11.9 % of crude fiber.

Table 10. Proximate analysis of the experimental diets. Values are means±S.D.[1]

Diet	%Protein	%Fat	%Fiber	%Moisture	%Ash	%Carbohydrate
1*	47.8±1.1	7.6±0.3	3.8±0.1	4.7±0.9	11.3±0.1	75.2±2.5
2	47.8±0.6	8.5±0.3	4.3±0.2	3.8±0.5	11.4±0.4	75.8±2.0
3	46.9±0.1	10.7±0.8	3.9±0.3	4.4±0.8	10.9±0.2	76.8±2.2
4	48.3±0.4	8.9±0.3	3.7±0.7	4.4±0.3	11.6±0.4	76.9±2.1
5	48.2±0.4	8.5±0.2	4.8±0.9	4.2±0.1	11.9±0.4	77.6±2.0
6	47.8±1.4	8.1±0.4	4.5±0.2	5.3±0.3	11.9±0.2	77.6±2.5
7	47.8±0.4	8.5±0.1	4.6±0.4	4.1±0.2	11.9±0.2	76.9±1.3

[1] Each diet was analysed triplicately.

* Control diet

The quality of rearing water was recorded and shown in Table 11. Water temperature was at 23-30°C, with pH range of 7.7-8.0. Ammonium, nitrate and nitrite concentrations were at 0-1, 0-25 and 0.05-0.25 ppm, respectively. Salinity was controlled as constantly at 30 ppt.

Table 11. The quality of rearing water during the experiment.[1]

Week	Temperature (°C)	NH_4^+ (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	pH	Salinity (ppt)
1	23-26	0-0.5	10	0.05-0.25	7.7	30
2	26-28	0-1	0-25	0.05-0.25	7.7-8.0	30
3	27-30	0-0.5	0-25	0.05-0.25	7.7-8.0	30
4	28-30	0-0.5	0-25	0.05-0.25	7.7-8.0	30

[1] Data shown are ranges or means of each factor.

All parameters in Table 11 are acceptable ranges for rearing aquatic animal. So growth and survival of postlarvae dependent conclusively on diet. Furthermore, all diets had the same levels of protein, fat, fiber, moisture and ash (Table 10). Only levels of n-3 HUFAs and ratios of EPA/DHA in experimental diets were varied accordingly to the protocol (Table 7).

Fatty acid composition in fish oil

Semi-refine oil was composed of 20:5n-3 and 22:6n-3 at 6.5% and 22.7%, respectively, whereas refine tuna oil contained the two respective fatty acids of 16.8% and 12.3%. In both oil, the total percentage of n-3 HUFAs was about 29% (Table 12).

Table 12. Fatty acid composition of two fish oil used in this experiment

Fatty acid	Semi-refined oil (%)	Refine tuna oil (%)
14:0	5.2	9.3
16:0	25.1	19.4
16:1	7.3	10.4
18:0	6.0	3.8
18:1	2.5	3.6
18:2	1.4	1.3
18:3	0.5	0.8
20:0	0.4	-
20:1	0.7	0.9
20:4	1.7	1.2
20:5	6.5	16.8
22:6	22.7	12.3
ratio[1]	0.3	1.4
Σn-3 HUFAs[2]	29.2	29.0

[1] EPA/DHA = eicosapentaenoic acid/ docosahexaenoic acid

[2] The total percentage of EPA and DHA in fish oil

Fatty acid composition of the experimental diets

Fatty acid composition of the diets is shown in Table 13. Diets 1-4 contained higher levels of 18:2, 18:1 and 16:0 than the other diets. These diets contained high level of 18:2, because corn oil used in these diets is rich in 18:2 fatty acid. Diets 5-7 contained mainly 16:0, 18:2 and 18:1, respectively. The levels of 16:0 and 18:0 in all diets were not significantly different (P>0.05). It

Table 13. Fatty acid composition of the experimental diets for *P. monodon* (mg/g dry weight)

Fatty acid	Diet no.						
	1	2	3	4	5	6	7
14:0	1.3	1.5	3.1	3.0	2.8	3.2	4.2
16:0	14.0	13.6	19.6	15.2	15.7	15.1	15.6
16:1n-7	1.4	2.4	4.3	3.4	4.1	4.5	5.4
18:0	2.4	3.9	5.1	4.1	4.5	4.4	3.8
18:1n-9	17.7	13.4	20.5	14.1	9.7	8.6	7.5
18:2n-6	32.6	22.5	33.1	23.1	10.8	7.5	8.4
18:3n-3	1.6	1.3	1.5	1.3	1.1	1.1	1.0
20:4n-6	0.6	0.8	1.0	0.9	1.2	1.1	1.0
20:5n-3	1.9^{d}	1.9^{d}	3.9^{bc}	4.1^{bc}	3.3^{c}	4.9^{b}	6.4^{a}
22:6n-3	3.0^{d}	6.4^{c}	7.9^{b}	5.5^{c}	10.1^{a}	9.6^{a}	7.4^{b}
ratio[1]	0.3^{d}	0.3^{d}	0.5^{c}	0.8^{b}	0.3^{d}	0.5^{c}	0.9^{a}
n-3HUFAs[2]	4.0^{d}	8.3^{c}	11.8^{abc}	9.6^{bc}	13.4^{ab}	14.5^{a}	13.8^{a}
$\sum$n-3[3]	5.6	9.6	13.3	10.9	14.4	15.6	14.8
$\sum$n-6[4]	32.2	23.3	34.1	24.0	12.0	8.6	9.4
$\sum$n-3/$\sum$n-6	0.2	0.4	0.4	0.5	1.2	1.8	1.6
$\sum$sat[5]	17.7	19.0	27.8	22.3	23.0	22.7	23.6

[1] EPA/DHA= eicosapentaenoic acid/ docosahexaenoic acid.

[2] The total percentage of EPA and DHA in diet.

[3] Groups of n-3 fatty acids are 18:3n-3, 20:5n-3 and 22:6n-3.

[4] Fatty acids in this group (n-6) are 18:2n-6, 20:2n-6 and 20:4n-6.

[5] Saturated fatty acids (sat) include 14:0, 16:0, 18:0, 20:0 and 24:0.

[a, b, c, d] Means (n=3) with different letters in row were significantly different (P<0.05).

could be noticed that levels of 18:2, 18:1 and 18:3 in diets 5-7 were less than those in diets 1-4.

The quantity of n-3 HUFAs in diets 5-7 was more than that in diets 1-4. Diets 1, 2 and 5 had the same ratio of EPA/DHA (0.3). Diets 3 and 6 had the EPA/DHA ratio of 0.5, and diets 4 and 7 had the ratio of 1. Saturated fatty acid content in each diet was not significantly different ($P>0.05$). It is expected that these saturated fatty acids would be used by shrimp as an energy source. Dietary n-3 HUFAs appear to enhance the utilization of dietary saturated fatty acids (Salhi et al., 1994). The digestibilities of saturated fatty acids generally decreased with increasing chain length up to C18 (Merican and Shim, 1994). Diets 1-4 contained the n-6 fatty acid more than diets 5-7 significantly ($P<0.05$), but diets 5-7 had higher ratio of n-3/ n-6 than diets 1-4 (Table 13).

Effect of fatty acids on growth and survival of *P. monodon*

Results of feeding experiment for 30 days in *P. monodon* are shown in Table 14. The effect of diet containing various levels of n-3 HUFAs on growth of *P. monodon* was significantly different ($P< 0.05$). The low final weight was obtained in postlarvae receiving diet 1 and diet 5, and the higher final weight of postlarvae was found in diet 4, diet 6 and diet 7. The postlarvae that fed the other diets gave no difference in weight. The weight of postlarvae fed on diet 4, diet 6 and diet 7 was superior to those fed on diet 1 and diet 5 significantly ($P<0.05$).

Table 14. Growth of *P. monodon* postlarvae fed diets containing different levels of n-3 HUFAs after 30 day feeding experiment[1]

Diet	Ratio[2]	%n-3 HUFAs[3]	Weight (mg)[4]	%Survival[5]
1*	1:3	0.35	184.9±100[d]	95±2.8
2	1:3	1.0	195.0±90[cd]	92±5.6
3	1:2	1.0	198.9±100[bcd]	91±3.2
4	1:1	1.0	210.4±110[abc]	85±3.7
5	1:3	1.5	180.2±110[d]	90±2.3
6	1:2	1.5	227.0±100[a]	92±5.5
7	1:1	1.5	219.6±110[ab]	89±3.5

[1] Each treatment group initially was consisted of 73 animals with 3 replicates.

[2] EPA/DHA = eicosapentaenoic/docosahexaenoic acids.

[3] Total amount (%, w/w) of EPA and DHA.

[4] Means±S.D of the final weight of postlarvae.

[5] Means±S.D of the survival of postlarvae.

[a, b, c, d] Means with different letters in column are significantly different ($P<0.05$).

* Control diet

The weight of postlarvae fed on 1% of n-3 HUFAs was likely to be increased with the increasing ratios of EPA/DHA in the diets. The ratio of EPA/DHA obtaining the highest weight was 1:1 (Figure 2). But in 1.5% n-3 HUFAs, the highest weight was found in EPA/DHA ratio of 1:2. At the same ratio, the weights of postlarvae fed on 1 %, 1.5 % of n-3 HUFAs showed no significant difference, though it tended to increase with an increasing content of n-3 HUFAs, exceptionally, the ratio 1:3, the weight of postlarvae was decreased when fed diets containing high amount of n-3 HUFAs.

In this experiment, *P. monodon* grew well on 0.35% of n-3 HUFAs diet 1 (control diet) with low content of n-3 HUFAs. The postlarvae were reported to be able to utilise body HUFAs for sometimes to substitute for dietary HUFAs (Sarac et al., 1993). For this reason, the percent survival of postlarvae fed on 0.35% of n-3 HUFAs was as good as that fed on higher contents of n-3 HUFA. It was found that not only the quantitative n-3 HUFAs that could improve growth but also other factors such as the ratio of EPA/DHA.

The effects of n-3 HUFAs dietary and ratio EPA/DHA on survival were not significantly different among treatments (Table 14).

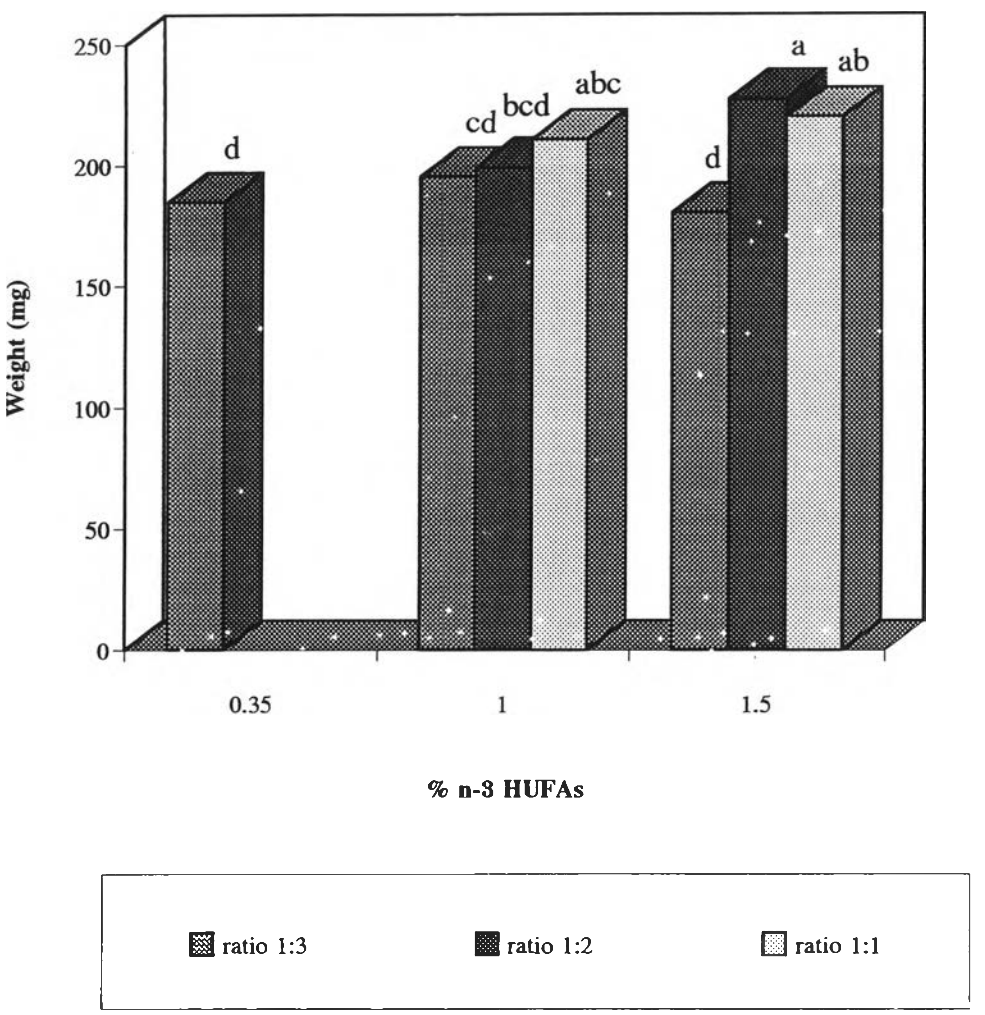

Figure 2. Effect of n-3 HUFAs and EPA/DHA ratio on the final weight of *P. monodon* postlarvae

Fatty acid of total lipids from the whole body of *P. monodon*

The influence of dietary fatty acids on the fatty acid composition of total, non-polar and polar lipids of shrimp tissue is shown in Tables 15, 16, and 17, respectively. Fatty acids of total lipids including 18:2, 16:0, 18:1 and 22:6 were main components in postlarvae that fed on diets 1-4 (Table 15). Postlarvae fed on diets 5-7 contained mainly 16:0, 22:6, 18:1, 18:0 and 20:5. However contents of fatty acids 16:0, 18:0, 18:1, 18:3, 20:1, 20:2 and 20:4 were not significantly different in all postlarvae (Table 15). Postlarvae fed on diets 1-4 had more 18:2 and 18:1 than those fed on diets 5-7 significantly ($P<0.05$). The quantity of n-6 fatty acids in all postlarvae was not different. But the ratio of n-3/n-6 fatty acid in the postlarvae fed diets 1-4 was less than those fed on diets 5-7.

The n-3 fatty acids were reported to be used for the biosynthesis of longer chain PUFAs to incorporate in tissue, whereas n-6 fatty acids were utilized as energy source (Sandifer and Joseph, 1976). In this experiment, the levels of 18:3 and saturated fatty acids in all postlarvae tissue were similar. Moreover, the levels of 22:6, n-3 HUFAs and Σn-3 in shrimp fed diet 6 were the highest, while these fatty acid levels were the lowest in shrimp fed diet 1.

The postlarval saturated fatty acid levels before and after experimental feeding were not significantly different ($P>0.05$). However, the fatty acid composition of the initial postlarvae had more 16:1 than those fed on diets 1, 2, 3 and 5, but it has similar 16:1 content to those fed on diets 4 and 7 (Table 15).

Table 15. Fatty acid composition of total lipids from the whole body of *P. monodon* postlarvae fed the experiment diets (mg/g dry weight)

Fatty acid	Diet no.							
	Initial*	1	2	3	4	5	6	7
14:0	0.3	0.2	0.4	0.4	0.5	0.4	0.6	0.6
16:0	5.7	7.5	9.7	10.7	9.8	8.5	8.4	7.0
16:1n-7	0.9	0.2	0.6	0.6	0.7	0.6	1.2	1.0
18:0	2.9	2.6	2.9	3.2	3.2	3.1	3.2	3.1
18:1n-9	5.3	5.4	5.1	6.2	5.6	3.7	4.1	3.9
18:2n-6	1.7	10.7	8.8	9.4	8.8	4.6	4.9	4.1
18:3n-3	4.1	0.3	0.3	0.3	0.3	0.4	0.4	0.3
20:0	0.2	0.1	0.3	0.3	0.3	0.3	0.4	0.3
20:1n-9	0.3	0.3	0.3	0.4	0.4	0.3	0.4	0.3
20:2n-6	-	0.3	0.3	0.4	0.4	0.4	0.4	0.2
20:4n-6	-	1.0	1.2	1.3	1.1	1.4	1.3	1.2
20:5n-3	3.7^{a}	1.6^{d}	2.6^{bc}	2.6^{bc}	2.9^{bc}	2.4^{c}	3.0^{bc}	3.3^{ab}
22:6n-3	ND	3.2^{c}	4.5^{ab}	4.5^{ab}	4.0^{bc}	4.9^{ab}	5.4^{a}	4.6^{ab}
ratio[1]	ND	0.5^{c}	0.58^{ab}	0.59^{ab}	0.72^{a}	0.5^{b}	0.6^{ab}	0.7^{a}
n-3HUFAs[2]	3.7^{c}	4.8^{c}	7.1^{b}	7.1^{b}	6.9^{b}	7.3^{ab}	8.4^{a}	7.9^{ab}
Σn-3[3]	7.8	5.1	7.4	7.4	7.2	7.7	8.8	8.2
Σn-6[4]	1.7	12.0	10.3	11.1	10.3	6.4	6.6	5.5
Σn-3/Σn-6	4.6	0.4	0.7	0.7	0.7	1.2	1.3	1.5
Σsat[5]	9.1	10.5	13.3	14.2	13.8	12.3	12.6	11.0

* Postlarvae was analysed fatty acid before feeding experiment. ; ND= not detected

[1] EPA/DHA= eicosapentaenoic acid/ docosahexaenoic acid.

[2] The total percentage of EPA and DHA in diet.

[3] Groups of n-3 fatty acids are 18:3n-3, 20:5n-3 and 22:6n-3.

[4] Fatty acids in this group (n-6) are 18:2n-6, 20:2n-6 and 20:4n-6.

[5] Saturated fatty acids (sat) include 14:0, 16:0, 18:0, 20:0 and 24:0.

[a, b ,c , d] Means (n=3) with different letters in row were significantly different ($P<0.05$).

The initial postlarvae had less 16:1 than postlarvae fed on diet 6. The initial postlarvae had higher 20:5n-3 than those fed on all diets significantly (P<0.05), because the initial postlarvae were fed with *Artemia* that had large amount of 20:5n-3. The results were similar to the study of O'Leary and Matthews (1990) that fed the postlarvae with *Skeletonema costatum, Chaetoceros sp.* containing low levels of 22:6n-3 and high levels of 20:5n-3.

The initial postlarvae are rich in palmitic acid and n-3 fatty acids, but poor in n-6 unsaturated fatty acids. These data imply that the fatty acids of the linolenic family are, perhaps, essential for *P. monodon* like *P. japonicus* (Guary, Kayama and Murakami, 1974).

Fatty acid of polar lipids from the whole body of *P. monodon*

Fatty acid composition of polar lipid (Table 16) was almost as the same as that of non-polar lipid and total lipid. But polar lipids has more n-3 HUFAs than non-polar lipid (Table 17). This experiment showed that dietary 20:5n-3 and 22:6n-3 were perferentially accumulated in polar lipid. It agreed well with the report in fish, although fish fed diets that contained no supplemental n-3 HUFAs (Lochmann and Gatlin III, 1993). Phospholipids are the major of polar lipid and they are also the main structural component of cell membranes and correct formation of these membranes is necessary in order that the cells may function effectively as osmoregulation, reproduction, nutrient assimilation and transport (O'Leary and Mathews, 1990; Borlogan and Benitez, 1992). The n-3 HUFAs has been known to be important in living, and postlarvae must conserve

Table 16. Fatty acid composition of polar lipids from the whole body of *P. monodon* postlarvae fed the experimental diets (mg/g dry weight)

Fatty acid	Diet no.						
	1	2	3	4	5	6	7
14:0	0.2	0.1	0.1	0.1	0.1	0.1	0.2
16:0	2.3	2.3	1.8	2.2	1.6	2.2	2.1
16:1n-7	0.2	0.2	0.1	0.2	0.2	0.2	0.4
18:0	0.8	0.8	0.7	0.8	0.6	0.8	0.7
18:1n-9	1.5	1.3	1.1	1.2	0.8	1.1	0.9
18:2n-6	2.8	2.1	1.7	2.1	1.2	1.3	1.1
18:3n-3	-	0.1	0.1	0.1	0.1	0.1	-
20:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
20:1n-9	0.1	0.2	0.1	0.1	0.2	0.1	0.1
20:2n-6	0.1	0.1	0.1	0.1	0.1	0.1	-
20:3n-6	-	-	-	0.9	0.1	-	-
20:4n-6	0.2	0.3	0.2	0.3	0.3	0.3	0.2
20:5n-3	0.4^{b}	0.6^{ab}	0.5^{ab}	0.7^{a}	0.5^{ab}	0.6^{ab}	0.6^{ab}
22:6n-3	0.7^{c}	1.0^{abc}	0.8^{abc}	1.1^{ab}	1.0^{abc}	1.2^{a}	0.7^{bc}
ratio[1]	0.61^{bc}	0.61^{bc}	0.64^{ab}	0.64^{ab}	0.52^{c}	0.54^{bc}	0.71^{a}
n-3 HUFAs[2]	1.1	1.6	1.3	1.8	1.5	1.8	1.3
$\sum$n-3[3]	1.1	1.7	1.4	1.9	1.6	1.9	1.3
$\sum$n-6[4]	3.1	2.5	2.0	3.4	1.7	1.7	1.3
$\sum$n-3/$\sum$n-6	0.4	0.7	0.7	0.6	0.9	1.1	1.0
$\sum$sat[5]	3.4	3.3	2.7	3.4	2.4	3.4	3.1

[1] EPA/DHA= eicosapentaenoic acid/ docosahexaenoic acid.

[2] The total percentage of EPA and DHA in diet.

[3] Groups of n-3 fatty acids are 18:3n-3, 20:5n-3 and 22:6n-3.

[4] Fatty acids in this group (n-6) are 18:2n-6, 20:2n-6, 20:3n-6 and 20:4n-6.

[5] Saturated fatty acids (sat) include 14:0, 16:0, 18:0, 20:0 and 24:0.

[a, b ,c , d] Means (n=3) with different letters in row were significantly different ($P<0.05$).

the n-3 HUFAs in polar lipid (Koven, Kissil and Tandler, 1989).

Koven et al. (1992) found that the EFA index (the ratio of 18:1n-9/ n-3 HUFAs in polar lipid fraction) was criterion EFA in marine species less than 1, indicating that it has adequate n-3 HUFAs in polar lipid of seabream. In this experiment, the EFA index was more than 1 only in diet 1, indicating that the n-3 HUFAs requirement should be inadequated for normal growth. But from the results, shrimps did not show any signs of lacking those fatty acids, i.e., growing slowly and having low survival rate. It might be that postlarvae could use other fatty acids in stead of n-3 HUFAs or that the 0.35 % of n-3 HUFAs may be adequated for growth of *P. monodon* postlarvae. Thus, the EFA index may not be applied for this experiment like in *Coregonas lavaretus maraena* (Thongrod et al., 1989).

All the experimental postlarvae did not have significant difference of 18:0 contents (Table 16). Moreover, postlarvae fed on diets 1-4 had more 18:1 and 18:2 than those fed on diets 5-7 (Table 16). In other hand, the 16:1 content of postlarvae feeding diets 1-4 was less than those fed on diets 5-7.

Fatty acid of non-plar lipids from the whole body of *P. monodon*

Fatty acid composition of non-polar lipid (Table 17) was nearly as the same as that of polar lipid and total lipid. But non-polar lipid has less n-3 HUFAs than polar lipid. Moreover, the ratio of EPA/DHA in non-polar lipid was not significantly different among treatments ($P>0.05$). Postlarvae

Table 17. Fatty acid composition of non-polar lipids from the whole body of *P. monodon* postlarvae fed the experimental diets (mg/g dry weight)

fatty acid	diet no.						
	1	2	3	4	5	6	7
14:0	-	0.1	0.1	0.1	0.1	0.2	0.1
16:0	0.8	1.3	1.0	1.3	1.0	1.2	1.2
16:1n-7	-	0.2	0.1	0.2	0.1	0.2	0.2
18:0	0.5	0.5	0.4	0.5	0.4	0.5	0.4
18:1n-9	0.7	1.1	0.9	1.0	0.6	0.7	0.6
18:2n-6	1.4	1.7	1.4	1.6	1.0	0.9	0.7
18:3n-3	-	0.1	0.1	0.1	0.1	0.1	-
20:0	-	0.1	0.1	0.1	-	trace	0.1
20:1n-9	-	0.1	-	0.1	-	0.1	-
20:2n-6	-	-	trace	0.1	-	trace	-
20:3n-6	-	0.1	-	-	0.1	-	-
20:4n-6	0.2	0.2	0.1	0.1	0.2	0.2	0.1
20:5n-3	0.1	0.2	0.2	0.3	0.2	0.3	0.3
22:6n-3	0.2	0.3	0.3	0.4	0.5	0.5	0.5
ratio[1]	0.56	0.49	0.69	0.85	0.43	0.52	0.70
n-3 HUFAs[2]	0.3	0.5	0.5	0.7	0.7	0.8	0.8
Σn-3[3]	0.3	0.6	0.6	0.8	0.8	0.9	0.8
Σn-6[4]	1.6	2.0	1.5	1.8	1.3	1.1	0.8
Σn-3/Σn-6	0.2	0.3	0.4	0.4	0.6	0.8	1
Σsat[5]	1.3	2.0	1.6	2.0	1.5	1.9	1.8

[1] EPA/DHA= eicosapentaenoic acid/ docosahexaenoic acid.

[2] The total percentage of EPA and DHA in diet.

[3] Groups of n-3 fatty acids are 18:3n-3, 20:5n-3 and 22:6n-3.

[4] Fatty acids in this group (n-6) are 18:2n-6, 20:2n-6, 20:3n-6 and 20:4n-6.

[5] Saturated fatty acids (sat) include 14:0, 16:0, 18:0, 20:0 and 24:0.

[a, b ,c , d] Means (n=3) with different letters in row were significantly different (P<0.05).

maintained the ratio of EPA/DHA only in polar lipid that is important in membranes.

However, in polar and non-polar lipids, the lowest n-3 HUFAs found in postlarvae fed on diet 1. Moreover, in this experiment, it was found that the levels of n-3 HUFAs and the ratio of EPA/ DHA in all the postlarvae reflected the levels of n-3 HUFAs and ratio of EPA/DHA in feeding diet. But postlarvae had generally higher levels of n-3 HUFAs than the diet.

Result of osmotic stress of *P. monodon*

After terminating the feeding experiments, *P. monodon* postlarvae were challenged to osmotic shock. There were marked differences in the ability of the postlarvae to survive from osmotic stress among the seven diets. Cumulative mortality index (CMI) was analyzed by probit analysis (SPSS-PC).

Median cumulative mortality (CM_{50}) of postlarvae in each treatment is shown in Table 18. It was found that postlarvae fed on diet 5 gave slow mortality, whereas those fed on diet 7 died gave a higher mortality.

Effects of n-3 HUFAs and ratio of EPA/DHA in diets on resistance to osmotic stress of *P. monodon* postlarvae are shown in Figure 3. At 1% n-3 HUFAs, the resistance of postlarvae that fed on diets containing an increasing ratio of EPA/DHA did not increase with the ratio. Furthermore, the resistance of

Table 18. CM_{50} of postlarvae fed each diet[1]

diet	CM_{50} (min)
1	86.36±7.69[bc]
2	89.13±5.52[bc]
3	92.54±3.62[ab]
4	88.25±1.84[bc]
5	103.77±7.22[a]
6	88.96±1.04[bc]
7	79.42±4.34[c]

[1] CM_{50} is the time when shrimp of each group has 50 % mortality after osmotic stress.

[a, b, c] Means (n=3) with different letters in column are significantly different ($P<0.05$).

postlarvae fed on diets containing an increasing ratio of EPA/DHA decreased at 1.5% n-3 HUFAs.

When the resistance of postlarvae that fed on 0.35%, 1%, 1.5% n-3 HUFAs was compared, it was not different at $P>0.05$ (Appendix C). It might be that the n-3 HUFAs affect postlarval osmotic stress more in early stages (PL5-PL15) than in later stages (PL-15). And PL-20 was used as an experimental animal, so the levels of n-3 HUFAs did not improve osmotic resistance of postlarvae. Whether this reflects changes in shrimp lipid metabolism or in their osmoregulatory physiology remains an open question (Ree et al., 1994).

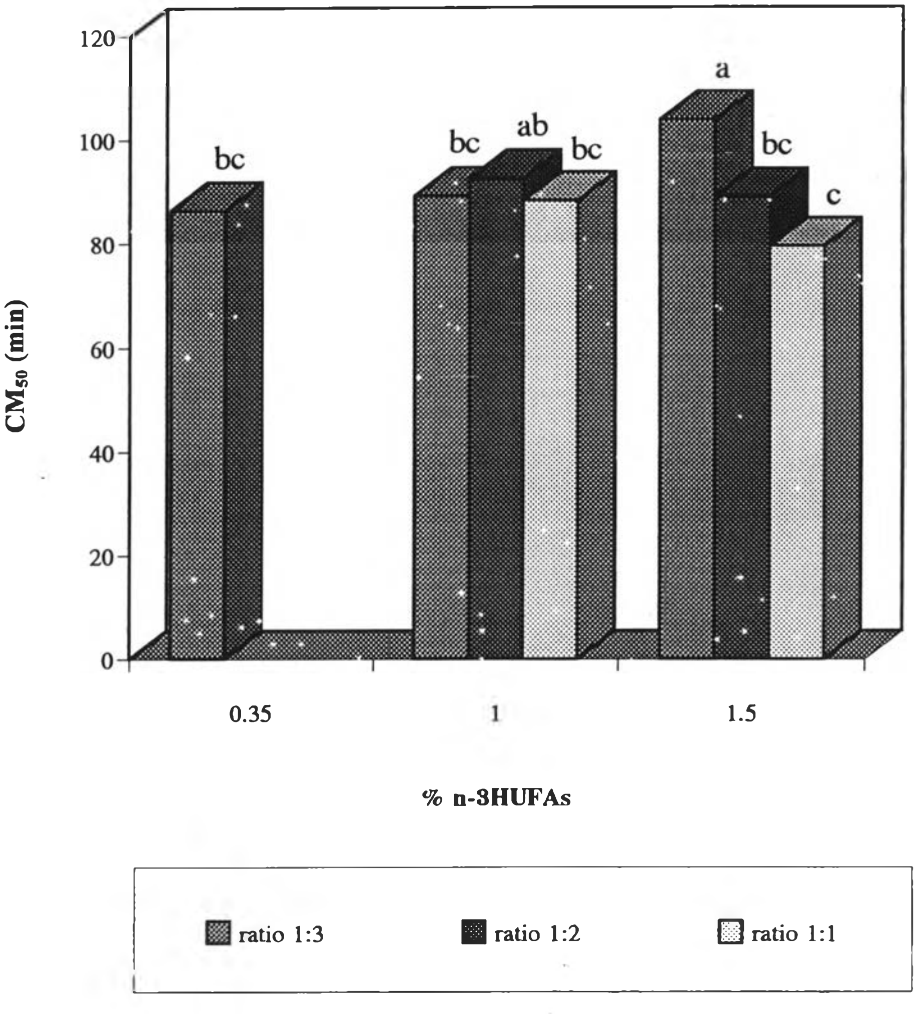

Figure 3. Effect of n-3 HUFAs and EPA/DHA ratio in diet on resistance to osmotic stress of *P. monodon* postlarvae

However, the ratio of EPA/DHA could improve resistance of postlarvae. By comparing the resistance of postlarvae (CM_{50}), it was found that shrimps fed diets containing the ratio of EPA/DHA 1:2, 1:1 had higher osmotic resistance those with the ratio of EPA/DHA 1:3. This mechanism underlying this protective effect of ratio of EPA/DHA on osmotic resistance in shrimp is not known. Erythrocytes of rats fed HUFA-rich cod river oil achieved a higher resistance to hypoosmotic shock, an effect which probably resulted from a higher incorporation of n-3 HUFAs in all membranes (Ree et al., 1994).

Considering the growth and the osmotic resistance of postlarvae (Figure 4), it was found that the growth of postlarave that fed on diets containing an increasing ratio of EPA/DHA at 1% n-3 HUFAs trended to increase, but the osmotic resistance did not increase. Whereas the osmotic resistance of those decreased at 1.5% n-3 HUFAs.

In summary, although the osmotic resistance of postlarvae fed on 1.5% n-3 HUFAs with the ratio of EPA/DHA at 1:2 was moderated, the growth was the highest. So diet containing 1.5% n-3 HUFAs with the ratio of EPA/DHA at 1:2 was suitable for improving growth.

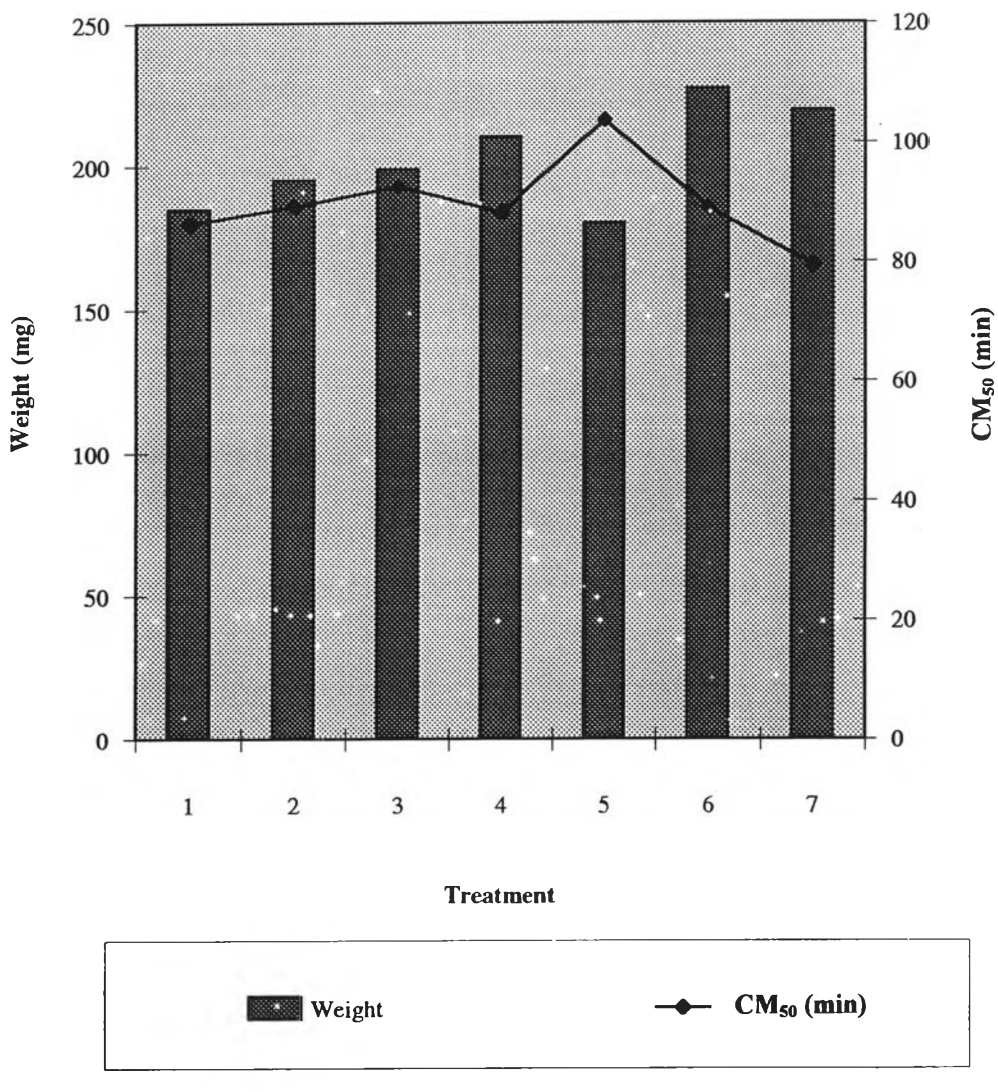

Figure 4. The final weight and resistance to osmotic stress of *P. monodon* postlarvae