CHAPTER IV

RESULTS AND DISCUSSION

Characterization of lipids in Lecithins

In the present study fish meal and soybean were used as sources of lecithins. Two grades of fish meals: Danish and Thai local fish meals employed in the study were supplied from the Department of Marine Science, Faculty of Science, Chulalongkorn University. Lecithins obtained from both sources contain high n-3 HUFA whereas soybean lecithin has high content of linoleic acid (18:2 n-6) and alpha-linolenic acid (18:3 n-3). Both fatty acids have been implicated as important to growth in lobsters and shrimps (Castell and Covey, 1976; Kanazawa et al. , 1977, 1979a). In addition, the soybean lecithin may act as phagostimulant (Thorsteinson and Nayar, 1963) or its presence may enhance uptake and solubilization of other nutrients within the diet (Lester et al., 1975).

Lecithin derived from soybean was brownish yellow, phopholipid content was 50% whereas those extracted from Danish fish meal and local fish meal were dark brown with phospholipid content of 25% and 20%, respectively. The picture of lecithins derived from soybean, Danish and local fish meal are shown in **Figure 5**.

Fatty acid in Oil portion of Lecithin

The concentrations of individual fatty acids and highly polyunsaturated fatty acid (HUFA; C 20:4 n-6, C 20:5 n-3, C 22:5 n-3, C 22:6 n-3) present in crude oil extracted from soybean, Danish and local fish meal are shown in **Tables 3, 5** and 7. Among three lecithins, those of Danish fish meal contains the highest HUFA content. This advantage is more apparent when DHA and EPA are taken into account since Danish fish meal lecithin contains both fatty acids the highest (DHA: 21.68 vs 19.25 vs 0 g/ 100 g; EPA: 7.51 vs 6.40 vs 0 g/ 100g for Danish, local fish meal and soybean lecithins, respectively).

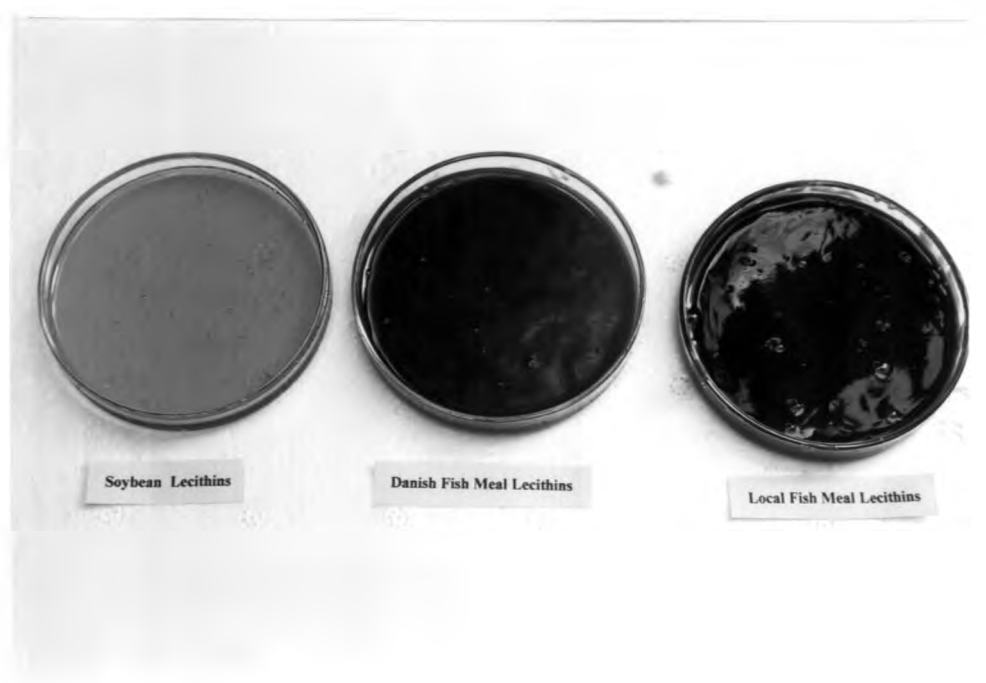

Figure 5 Soybean lecithin (left) was supplied from the commercial manufacturer whereas Danish fish meal lecithin (middle) and local fish meal lecithin (right) were prepared at FORC as described in the text.

Fatty Acid Moieties of Triglycerides and Phospholipids

The fractions of triglycerides (TG) or triacylglycerols and phospholipids (PL), the latter so-called in this experiment as lecithin, were purified from crude oils by chromatographic technique of one– dimentional TLC as described earlier. The actual concentrations of individual and total HUFA of TG and PL fractions of all lecithins employed in the study are shown in **Tables 3 , 5** and 7. The total concentration of HUFA was found in Danish fish meal lecithin higher than other lecithins. Among those HUFA, DHA is the highest (for TG fraction: 17.56 vs 8.38 vs 0 g/100 g and for PL fraction: 28.32 vs 12.40 vs 0 g/ 100g for DHA in Danish, local fish meal and soybean lecithins, respectively). Furthermore, EPA is also highest in Danish fish meal lecithin (for TG fraction: 7.32 vs 3.62 vs 0 g/100g; for PL fraction: 7.85 vs 3.47 vs 0 g/100 g for EPA in Danish, local fish meal and soybean lecithins, respectively).

Thus Danish fish meal lecithin shows its prominent character of having highest HUFA content especially EPA and DHA compared to other lecithins.

Fatty Acid Composition in Fish Oils

The composition of fatty acid in fish oil used as fat source in the shrimp's diets is shown in **Table 9**. Compared to Danish fish meal lecithin, local fish meal lecithin and soybean lecithin, it found that total HUFA of fish oil was the highest (37.18 vs 33.78 vs 31.43 vs 0 g/100g for fish oil, Danish fish meal lecithin, local fish meal lecithin and soybean lecithin, respectively).

Table 3 Fatty acid composition of total lecithin derived from soybean as well as those after separating into triglyceride and phospholipid fractions.

Fatty acid	Soybean lecithin		
	Total lecithin	TG fraction	PL fraction
C 16:0	17.21 ± 2.26	13.94 ± 1.68	20.59 ± 0.31
C 18:0	3.88 ± 0.27	4.19 ± 4.25	4.26 ± 4.21
C 18:1	15.49 ± 0.43	25.03 ± 2.61	10.96 ± 0.55
C 18:2 n-6	55.59 ± 1.28	50.57 ± 2.60	57.00 ± 0.67
C 18:3 n-3	7.81 ± 0.26	6.28 ± 1.44	7.17 ± 0.24

The data are expressed as g/100 g total fatty acids.

The results of individual fatty acids are Mean ± S.D. of three determinations.

Table 4 Saturated, monoenes and polyenes fatty acids in total lecithin, TG fraction and PL fraction of soybean (g/100 g total fatty acid)

Fatty acid	Soybean lecithin		
	Total lecithin	TG fraction	PL fraction
Saturated	21.09 ± 1.99	18.14 ± 1.53	24.85 ± 0.42
Monoenes	15.49 ± 0.43	25.09 ± 2.61	10.96 ± 0.55
Polyenes	63.40 ± 1.56	56.83 ± 4.04	64.19 ± 0.93
n-3	7.81 ± 0.26	6.28 ± 1.44	7.17 ± 0.24
n-6	55.59 ± 1.28	50.57 ± 2.60	57.00 ± 0.67
n-3/n-6	0.141 ± 0.002	0.123 ± 0.022	0.126 ± 0.003

The results of fatty acids are expressed as Mean ± S.D. of three determinations.

Table 5 Fatty acid composition of total lecithin derived from Danish fish meal as well triglyceride and phospholipid fractions of total lecithins.

Fatty acid	Danish fish meal lecithin		
	Total lecithin	TG fraction	PL fraction
C 14:0	4.94± 0.21	6.18± 0.50	1.93± 0.18
C 16:0	24.40± 0.52	25.17±0.83	21.55± 0.67
C 16:1 n-7	4.36± 0.17	5.33± 0.21	1.85± 0.12
C 18:0	8.18± 0.16	6.82± 0.23	9.25± 0.19
C 18:1 n-9	11.49± 0.47	12.78± 0.74	9.80± 0.57
C 18:1 n-7	2.96± 0.15	2.56± 0.14	2.99± 0.21
C 18:2 n-6	2.12± 0.04	2.55± 0.09	1.97± 0.06
C 18:3 n-3	1.03± 0.05	1.48± 0.06	0.47± 0.07
C 20:4 n-6	2.93± 0.25	2.41± 0.30	3.72± 0.29
C 20:5 n-3	7.51± 0.36	7.32± 0.13	7.85± 0.24
C 22:5 n-3	1.66± 0.11	1.52± 0.13	1.85± 0.22
C 22:6 n-3	21.68± 0.65	17.56± 0.96	28.32± 0.73
C 24:1	1.57± 0.09	1.34± 0.14	1.73± 0.11
Others	5.17± 0.36	6.98± 0.76	6.72± 0.44
$\sum$ HUFA*	33.78±0.43	28.80± 0.65	41.74±1.04

The data are expressed as g/100g total fatty acids.

The results of individual fatty acids are Mean ± S.D. of three determinations.

*Sum of C 20:4 n-6, C 20:5 n-3, C 22:5 n-3, C 22:6 n-3

Table 6 Saturated, monoenes and polyenes fatty acids in lecithin, TG fraction and PL fraction of Danish fish meal (g/100 g total fatty acids).

Fatty acid	Danish fish meal lecithin		
	Total lecithin	TG fraction	PL fraction
Saturated	37.52 ± 0.63	38.17 ± 0.97	32.73 ± 0.75
Monoenes	20.38 ± 0.48	22.01 ± 0.83	16.37 ± 0.64
Polyenes	36.93 ± 0.77	32.84 ± 1.15	44.18 ± 0.93
n-3	31.88 ± 0.75	27.88 ± 1.07	38.49 ± 0.87
n-6	5.05 ± 0.31	4.96 ± 0.25	5.69 ± 0.19
n-3/n-6	6.31 ± 0.08	5.62 ± 0.15	6.76 ± 0.09

The results of fatty acids were expressed as Mean ± S.D. of three determinations.

Table 7 Fatty acid composition of total lecithin derived from local fish meal and those separated into triglyceride and phospholipid fractions.

Fatty acid	Local fish meal lecithin		
	Total lecithin	TG fraction	PL fraction
C 14:0	3.82 ± 0.23	9.88 ± 5.88	4.27 ± 1.18
C 15:0	0.90 ± 0.001	1.50 ± 0.62	1.22 ± 0.30
C 16:0	26.42 ± 0.82	34.60 ± 1.17	36.28 ± 5.93
C 16:1 n-7	4.39 ± 0.08	5.33 ± 2.44	3.51 ± 0.53
C 18:0	10.46 ± 0.12	9.34 ± 3.05	12.11 ± 0.40
C 18:1 n-9	6.54 ± 0.11	8.57 ± 0.70	7.89 ± 0.63
C 18:1 n-7	2.55 ± 0.03	2.35 ± 0.56	2.62 ± 0.25
C 18:2 n-6	1.85 ± 0.78	6.09 ± 2.60	1.67 ± 0.17
C 18:3 n-3	0.45 ± 0.09	0.61 ± 0.35	trace
C 20:4 n-6	3.99 ± 0.05	2.08 ± 1.30	2.80 ± 0.82
C 20:5 n-3	6.40 ± 0.23	3.62 ± 0.84	3.47 ± 0.98
C 22:5 n-3	1.80 ± 0.08	0.68 ± 0.40	0.79 ± 0.53
C 22:6 n-3	19.25 ± 0.90	8.38 ± 7.79	12.40 ± 6.19
C 24:1	0.87± 0.11	0.92 ± 0.16	1.09 ± 0.85
Others	1.91 ± 1.81	1.48 ± 1.06	1.97 ± 1.76
∑ HUFA*	31.43 ± 1.11	14.77 ± 10.31	19.46 ±8.49

The data are expressed as g/100 g total fatty acids

The results of individual fatty acids are Mean ± S.D. of three determinations.

*Sum of C 20:4 n-6, C 20:5 n-3, C 22:5 n-3, C 22:6 n-3

Table 8 Saturated, monoenes and polyenes fatty acids in lecithin, TG fraction and PL fraction of local fish meal (g/100 g total fatty acids)

Fatty acids	Local fish meal lecithin		
	Total lecithin	TG fraction	PL fraction
Saturated	41.60 ± 0.92	55.32 ± 4.16	53.89 ± 7.25
Monoenes	14.34 ± 341	17.17 ± 1.96	15.11 ± 0.79
Polyenes	33.73 ± 0.94	21.47 ± 7.39	21.13 ± 8.62
n-3	27.90 ± 1.10	13.30 ± 8.68	16.66 ± 7.69
n-6	5.84 ± 0.74	8.17 ± 1.30	4.47 ± 0.97
n-3/n-6	4.84 ± 0.69	1.79 ± 1.48	3.61 ± 0.91

The result of fatty acids were expressed as Mean ± S.D. of three determinations.

Table 9 Fatty acid composition of fish oil (data are expressed as g/100 g total fatty acids).

Fatty acid	g/100 g total fatty acids
C 14:0	4.40 ± 0.33
C 15:0	1.12 ± 0.05
C 16:0	22.34 ± 1.24
C 16:1 n-7	5.22 ± 0.33
C 18:0	6.04 ± 0.11
C 18:1 n-9	13.13 ± 0.13
C 18:1 n-7	2.64 ± 0.28
C 18:2 n-6	1.45 ± 0.03
C 18:3 n-3	0.80 ± 0.05
C 20:0	1.12 ± 0.01
C 20:4 n-6	2.15 ± 0.15
C 20:5 n-3	6.72 ± 0.36
C 22:5 n-3	1.49 ± 0.01
C 22:6 n-3	26.82 ± 1.43
C 24:1	0.45 ± 0.14
Others	4.11 ± 0.13
$\sum$ HUFA *	37.18 ± 1.93

The results of individual fatty acids are Mean ± S.D. of three determinations.

* Sum of C 20:4 n-6, C 20:5 n-3, C 22:5 n-3, C 22:6 n-3.

Table 10 Saturated, monoenes and polyenes fatty acids in fish oil (g/100 g total fatty acids)

Fatty acids	g/100 g total fattyacids
Saturated	35.02 ± 1.62
Monoenes	21.44 ± 0.56
Polyenes	39.42 ± 1.97
n-3	35.83 ± 1.84
n-6	3.60 ± 0.14
n-3/n-6	9.96 ± 0.23

The results of fatty acids were expressed as Mean ± S.D. of three determinations

Lecithin in the Shrimp ' s Diets

A. Physical properties of Shrimp ' s Diets

Four diets: soybean lecithin – added diet (SAD), Danish fish meal lecithin – added diet (DAD), local fish meal lecithin – added diet (LAD) and lecithin-free diet or control diet (CD) were prepared for the present experiment. The color of diets varies from yellowish to orange depending on the source of lecithin (**Figure 6**). It was found that all shrimp's diets suspending in water did not change their appearance during 1 hour as shown in **Figure 7.**

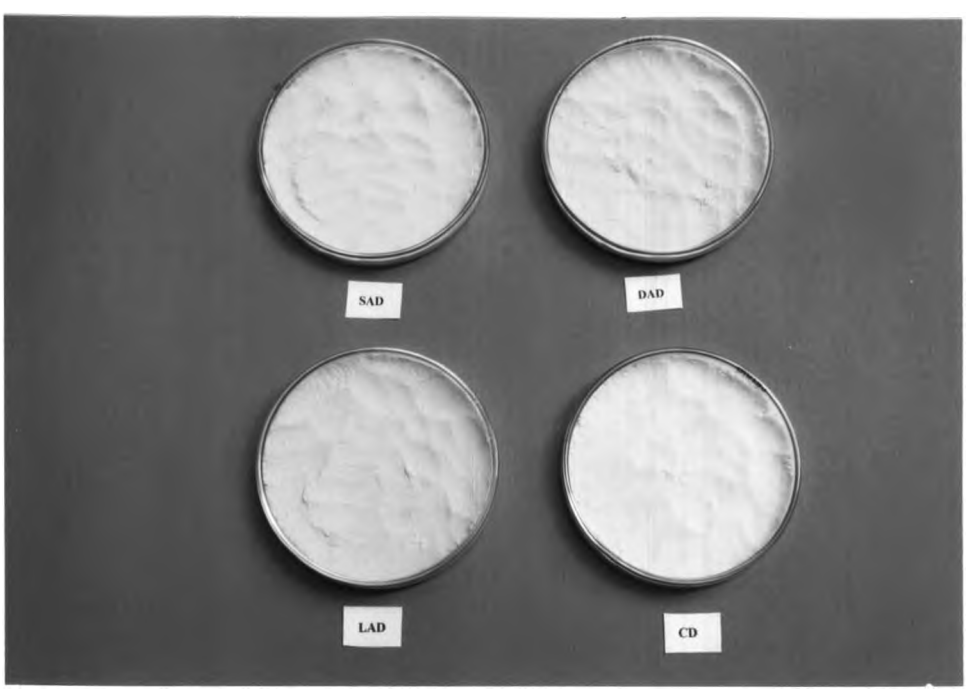

Figure 6 Microparticulated diets for shrimp larva ; soybean lecithin –added diet (SAD), Danish fish meal lecithin added diet (DAD), local fish meal lecithin - added diet (LAD) , control diet (CD).

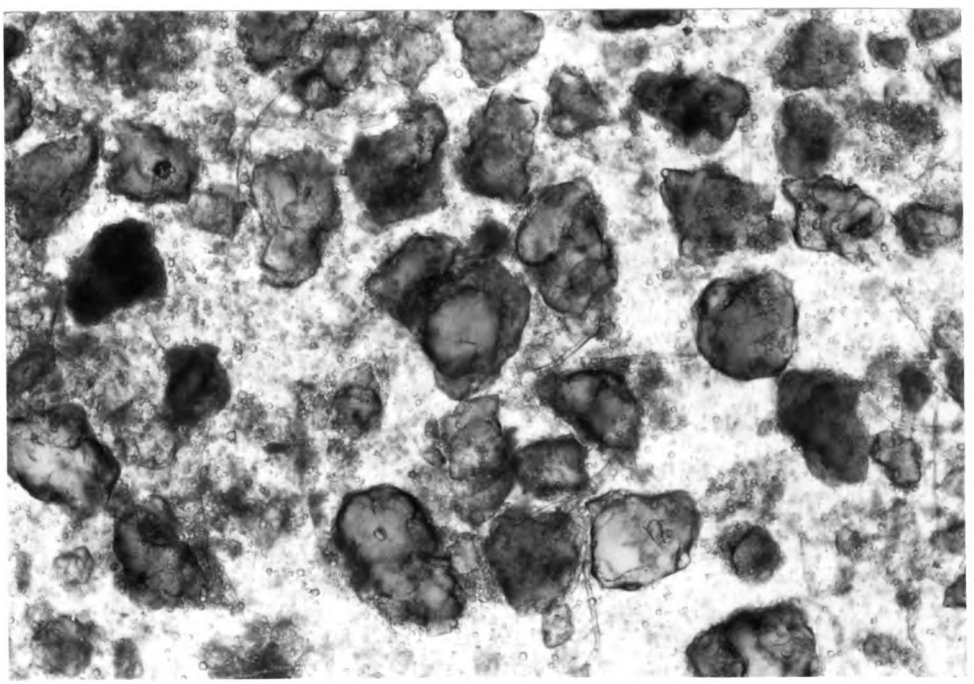

Figure 7 Appearance of shrimp's diets after 1 hour suspended in water (163x by objective lens 4x, photo-eyepiece 3.3x, magnification 12.3x)

B. Fatty acid composition in Shrimp's Diets

Shrimp ' s diets were analysed and shown in **Table 11.** DAD contains HUFA higher than those of other diets (28.74 in comparison to 23.81, 21.32, 19.91 g/100 g for SAD, LAD, CD, respectively). This high content is prominent especially among two fatty acids of interest, DHA and EPA (DHA: 19.97 in comparison to 16.90, 14.39, 13.25 g/100 g; EPA: 5.94 in comparison to 4.64 , 4.57 , 4.56 g/100g for SAD, LAD, CD, respectively).

Paibulkichakul (1996) reported that the quantity of lecithin for best survival and growth of *P. monodon* larvae was found as 1.5 g/100 g of diet. Therefore, the present study employed similar content of lecithin. One and a half per cent of lecithins from 3 sources was supplemented for the preparation of 3 diets (**Table 11**). Regarding n-3 HUFA concentration, DAD contains the highest followed by SAD, LAD and CD. Despite of the absence of lecithin, CD still contains n-3 HUFA derived from fish oil as previously shown in **Table 9**. Actually, refined fish oil used in CD supplied n-3 HUFA to this lecithin-free diet which explain that why its content of these crucial fatty acids similar to those found in LAD (19.91 in comparison to 21.32 g)

Effect of lecithin on Survival and Growth of *Penaeus monodon* Larvae at salinity 25 and 30 ppt

A. Survival rate of larvae

Survival rate of shrimp larva (3 stages): zoea , mysis and postlarva 15 fed with different diet and growth in different salinity are shown in **Tables 13, 14** and **Figures 8, 9.** At salinity of 30 ppt, survival rate of zoea fed with all diets was significant higher than that of zoea at 25 ppt. This might be explained by salinity 30 ppt which is the salinity closed to broodstock acclimation in hatchery. In nature, young stages of shrimp live in open sea where its salinity is as high as 35 ppt. Salinity 25 ppt is too low for shrimp zoea to survive in comparison to 30 ppt. However, this finding on survival rate of different salinity was not observed in mysis and postlarva stages. This might be explained by the fact that free-living, young shrimp stages can migrate from the open sea to the lower salinity area of esturine or coast. Mysis and postlarvae shrimp may adapt to survive in low salinity better than zoea stages. This

might be explained by the higher health strength of both latter stages after zoea. The calculation on this matter is shown in detail in Appendix II.

Table 11 Fatty acid composition of shrimp's diets.

Fatty acid	SAD	DAD	LAD	CD
C 14:0	4.82 ± 0.16	5.14 ± 0.15	8.49 ± 1.26	9.32 ±1.27
C 15:0	1.08 ± 0.06	1.21 ± 0.03	1.64 ± 0.21	1.72 ± 0.15
C 16:0	23.81 ± 0.48	25.18 ± 0.18	31.88 ± 3.88	31.24 ±2.24
C 16:1 n-7	4.61 ± 0.01	5.38 ± 0.04	6.57 ± 0.52	6.86 ± 0.77
C 18:0	5.75 ± 0.15	6.80 ± 0.38	6.51 ± 0.35	6.18 ± 0.29
C 18:1 n-9	14.05 ± 0.38	14.31 ± 0.63	13.10 ± 0.03	13.99 ± 0.20
C 18:1 n-7	2.14 ± 0.04	2.23 ± 0.39	2.21 ± 0.06	2.22 ±0.12
C 18:2 n-6	12.92 ± 0.24	2.95 ± 0.81	2.57 ± 0.18	2.58 ± 0.08
C 18:3 n-3	1.69 ± 0.18	0.55 ± 0.08	0.45 ± 0.04	0.76 ± 0.01
C 20:0	0.58 ± 0.06	0.62 ± 0.16	0.77 ± 0.17	0.51 ± 0.24
C 20:4 n-6	1.41 ± 0.21	1.69 ± 0.53	1.47 ± 0.38	1.37 ± 0.14
C 20:5 n-3	4.57 ± 0.42	5.94 ± 0.68	4.64 ± 0.76	4.56 ± 0.53
C 22:5 n-3	0.93 ± 0.07	1.14 ± 0.25	0.82 ± 0.17	0.73 ± 0.05
C 22:6 n-3	16.90 ± 0.05	19.97 ± 2.04	14.39 ± 3.76	13.25 ±2.37
Others	5.15 ± 0.64	6.24 ± 0.47	4.51 ± 0.11	5.08 ± 0.61
∑ HUFA*	23.81 ± 0.68	28.74 ± 1.92	21.32 ± 5.08	19.91 ± 2.91

The data are expressed as g/100 g of total fatty acids in diet's fats.

The results of individual fatty acids are Mean ± S.D. of three determinations.

* Sum of C 20:4 n-6, C20:5 n-3, C 22:5 n-3, C 22:6 n-3.

Table 12 Saturated, monoenes and polyenes fatty acids in shrimp's diet (g/ 100 g total fatty acid).

Fatty acid	Shrimp's diets			
	SAD	DAD	LAD	CD
Saturated	36.04 ± 0.37	38.95 ± 0.48	49.28 ± 4.83	48.97 ± 3.37
Monoenes	20.80 ± 0.42	21.96 ± 1.05	21.87 ± 0.49	23.07 ± 0.53
Polyenes	38.42 ± 0.63	32.24 ± 1.15	24.34± 5.21	23.25 ± 2.89
n-3	24.09 ± 0.65	27.60 ± 2.03	20.30 ± 4.66	19.30 ± 2.95
n-6	14.33 ± 0.07	4.66 ± 0.90	4.04± 0.56	3.96 ± 0.08
n-3/n-6	1.68 ± 0.05	6.16± 1.58	4.99 ± 0.43	4.88 ± 0.82

The results of fatty acids were expressed as Mean ± S.D. of three determinations.

Table 13 Percentage survival of larval stages fed with different diets at salinity of 25 ppt

	Survival of larval stages (%)		
Diets	Protozoea	Mysis	Postlarva
SAD	15.1± 26.51	56.7 ± 15.14	58.7 ± 23.18
DAD	25.4± 26.20	67.3 ± 20.50	21.3 ± 18.90
LAD	24.0± 27.07	62.7 ± 6.81	39.3 ± 9.45
CD	36.1± 14.07	69.0 ± 15.39	38.7 ± 11.37

Table 14 Percentage survival of larval stages fed with different diets at salinity of 30 ppt

	Survival of larval stages (%)		
Diets	Protozoea	Mysis	Postlarva
SAD	70.6 ± 15.93	52.7 ± 9.24	66.0 ± 23.07
DAD	49.1 ± 9.66	65.0 ± 13.08	53.3 ± 6.11
LAD	65.7 ± 7.34	75.0 ± 13.08	44.0 ± 24.33
CD	46.0 ± 20.64	58.0 ± 5.00	42.7 ± 11.37

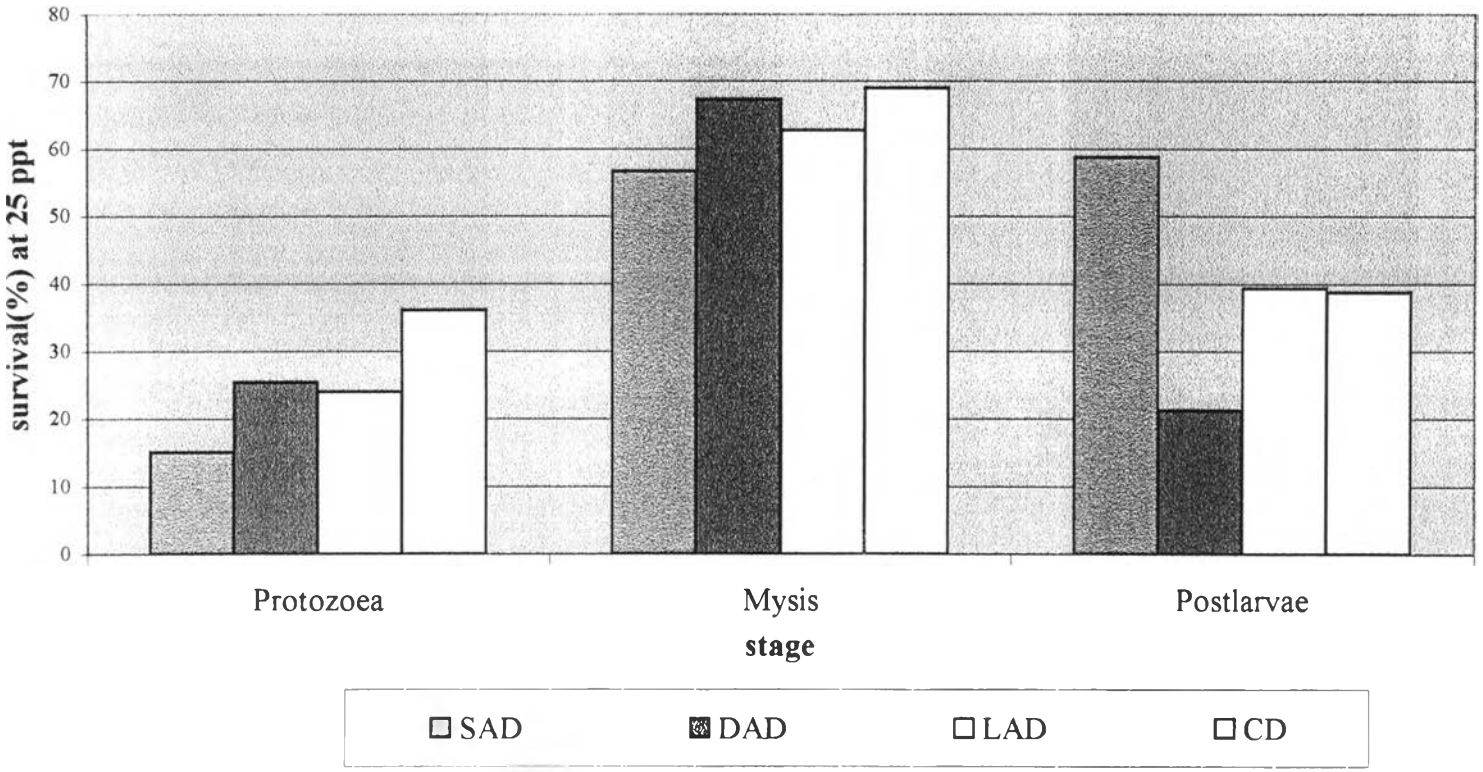

Figure 8 Percent survival of larval stages fed different diets at salinity 25 ppt.

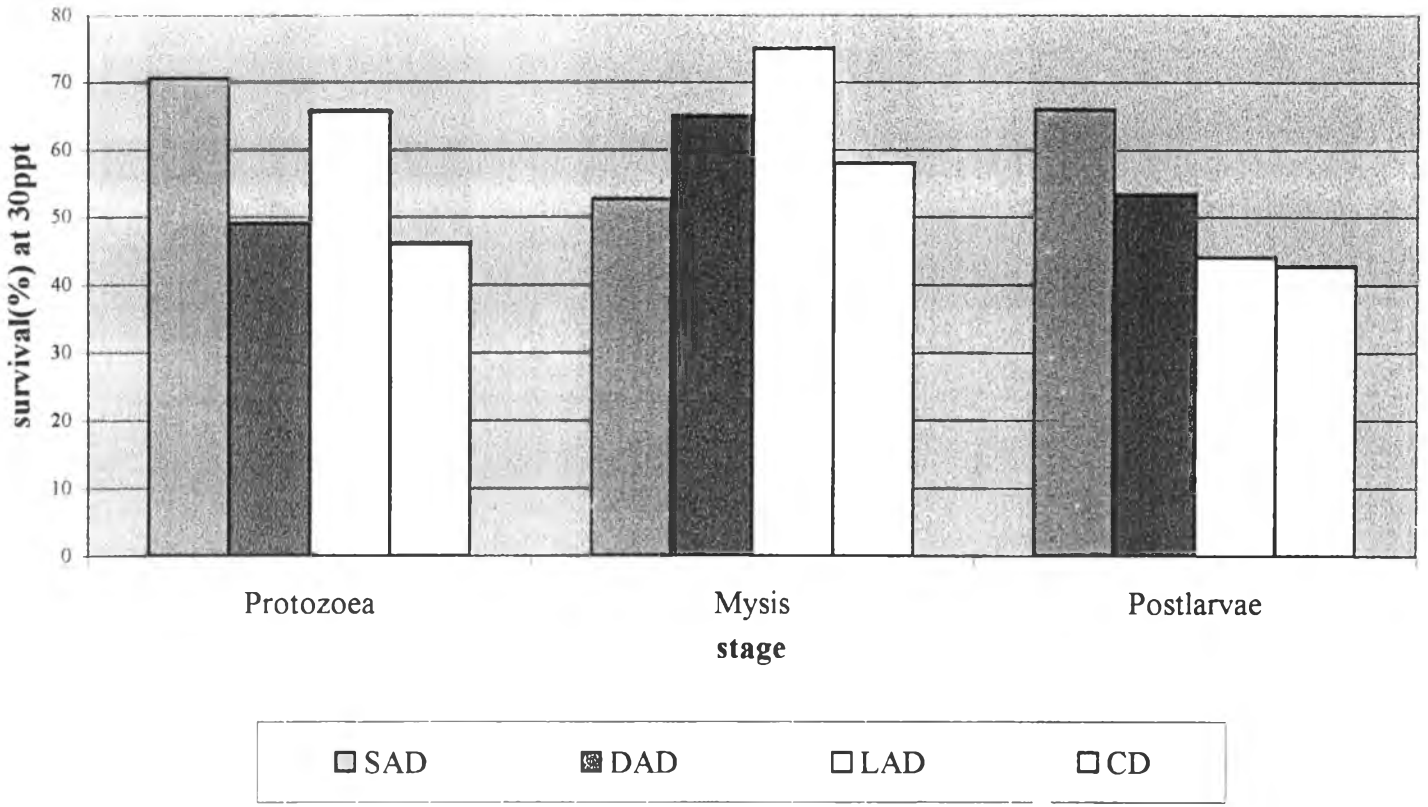

Figure 9 Percent survival of larval stages fed different diets at salinity 30 ppt.

B. Growth of postlarvae fed with different diets at salinity 25 and 30 ppt

The growth in term of total length of postlarva 15 fed diets with different sources of lecithin were shown in **Tables 15** and **16.** At salinity 25 ppt, the length of the larvae fed with SAD was significantly longer than those of larvae fed DAD and LAD. It is well established that essential fatty acids: linoleic and alpha-linolenic acids play the crucial role on growth and development (Dahlan 1989). High content of both fatty acids present in SAD in comparison to those of all other diets as shown in **Table 11** thus possibly explain this phenomenon. However, at salinity 30 ppt the larvae fed with LAD, DAD have similar length but not significantly different from larvae fed SAD. Noticeably, larvae fed CD at salinity 25 ppt has its length not different from larvae fed other diets. It indicates that the fortification of lecithin into shrimp culturing diet could be omitted at low salinity level. Statistic analysis of these data is shown in Appendix II.

Table 15 Length of postlarva 15 fed with different diets at salinity 25 ppt.

Diets	Length of PL 15 (mm)
SAD	$11.419^{a} \pm 1.29$
DAD	$10.812^{b} \pm 0.94$
LAD	$10.631^{b} \pm 1.11$
CD	$11.060^{ab} \pm 0.89$

Values are Mean ± S.D.

Means with different superscripts are significantly different at $P < 0.05$

Table 16 Length of postlarva 15 fed with different diets at salinity of 30 ppt.

Diets	Length of PL 15 (mm)
SAD	$11.042^{a} \pm 0.99$
DAD	$10.862^{ab} \pm 0.82$
LAD	$11.158^{a} \pm 0.77$
CD	$10.551^{b} \pm 1.76$

Values are Mean ± S.D.

Means with different superscripts are significantly different at $P < 0.05$

Eicosapentaenoic (20:5 n-3) and docosahexaenoic (22:6 n-3) acids have been considered as essential substances for penaeid nutrition and physiology and must be supplied in the diets of several penaeid species such as *P. japonicus* (Kanazawa et al.,1979a), *P. indicus* (Read, 1981) and *P. chinensis* (Xu et al., 1994).

Although crustaceans have ability to synthesize phospholipid de novo (Shieh,1969), the slow rate of endogenous biosynthesis of phospholipid may explain the requirements of this lipid from exogenous source particularly from diet (D'Abramo et al., 1981, Kanazawa et al., 1985).

The present study confirms the previous observation performed in our research unit that diet supplemented with soybean lecithin promoted the highest growth rate compared to other shrimp diets. Additionally, diet and salinity may cooperate in facilitating the growth. At salinity of 25 ppt, soybean lecithin promoted the highest growth whereas at salinity of 30 ppt the similarity of growth promotion are shown among lecithin supplemented diets and significantly higher than that of lecithin-free diet. At this point, we reconfirmed the advantage of lecithin supplementation in diet for *P. monodon* larval culture especially at high salinity (> 25 ppt). Nevertheless, lecithin-free diet is an alternative at low salinity. Regarding survival rate as shown in **Tables 13 and 14**, both salinity and the presence of lecithin did not show any effect, however, at high salinity zoea fed with all diets shows apparently significantly greater survival rate than those of the low salinity one.

The proportion of lecithin in diet has to be contemplated. Chen et al.(1991) employed purified phosphatidylcholine derived from soybean lecithin and found that it could improve growth and survival in *P. penicillatus*. In several experiments, Chen fortified soybean phosphatidylcholine with 80% purity into shrimp diet and found that the appropriate lecithin proportion for promoting significant growth of *P. monodon* juvenile and *P. penicillatus* was 1.25% (Chen 1993, Chen and Jenn, 1991). Coutteau

et al. employed pure phosphatidylcholine content of 1.5% for the diet to maximize growth of *P. vannamei* postlarval under similar experimental conditions (Coutteau et al, 1996). Kanazawa et al. (1985) criticized that the subclass of phospholipids could yield different effects. Among them, soybean phosphatidylcholine and phosphatidylinositol were the most effective in promoting growth and survival of larval *P. japonicus*. They also mentioned a possible interaction between dietary soybean lecithin and n-3 HUFA for survival and growth of larval *P. japonicus*. In addition, D'Abramo et al. (1981) exhibited that refined soy phosphatidylcholine was effective in reducing *Homerus americanus* mortality than bovine phosphatidylethanolamine, soy-phosphatidylinositol and non-phospholipids. Hitherto, the benefit of lecithin fortification in shrimp diet has been well recognized. The explanation for the advantage of exogenous or dietary phospholipid once located inside the shrimp's body may due to a more efficient transport and utilization of neutral lipids expressing by better lipid mobilization, which results in enhanced lipid deposition as well as an increase of the energy available for growth (Teshima et al, 1986c; Kontara et al, 1997).

Regarding the fatty acid species present in diet, Merican et al., (1996) demonstrated the benefit of adding 22:6 n-3 and 20:5 n-3 in diet on enhancing the growth of *P. monodon* juvenile. Kontara et al. (1997) demonstrated a higher growth and survival of *P. japonicus* postlarvae fed with 1% n-3 HUFA compared to those fed with the diet absent of HUFA. Lim et al. (1997) confirmed the essentiality of both n-6 and n-3 fatty acids for juvenile *P. vannamei* and manifested that n-3 fatty acids promoted growth faster than those of n-6. Among n-3 fatty acids, HUFA (20:5 n-3 and 22:6 n-3) provided better growth promoting effect than 18:3 n-3 due probably to the limited ability of shrimp to bioconvert fatty acids to polyenoic forms of longer chain length or upper derivatives. Kanazawa et al (1978, 1979e) strengthened

this fact by showing in their investigations that 20:5 n-3 and 22:6 n-3 provided better growth of *P. japonicus*. The substantiation of HUFA benefit was also performed in *P. indicus* by Read (1981).

Several studies which document the fatty acid composition of the organs of different shrimp species have demonstrated that, throughout ovarian maturation, ovarian lipid contained higher proportions of 20:5 n-3 and 22:6 n-3 than the hepatopancreas (Teshima and Kanazawa, 1983; Jeckel et al, 1989; Ji and Xu, 1992). Mourente et al. (1990) showed that 65% of the fatty acids of the total ovarian lipids are incorporated into egg and embryos during ovarian maturation of *P. kerathurus*. Xu et al .(1994) showed that the content of 20:5 n-3 in the egg exhibits a close correlation with the hatching rate of the egg of *P. chinensis*. These relationships suggest that 20:5 n-3 may play some specific role in ovarian development process relating to fecundity, whereas 22:6 n-3 may play some other role in early embryogenesis which is related to egg hatchability of *P. chinensis* larval. It has been known that the growth and development of the central nervous system is a critical component of embryonic development of all animals. The n-3 HUFAs, especially 22:6 n-3, have also been shown to be critical in brain and neurological development during embryogenesis of fish (Mourente and Tocher,1992; Tocher et al.,1992) as well as mammals (Neuringer et al.,1986; Kanazawa et al., 1991; Bourre et al., 1992) and is also certained to be a critical factor in the embryonic development of crustaceans. These aspects of the role of 22:6 n-3 in embryonic development may help account for the apparent relationship between 22:6 n-3 content in the egg and hatchability.

C. Tolerance of postlarva 15 fed with different diets, two salinity on stress test

Postlarva 15 was tested for their tolerance employiong sudden salinity stress test by transferring them from natural salinity level of 30 ppt to hypotonic shock level at 0 ppt and keeping there for 2 h. The similar test was performed also at 25 ppt transferred to 0 ppt. The percent survival of larvae on the stress test was recorded and shown in **Figures 10** and **11**. At salinity of 0 ppt, the decease of larvae was found at 25 minutes for the transferred larvae fed all diets at 25 ppt. At time 120 minutes, the highest number of dead shrimp was found in the larvae fed CD, followed by the larvae fed LAD, SAD, DAD (**Figure 10**). For salinity of 30 ppt, the decease of larvae fed all diets was observed at 20 minutes. At the time 120 minutes, the highest number of dead shrimp was found in the larvae fed CD, followed by the larvae fed SAD, DAD, LAD (**Figure 11**). After 120 minutes, shrimps survived in both 25 and 30 ppt were considered to resist this hypotonic shock. The study was finally terminated at 140 minutes. From the results, it should be noted that shrimps fed diets fortified with lecithins derived from any sources could tolerate low salinity stress better than those fed diet without lecithin supplementation.

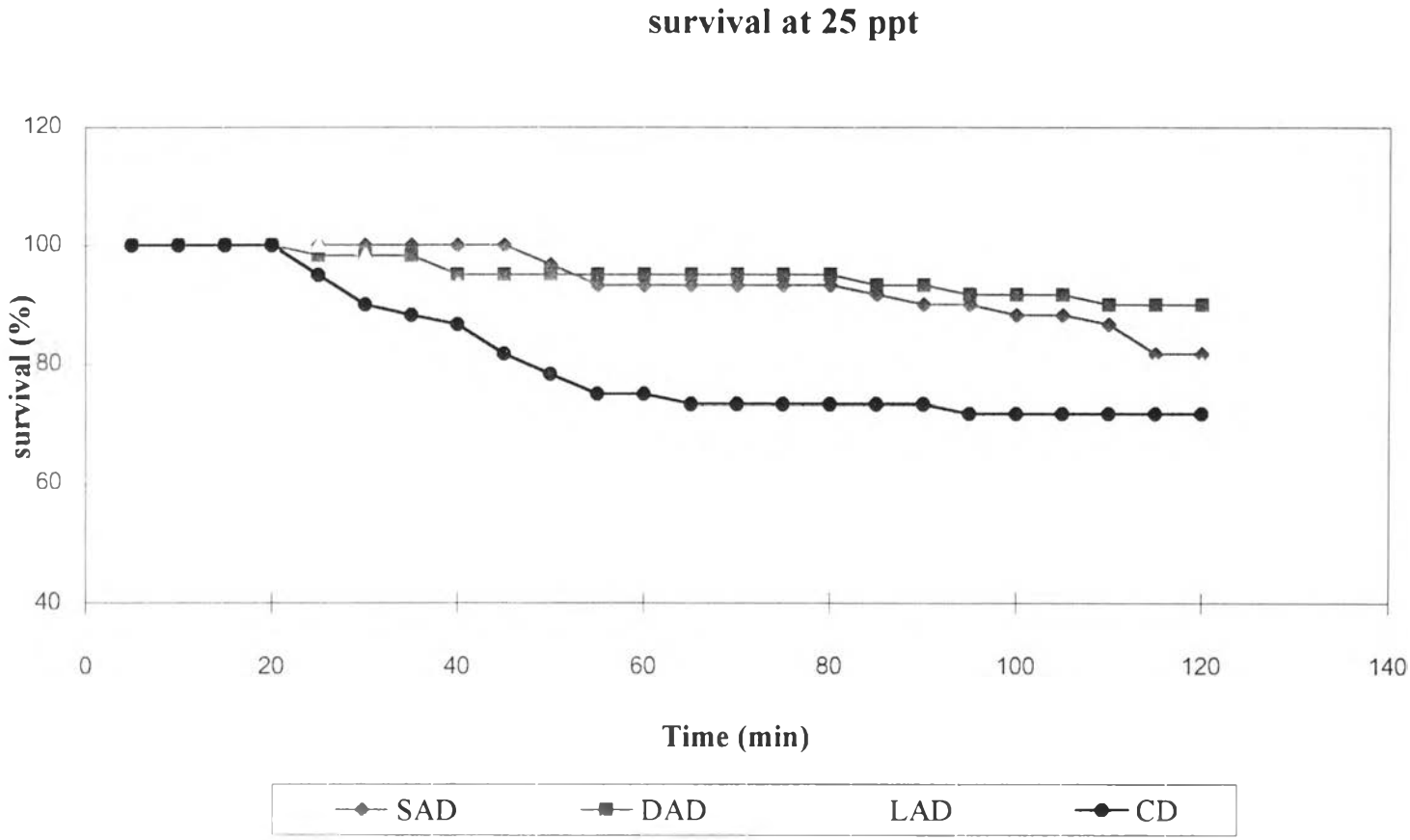

Figure 10 Percent survival of larvae on stress test from salinity 25 ppt to 0 ppt.

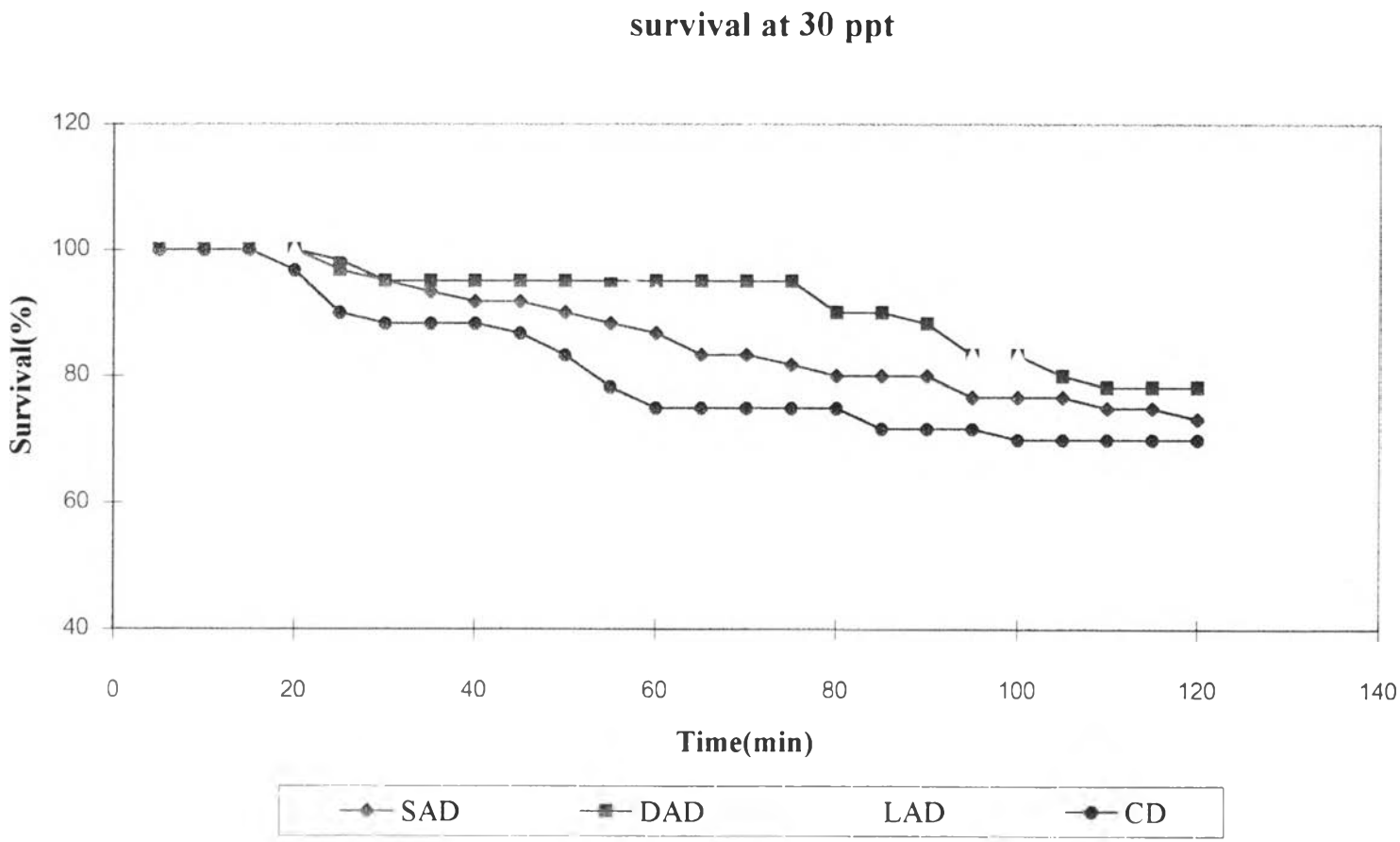

Figure 11 Percent survival of larvae on stress test from salinity 30 ppt to 0 ppt .

The results of the present investigation indicate that the resistance of *P. monodon* postlarvae 15 to osmotic shock was improved when the shrimp were fed lecithin fortified diets compared to lecithin free diet. Ree et al. (1994) demonstrated that the resistance of *P. monodon* postlarvae to osmotic shock was markedly improved when the shrimp were fed brine shrimp with only 0.27% of n-3 HUFA (on dry matter basis) compared to controls fed brine shrimp without n-3 HUFA enrichment. Kanazawa et al. (1985) observed that the sensitivity to osmotic stress of 32- and 52-day-old postlarvae of *P. japonicus* was significantly when n-3 HUFA content was raised in shrimp's regular diet from 0 to 0.5% (with PC in the diet) or from 0.5 to 1% (PC-free diet). Their study implies that n-3 HUFA possibly involves in the adaptation process on salinity of shrimp. Furthermore, the dietary DHA and soybean lecithin were effective in increasing the tolerance of red sea bream (Kanazawa,1997) and postlarval *P. japonicus* fed a PC- supplement diet exhibited a higher resistance to a salinity stress than *P. japonicus* fed a PL- deficient diet (Camara,1994).

It remains unclear whether improved resistance to osmotic stress due to dietary n-3 HUFA is resulting from changes in the shrimp lipid metabolism and the overall physiology condition by the provision of EFA, or from the direct effect of HUFA on the osmoregulatory physiology by changing the membrane composition and/or structure of the gills. The interaction of dietary PL on the HUFA requirement for reducing stress sensitivity may thus be due to the effect of PL on lipid metabolism /or the improved incorporation efficiency of n-3 HUFA compensating for a deficiency in diet. In the present study, we incorporated both benefits together: lecithins and n-3 HUFA, as mentioned by many investigators, into one: n-3 HUFA-rich lecithin. As described earlier, if n-3 HUFA facilitates the appropriation of membrane lipid composition for osmoregulatory physiology thus the presentation of these fatty acids as lecithin moiety should provide the faster track for n-3 HUFA to the membranes. It

should be emphasized that n-3 HUFA present in membranes is in the form of n-3 HUFA-rich lecithin.

Water quality

It should be emphasized that water quality used in the present study was similar among four diets and was in normal ranges throughout the whole period of the study as shown in **Table 21** and Appendix I.

Fatty Acid Composition in Shrimps

Cultured shrimp at postlarva 15 fed all diets was grouped, blended, lipid extracted, transesterified and the content of fatty acids was determined as shown in **Tables 18, 19.** One can see that shrimps collected HUFA in their flesh even in CD group. This observation can be explained by the fact that despite of the absence of lecithin, CD still contains fish oil with high content of HUFA as previously shown in **Table 9**. However, the shrimp fed CD finally collected HUFA the least compared to those fed with lecithin-fortified diets. Considering the percentage of individual fatty acids as shown in **Table 20**, it was found that shrimp fed LAD accumulated HUFA the most whereas those fed with SAD collected these fatty acids the least. However, no statistical significant was found.

The present study indicates that the fatty acid in *P. monodon* larvae fed LAD, accumulated n-3 HUFA in their fats higher than shrimp fed other diets. Chen (1993) found total lipid content of muscle in *P. monodon* increased in relation with the increase of phosphatidylcholine supplementation. Work with *P. japonicus*, Teshima et al (1986) found total lipid and phospholipid concentrations of the hemolymph were

higher in the shrimps receiving phospholipid in diet than in these receiving phospholipid-deficient diet. Similary, Xu et al (1994) found a higher total body lipid content in *P. chinensis* fed diets with phosphatidylcholine. Kontara et al. (1997) concluded that soybean phosphatidylcholine in diet resulted in an increased retention of lipid and n-3 HUFA in the shrimp tissue compared to that of shrimp fed with phospholipid-free diets containing similar levels of total lipid and n-3 HUFA. In the study of Dahlan et al (1996), fish meal which was the same grade as local fish meal utilized in the present study contained phosphatidylcholine 52.2 mole/100 mole total phospholipids. This content is much higher than phosphatidylcholine contained in soya which is in the range of 32-40 (Hawthorne and Lekim 1982). Hence, the higher phosphatidylcholine content in local fish meal in comparison to soya is probably the possible explanation for the higher accumulation of HUFA in flesh of shrimp fed diet supplement with lecithin derived from local fish meal. Therefore, dietary lipid composition especially lecithin may influence the accumulation of total lipid level and some lipid class compositions in the muscle of shrimp.

Table 17 Fatty acid composition of shrimps fed different diets.

Fatty acid	SAD	DAD	LAD	CD
C 14:0	0.231	0.137	0.082	0.131
C 16:0	3.078	1.501	0.963	1.364
C 16:1 n-7	0.245	1.245	0.087	0.086
C 18:0	1.170	0.713	0.649	0.660
C 18:1 n-9	2.274	1.181	0.797	0.997
C 18:1 n-7	0.743	0.451	0.405	0.339
C 18:2 n-6	1.159	0.425	0.261	0.778
C 18:3 n-3	0.834	0.455	0.417	0.450
C 20:4 n-6	0.231	0.141	0.158	0.115
C 20:5 n-3	0.721	0.474	0.536	0.415
C 22:5 n-3	0.078	0.070	0.123	0.073
C 22:6 n-3	0.377	0.307	0.303	0.240
Σ HUFA	1.406	0.992	1.119	0.843
Total	11.139	7.098	4.781	5.648

The data are expressed as mg/g by weight of shrimps.

Table 18 Saturated, monoenes and polyenes fatty acids in Shrimp's diets.

Fatty acid	SAD	DAD	LAD	CD
SAFA	4.479	2.350	1.694	2.156
MUFA	3.261	2.877	1.290	1.422
HUFA + PUFA	3.399	1.872	1.797	2.071
n-3	2.009	1.306	1.378	1.178
n-6	1.390	0.566	0.419	0.893
n-3/n-6	1.446	2.305	3.286	1.319

The data are expressed as mg/g by weight of shrimps.

Table 19 Fatty acid composition of shrimps fed different diets (g/100 g total fatty acids)

Fatty acid	SAD	DAD	LAD	CD
C 14:0	2.071	1.926	1.715	2.324
C 16:0	27.635	21.138	20.148	24.149
C 16:1 n-7	2.198	17.537	1.827	1.521
C 18:0	10.502	10.037	13.567	11.687
C 18:1 n-9	20.411	16.641	16.676	17.649
C 18:1 n-7	6.670	6.351	8.477	6.003
C 18:2 n-6	10.404	5.987	5.466	13.776
C 18:3 n-3	7.487	6.406	8.715	7.967
C 20:4 n-6	2.071	1.992	3.305	2.033
C 20:5 n-3	6.471	6.681	11.210	7.344
C 22:5 n-3	0.699	0.980	2.566	1.300
C 22:6 n-3	3.383	4.325	6.330	4.246
Σ HUFA	12.623	13.978	23.411	14.924

Table 20 Saturated, monoenes and polyenes fatty acids in shrimp's diets (g/100 g total fatty acids)

Fatty acid	SAD	DAD	LAD	CD
SAFA	40.208	33.102	35.430	38.160
MUFA	29.278	40.528	26.980	25.173
HUFA + PUFA	30.515	26.370	37.591	36.667
n-3	18.039	18.391	28.820	20.858
n-6	12.475	7.979	8.770	15.809
n-3/n-6	1.446	2.305	3.286	1.319