Growth, Molting and Survival Response of Juvenile Narrow Clawed Crayfish, *Astacus leptodactylus*, Fed Two Sources of Dietary Oils

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Abstract

The influence of five pure diets of isonitrogenous and isoenergic with different ratios of *Clupeidae* fish oil (F) and soybean oil (S) on growth, molting and survival of narrow-clawed crayfish, *Astacus leptodactylus*, was evaluated during an 8-weeks trial. As soybean oil replaced with fish oil in each treatment, gently. Test diets were inclusive F0/S100, F30/S70, F50/S50, F70/S30 and F100/S0 fish oil per soybean oil percent. The test organism housed in fiber-glass tanks of 100 l capacity with flowing water and aeration in tanks are supplied. The test followed a Randomized complete design. Each treatment organized with 6 replicates and totally 30 rearing tanks involved. At the end of the trial, the crayfish offered diet containing *Clupeidae* fish oil (rich in n-3 fatty acids) showed the lowest food conversion ratio and highest weight gain performance as well as molting and survival rates. In contrast, the diet containing soybean oil (rich in fatty acids n-6) contributed least to growth and other indices. Statistical test showed the differences between this two formulated diets were significant ($P<0.05$). Results indicated that n-3 series of fatty acids are required in the diet of *A. leptodactylus*.

Keywords: *Astacus leptodactylus*, Diet, Fish oil, Soybean oil , n-3, n-6 , Growth, Survival, molting

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Introduction

High nutritional value and significant market demand, versatile feeding habit of not costly dietary regime, reduction in wild populations as results of unsustainable fishing and outbreaks of epidemic diseases are considered as major reasons for breeding of crayfish in the world. Among various species of crayfish, *Astacus leptodactylus* is highly appreciated and enjoys high commercial rank in the world market. Major advantages of *A. leptodactylus* over other crayfish species are its higher fecundity, robust adaptability to various water bodies, rapid growth, resistance to disease, and high degree of elasticity in feed demand (Kolmykov, 2002; Holdich, 2002). Dadgar (2010) could replace soybeen with cotton seed in feeding Rainbow Trout due to low level of lysine and methionine. *A. leptodactylus* mostly distributed in south-eastern European states and parts of mid-Asian countries (Holdich, 2002), it also inhabits Anzali wetland, and Arass water reservoir in the southern reach of the Caspian coast along Iran (Karimpour, 2000). Although various crayfish species in several countries have become aquaculture species (Skurdal et al., 1987), its yield in Iran has so far been solely dependent on fishing in its natural habitat. *A. leptodactylus* possesses abundant potential to be a candidate for farming. It could function to enhance aquaculture items and boost aquatic products of export value. The first step for commercial crayfish cultivation is provision of adequate feed to ensure its biological requirements. Fatty acids, particularly the long-chained unsaturated ones (HUFA & PUFA), are important ingredients of a formulated diet, which contribute to growth and other biological parameters (Goddard, 1996). Lipids are the source of fatty acids and more than 40 types of fatty acids are found (Goddard, 1996). In general, four types of fatty acids are recognized as essential for shrimps growth, which comprise linoleic acid (LOA, 18:2n-6), linolenic acid (LNA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexsaenoic acid (DHA, 22:6n-3) (Kanazawa, 1984). Unsaturated fatty acids of n-3 and n-6 series exert positive effects on the biological activities of animals, as they are not able to synthesis de novo, so must be supplied by feeding regime (Tacon, 1990). A good deal of literature on the importance of fatty acids in some aquatic organisms is available, dietary needs of crustaceans (Castell, 1981), on growth and survival of fish, shrimps and other shellfish larvae (Mourente & Rodriguez, 1997), fatty acids profile in tissues in of milkfish larvae and juvenile (Borlongan and Benitz, 1992), growth and survival of *Ostrea edulis,* reproduction stage of *Penaeus monodon* (Millamena et al., 1993), *Penaeus chinensis* (Xu et al., 1994), and the crab (*Paratelphusa hydrodromus*) (Adiodi and Adiodi,1979). Thus, determining the type and the ratio n-3/n-6 in the diet of commercially cultivated aquatic animals are indispensable. Since marine fish oil is a rich source of essential n-3 fatty acids and the plant oil is the source of n-6 fatty acids (Goddard, 1996), in this study we used *Clupeidae* (*Clupeonella cultriventris caspia*) (Abbasi etal., 1999) and soybean oils- normally applied in formulated feed manufacturing- to evaluate the effect of

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different ratios of these fatty acids on the growth and survival of *A. leptodactylus.*

Material and methods

Berried crayfish brooders trapped in Aras reservoir and transferred to Sefidroud Fisheries Research Station for a semi-controlled breeding. Released juvenile crayfish kept in cement tanks and fed with artificial food and *Clupeidae* fish until reaching 100-200 mg. Consequently, the juvenile crayfish transferred into earthen pond until arrival about one gram weight, during this period crayfish consumed natural food produced in earthen pond. The test young crayfish at the beginning of trial selected based on its health condition and body weight, biometric data such as total weight (g) and total length including carapace length (mm) are recorded. Average of initial body weight measured 1.22 ± 0.58 g. The experimental animals were fed once a day at 4% of their body weight for a 8-week period and the test organism housed in fiber-glass tanks of 100 l capacity with flowing water and aeration in tanks were supplied. All animals were individually weighed and measured every 14 days to collect growth data. Dead animals were removed and recorded. The test followed a Randomized complete design. Each treatment organized with 6 replicates and totally 30 rearing tanks involved.

Based on the proximate analysis of all ingredients, the experimental diets were formulated. The formulated diets were analyzed, too. Moisture was measured by placement of a 5 g sample into a convection oven (WT-BINDER) (105^{o}C) for 12 h until reaching a constant weight (AOAC, 1990, procedure 930.15); protein was determined by combustion (AOAC, 1990, procedure 930.03); lipid was determined by the acid hydrolysis method (AOAC, 1990, procedure 954.02); fiber was determined using the fritted-glass crucible method (AOAC, 1990, procedure 962.09); and ash was determined by placing a 2 g sample in a muffle furnace (600^{o}C) for 5 h (AOAC, 1990, procedure 942.05). The nitrogen-free extract (NFE) was determined by NFE= 100-(%protein + %lipid + %fiber + %ash). Fatty acids profile was measured by GC procedure (UNICAM Gas Chromatograph 4600). Crude energy was determined by automatic calorimetric bomb unit (GALLENKAMP AUTOBOMB2003, England). Fatty acids profile of the oils presented in Table 1.

Five isonitrogenus and isocaloric diets with 35% crude protein and different ratio of *Clupeidae* oil (F) to soybean oil (S) (different ratio of n-3/n-6) as the order of F0/S100, F30/S70, F50/S50, F70/S30 and F100/S0 percent based on wet weight) were prepared. Diets were formulated by LINDO program (copy right 1999, release 6.1). For preparation of diets, dry ingredients were weighed and mixed together by mixer. Tap water was then added to get 20% moisture level. Diets were single extruded through a 2 mm die to form "spaghetti-like" strands and air-dried in a convection oven (WT-BINDER) at temperature 70^{o}C for 6 h. After drying, all diets were broken into pellets of 1-2 mm size. Casein and gelatin (Merk) as the main source of protein and starch and sucrose (Merk) to supply carbohydrate were used in experimental diets. The sources for lipid were *Clupeidae* and soybean oils.

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Table 1: Fatty acids composition (total weight percentage) of oils

Fatty acids	*Clupeidae* oil	Soybean oil	LOA[1]	LNA[2]	EPA[3]	DHA[4]
C12:0	-	-	-			
0:C13	-	-	-	-	-	-
0:C14	4.13	0.347	-	-	-	-
0:C15	1.03	0.079	-	-	-	-
0:C16	22.56	15.36	-	-	-	-
7-C16:1n	5.65	0.288	-	-	-	-
0:C17	0.299	0.066	-	-	-	-
0:C18	5.205	4.348	-	-	-	-
7-C18:1n	23.5	2.055	-	-	-	-
C18:1n-9	2.402	22.66	-	-	-	-
C18:2n-6	3.224	49.94	100	-	-	-
C18:3n-3	1.747	4.054	-	100	-	-
0:C19	0.265	Trace	-	-	-	-
0:C20	0.418	0.371	-	-	-	-
C20:5n-3	7.67	Trace	-	-	100	-
0:C21	0.437	0	-	-	-	-
0:C22	0.330	0.401	-	-	-	-
C22:1n erosic	0.204	Trace	-	-	-	-
C22:6n-3	20.91	Trace	-	-	-	100
SFA[5]	40.34	26.27	-	-	-	-
MUFA[6]	31.56	25.00	-	-	-	-
PUFA[7]	4.98	53.99	100	100	-	-
HUFA[8]	28.59	Trace	-	-	100	100
∑n-3	30.33	4.054				
∑n-6	3.224	49.94				
N3/n6	9.41	0.081				
Sum	100	100				

[1] Linoleic Acid, [2] Linolenic Acid, [3] Eicosapentaenoic Acid, [4] Docosahexaenoic Acid ,
[5]SFA= Saturated Fatty Acid (C14:0, C15:0, C16:0, C17:0, C18:0, C19:0, C20:0, C21:0, C22:0),
[6]MUFA= Mono Unsaturated Fatty Acid (C16:1, C18:1, C22:1), [7]PUFA= Polyunsaturated Fatty Acid (C18:2, C18:3), [8]HUFA= Highly Unsaturated Fatty Acid (C20:5, C22:6)

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Table 2: Ingredient composition (percent dry weight) and proximate analysis of the experimental diets

Ingredients	Diets (%)				
	F0/S100	**F30/S70**	**F50/S50**	**F70/S30**	**F100/S0**
Casein	34	34	34	34	34
Gelatin	4	4	4	4	4
Dextrin	9	9	9	9	9
Starch	15	15	15	15	15
Sucrose	4	4	4	4	4
Soybean oil	9	2.7	4.5	6.3	0
Clupeidae oil	0	6.3	4.5	2.7	9
Vitamin mix.[1]	2	2	2	2	2
Mineral mix.[2]	4	4	4	4	4
Cholesterol	0.1	0.1	0.1	0.1	0.1
Lecithin	0.3	0.3	0.3	0.3	0.3
Vitamin C	0.5	0.5	0.5	0.5	0.5
Collin chloride	0.1	0.1	0.1	0.1	0.1
Cellulose	18	18	18	18	18
Proximate composition					
Moisture	2.5	2.39	4.2	2.72	2.14
Crude protein	33	32.4	32.7	32.1	32.9
Crude fat	10.1	10	9.9	10.4	9.8
Ash	4.6	4.8	4.5	4.4	4.2
Crude fiber	12.1	12.3	12	12.4	12.6
NFE[3]	37.7	38.1	36.7	37.9	37.6
Crude Energy (kcal/kg)	3681	3627	3658	3611	3543
F/P	0.31	0.31	0.3	0.3	0.3
P/E (kcal /g)	11.7	11.2	11.2	11.3	10.8

[1] Vitamin Mix: Vit A, 1600000 IU; Vit D3, 400000; Vit E, 40g; Vit k3, 2g; Vit B_1 (Thiamin), 6g; B_2 (Riboflavin), 8g; B_3 (Pantothenic acid), 12g; B_5 (Niacin), 40g; B_6 (Pyridoxine), 4g; B_9 (Folic acid), 12g; Vit B_{12}, 8g; Vit H_2, 0.24g; Vit C, 60g; Inositol, 20g; BHT, 20g; Choline chloride (added to Mineral Mix), 12g (There is 0.5% from up amounts in per kg of Vitamin Mixture).

[2] Mineral Mix: Fe, 26g; Zn, 12.5g; Se, 2g; Co, 480mg; Cu, 4.2g; Mn, 15.8g; I, 1g (There is 0.5% from up amounts in per kg of Mineral Mixture).

[3]NFE (Nitrogen – Free Extract) = 100- (Moisture+ Crude protein+ Crude fat+ Ash+ Crude fiber).

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Table 3: Fatty acids composition of experimental diets on based used *Clupeidae* and soybean oils

Fatty acids	Diets (%)				
	F0/S100	**F30/S70**	**F50/S50**	**F70/S30**	**F100/S0**
C12:0	0	0	0	0	0
C13:0	0	0	0	0	0
C14:0	0.347	1.483	2.240	2.997	4.133
C15:0	0.079	0.367	0.559	0.751	1.039
C16:0	15.359	17.520	18.960	20.401	22.561
C16:1n-7	0.288	1.896	2.969	4.041	5.650
C17:0	0.066	0.136	0.183	0.229	0.299
C18:0	4.384	4.630	4.795	4.959	5.205
C18:1n-7	2.055	8.488	12.776	17.065	23.497
C18:1n-9	22.658	16.581	12.530	8.479	2.402
C18:2n-6	49.939	35.924	26.581	17.238	3.224
C18:3n-3	4.054	3.362	2.901	2.439	1.747
C19:0	Trace	0.079	0.132	0.185	0.265
C20:0	0.371	0.385	0.395	0.404	0.418
C20:5n-3	Trace	2.302	3.836	5/371	7.672
C21:0	0	0.131	0.219	0.306	0.437
C22:0	0.401	0.380	0.365	0.351	0.330
C22:1n erosic	Trace	0.061	0.102	0.143	0.204
C22:6n-3	Trace	6.274	10.457	14.639	20.913
Sum	100	100	100	100	100
∑n3	4.05	11.94	17.19	22.45	30.33
∑n6	49.9	35.92	26.58	17.24	3.22
n3/n6	0.08	0.33	0.65	1/30	4.41

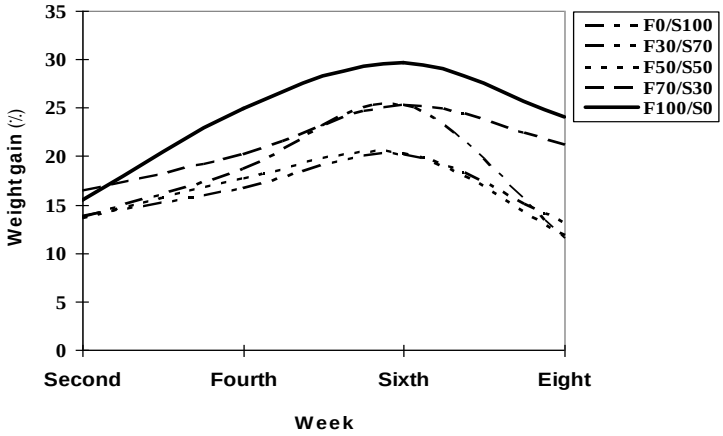


Figure 1: Average Weight Gain of juvenile *A. leptodactylus* in different ratio of oil in diet

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Table 4: Growth and survival rates of *A. leptodactylus* fed with diets of different n-3/n-6 ratios in an 8-weeks feeding trial

Diets	F0/S100	F30/S70	F50/S50	F70/S30	F100/S0
WG (%)	17.5 ± 1.6^{b}	17.5 ± 1.8^{b}	20.05 ± 2.2^{b}	23.3 ± 2.4^{ab}	24.6 ± 2.6^{a}
SGR(%/day)	0.26 ± 0.02^{b}	0.26 ± 0.03^{b}	0.29 ± 0.03^{b}	0.33 ± 0.03^{ab}	0.35 ± 0.03^{a}
FCR(g/g)	4.7 ± 0.77^{a}	4.4 ± 0.74^{ab}	4.13 ± 0.58^{ab}	3.90 ± 0.45^{ab}	3.20 ± 0.52^{b}
Molting (n)	1.46 ± 0.18^{b}	1.60 ± 0.17^{ab}	1.66 ± 0.22^{ab}	1.73 ± 0.21^{ab}	2.03 ± 0.20^{a}
Survival (%)	58.9 ± 4.9^{ab}	50.5 ± 6.0^{b}	55 ± 6.4^{ab}	56.7 ± 5.5^{ab}	69.4 ± 6.4^{a}

Mean ± SE Values in any row with different superscript are significantly different (P<0.05).

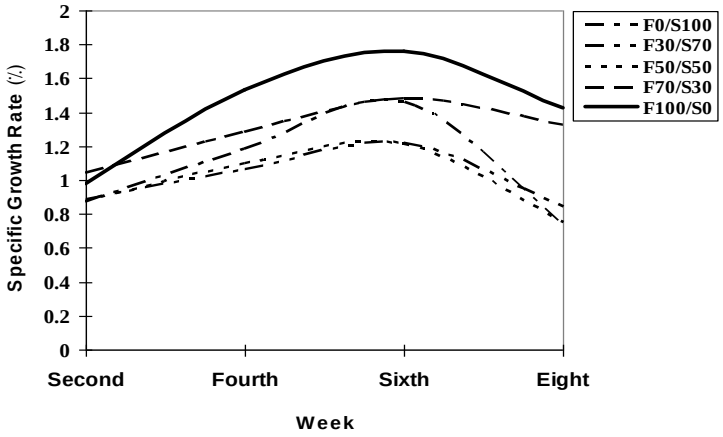


Figure 2: Average Specific Growth Rate of juvenile *A. leptodactylus* in different ratio of oil in diet

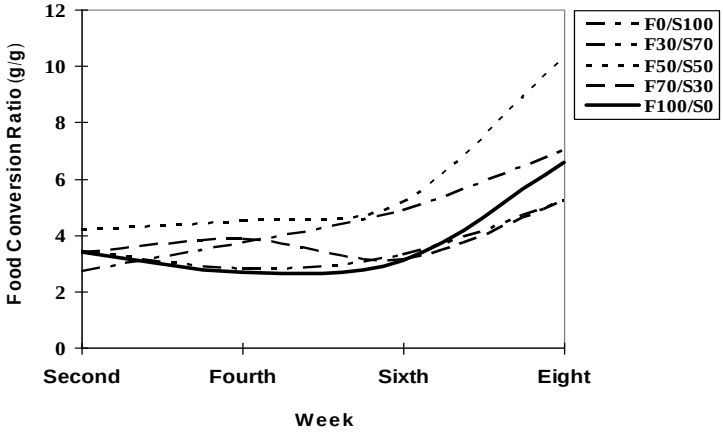


Figure 3: Average Food Conversion Ratio of juvenile *A. leptodactylus* in different ratio of oil in diet

Cellulose as a filler to replace oil as well as a binding material was applied. The vitamin-mineral mix (Science Company) in the order of 4%, 2% was also added. Except oil and cellulose, other ingredients in all feed treatments were added on equal basis. To avoid oil-oxidation, diets were stored in plastic container at -24°C until fed. Proximate composition of each of the five practical diets and their fatty acids

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composition presented in Table 2 and Table 3, respectively.

At the end of the feeding trial, each crayfish was individually weighed on an electronic scale (AND, Japan). Growth parameters and survival were calculated as follows:

Specific Growth Rate(SGR) (%/day) = 100 [(ln Wt –ln Wi)/ T];

Where Wt and Wi are the final and initial individual weights, respectively, and T is the length of the culture period in days (Goolish and Adelman ,1984) ;

Weight gain (WG) (%) = 100 [(Wt – Wi)/ Wi];

Feed Conversion Ratio (FCR) = total diet fed (kg)/ total wet weight gain (kg);

Molting (n) = number of molting in test period;

Survival Rate (SVR) (%) = (final individual numbers/ initial individual numbers) × 100.

Data were calculated for percent weight gain, specific growth rate (SGR), feed conversion ratio (FCR), molting and survival. Results were compared with one-way analysis of variance (ANOVA) and considered significant at $p \leq 0.05$ by using the SPSS 9.0. LSD test was used to compare differences among treatment means at the p=0.05 level of significance.

Results

Effects of various ratios of n-3/n-6 in formulated diet in relation to growth index and survival rate of juvenile crayfish are shown in Table 4. With increase of n-3/n-6 ratio (increasing *Clupeidae* oil in proportion to soybean oil), growth indices including weight gain, specific growth rate and food conversion ratio were improved. Results showed the crayfish fed with diet F100/S0 and F0/S100 produced the highest and lowest weight gains, respectively, and the difference was significant ($P<0.05$).

The crayfish fed with the diet of F100/S0 including F30/S70 and F50/S50 demonstrated significantly difference in growth (Table 4). Comparison of growth increment during trial period has been shown in Figure 1. In general, during test period gradually weight increased based on different proportion of n-3/n-6 in diet. Peak of weight gain was in the third two-weeks, but it reduced in the final two-weeks, again. Also, crayfish fed by the diet of F100/S0 showed highest growth than to other diets in two weeks internal. Specific growth rate during the whole period of experiment (Table 4) and its trend of changes (Figure 2) were quite similar to the variation of weight increment. SGR showed a gradual increase and reached its peak in the third two weeks, but in the two final weeks again reduction trend was witnessed.

Considering Table 4, with increase in the ratio of fatty acids n-3/n-6 in the diet, FCR relatively improved; the best FCR average were relevant to the experimental diets of F100/S0 and F0/S100, respectively, and their difference was significant ($P<0.05$). Other test diets did not shown significant differences ($P<0.05$). The two weeks variation of FCR showed, during the test period its rate increased gradually. It was also FCR in experiment diets such as F0/S100, F30/S70 and F50/S50 from being of test until final trial course slowly showed enhancement, meanwhile in diets of F70/S30 and F100/S0 until sixth weeks

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reduced and the end of study increment were witnessed (Figure 3).

Molting displayed that increase in n-3 fatty acids in proportion to n-6 contributed to increased molting; the most and the least frequent molting were, respectively, observed in diets F100/S0 and F0/S100 and the differences was significant ($P<0.05$), but the differences among other test diets were not significant ($P<0.05$) (Table 4). Heavy variation of molting in 14-days intervals of the test period showed that the highest molting frequency occurred in the first and second two-weeks and in the eighth week the lowest molting frequency was observed. In the earlier stages of test, the diets F0/S100 and F30/S70 containing higher proportion of soybean oil (n-6) demonstrated more frequent molting, but in the later stages until the termination of the experiment, diets F70/S30 and F100/S0 containing higher proportion of *Clupeidae* oil (n-3) brought more frequent molting (Fig. 4). In general, the crayfish fed with the diet F100/S0 exhibited the greatest survival rate while the diet F30/S70 showed the lowest survival rate, and the difference was statistically significant ($P<0.05$), but this significant differences were not found among other experimental diets ($P<0.05$). The trend of variation indicated the survival rate of juvenile crayfish improved when *Clupeidae* oil (n-3) replaced soybean oil (n6) in the diet (Table 4).

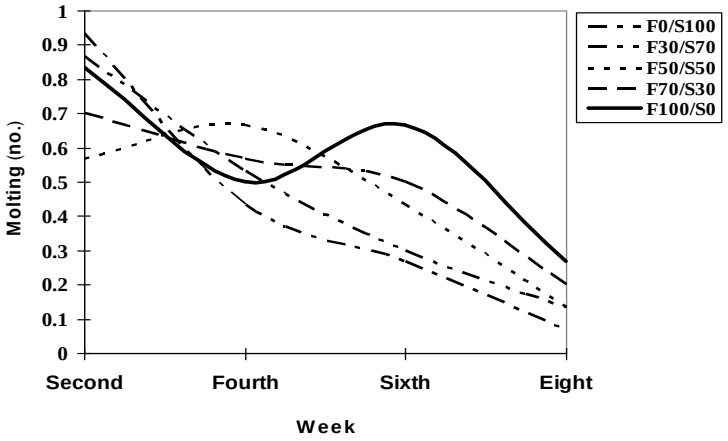


Figure 4: Average of Molting in juvenile *A. leptodactylus* in different ratio of oil in diet

Comparison of survival rate of juvenile crayfish in different periods of test indicated the trend of changes approximately was constant, and in each stage of biometry between different diets little discrepancy was observed. No significant differences in the survival rates among the crayfish fed on various diets in the first two weeks of the test were found. In the first two-week, the diet F0/S100 caused significantly lowest survival rate ($P<0.05$) while the diets F100/S0 brought about highest survival rate. Although in the third two-weeks the diets containing higher percent of n-3 showed more survival, the differences were not significant ($P<0.05$). Finally, in the fourth two-weeks, the diets F50/S50 and F70/S30 resulted into the greatest survival rate that

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was significantly higher (P<0.05) than the diet F30/S70 (Figure 5).

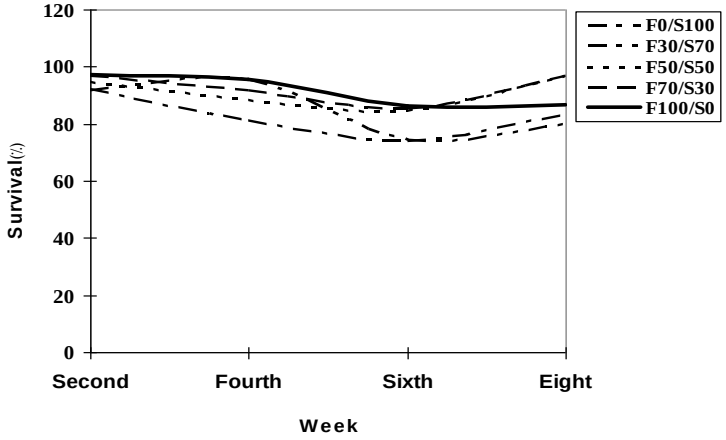


Figure 5: Average of Survival in juvenile *A. leptodactylus* in different ratio of oil in diet

Discussion

It has been demonstrated the crustaceans have the ability to synthesize saturated and monounsaturated fatty acids from carbohydrate precursors (Castell, 1983), but unsaturated fatty acids should be gained through diets (Tacon, 1990). Aquatic animals need more n-3 series fatty acids as compared to terrestrial animals. Many studies have also reported the superior nutritional value of marine oils such as sardine, pollack, short-necked clam and cod liver oil over plant oils or animal fats for *P. japonicus, P. monodon* and *P. vannamei* (Guary et al., 1976; Kanazawa et al., 1977a; Dominy and Lim, 1989; Catacutan , 1991). However, there have been no studies on the EFA requirements for the narrow-clawed crayfish. In present study, n-3 fatty acids exerted desirable effects on the growth performance and survival. The test diet F100/S0 (containing 100% *Clupeidae* oil) exhibited better effect on growth indices such as weight gain, specific growth rate, food conversion ratio, molting and survival rates in the crayfish. Thus, oil from marine fish species (*Clupeidae* fish) showed better metabolic function in comparison with plant oil (soybean) in the diet of crayfish. Similar results have been achieved in some species of crustaceans. Studies on the nutritional requirement of marine fish and crustaceans have shown that fatty acids of n-3 family have higher values than the fatty acids of the n-6 family, and have also demonstrated that marine fish and crustacean lack the ability for *de novo* synthesize of n-6 and n-3 fatty acids (Sargent et al., 1999). Qualitative requirements for linolenic series fatty acids (18:3n-3; 20:5n-3; 22:6n-3) have been identified for shrimps, *Penaeus aztecus* (Shewbart and Mies, 1973), *P. japonicus* (Guary et al., 1976; Jones et al., 1979; Kanazawa et al., 1985), *P. indicus* (Read, 1981), *Artemesia loginaris* (Patriella et al.,1984), *P. monodon* (Catacutan,1991) and *P. esculentus* (Dall, 1992). Kanazawa et al. (1978) showed improved growth of *P. japonicus* fed diets rich in 20:5n-3 and 22:6n-3 compared with other fatty acids sources. Positive relationship between

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22:6n-3 in diet with percentage of larvae yield hatched from female brooder of Chinese shrimp, *P. chinensis*, is recognized (Xu et al., 1994) and clear and definite effect of these series of fatty acids on growth and survival in crustaceans, and marine fish species being demonstrated by Berntsson et al.(1997).

The feed requirement of *A. leptodactylus* has nearer affinity to estuary and marine fish species. One of the reasons of further needs to n-3 fatty acids in *A. leptodactylus* could be fairly attributed to its adaptation to brackish water. This species has, however, been able to live in waters with 14 to 30 ppt salinity (Holdich, 2002). Furthermore, as has been said, this crayfish is almost carnivorous, which indicates it is more dependent on marine fish oil sources (n-3 fatty acids) than plant oil (n-6 fatty acids).

The oil from marine fish sources with 20:5n-3 and 22:6n-3 in *Homarus americanus* (Kanazawa et al., 1977a) and *Carcinus maenas* (Ponat and Adelung, 1983) showed better results on growth. Cod liver oil promoted better growth of P. monodon than soybean oil, corn oil, pork lard, beef tallow and coconut oil (Catacutan, 1991). The best growth of *Penaeus vannamei* was achieved when fed a cod liver oil based diet (Dominy and Lim, 1989). Based on the growth performance, menhaden oil was better utilized by juvenile *P. vannamei* than linseed, soybean, corn, sunflower, and coconut oils and stearic acid, which could possibly be attributed to the existence of fatty acids HUFA especially 20:5n-3 and 22:6n-3 in the applied fish oil (Lim et al., 1997).

Essential fatty acid (EFA) requirements for *P. japonicus* have been reported to be 0.5 to 1.0% (Jones et al., 1979) and those for freshwater prawns can be as low as 0.075% (D'Abramo and Sheen, 1993). In general, the EFA requirement of crustacean is not greater than 1% of the diet (D'Abramo et al., 1997). The levels of 20:5n-3 and 22:6n-3 0.5 to 1.3% found in the diets supplemented with 4 to 12% oil supported good growth of the mud crabs (D'Abramo et al., 1997).

This study demonstrated that molting frequency in animals fed with fish oil in diet was higher than soybean oil, as it's maximum was observed in diet with 100% *Clupeidae* fish oil. Also, in during of experimental period highest molting occurred in crayfish fed with of n-3 HUFA enriched diets. In totally, unsaturated fatty acids are effective in crustaceans molting. Crabs fed diets supplemented with oil had higher molting frequency than those fed the lipid-free diet. The low molting frequency may in some way be related to the synthesis of some hormone-like components such as the eicosanoids. Eicosanoids are derivatives of polyunsaturated fatty acids such as 20:4n-6, 20:5n-3 and 22:6n-3 (Rowley et al., 1995). Koskela et al. (1992) indicated that *Penaeus esculentus* injected with prostaglandin E2, a type of eicosanoid, displayed a shorter molt cycle than the control animals. Castell and Covey (1976) also indicated that American lobster fed a lipid-free diet had a lower molting frequency.

In this study the survival rate of the crayfish was enhanced when higher proportion of *Clupeidae* oil was added to the diet. The effect of fatty acids on the level of survival in crustaceans is corroborated by other studies as well. Diets containing marine source oil such as sardine or clam that have high level of series n-3 fatty acids with 20 and 22-

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carbon, contributed better to the survival rate of *P. japonicus* as compared with diet containing plant oil (Guary et al., 1976). Diet with soybean oil in its content, in comparison with fish oil of pollack caused lower survival in *P. japonicus* (Kanazawa et al., 1977a).

Variation in the growth process in regard to the test diets showed that with increase in the proportion of n-3 in the n-3/n-6 fatty acids ratio, growth indices, molting and survival of *A. leptodactylus* were improved. Various data gathered during the experiment trial, including biological parameters, indicated a constant and gradual growth from the beginning until the end of the trial. Meanwhile, different test diets in each stage rearing period showed equal trend, so that in all stages the diet of F100/S0 contributed the greatest and F0/S100 the lowest effect to growth indices of the juvenile crayfish. On the other hand, the crayfish fed with higher content of n-3 in ratio to n-6, until the end of culture the growth showed rising trend. Our data, therefore, indicated the advantages of *Clupeidae* oil (n-3) to soybean oil (n-6) in *A. leptodactylus* diet.

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