Effects of enriched *artemia* on growth and survival of juvenile freshwater crayfish (*Astacus leptodactylus* Esch. 1823)

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Abstract

The experiment was conducted to investigate the effects of *artemia* enriched with lipid emulsions containing highly unsaturated fatty acids on growth and survival of juvenile freshwater crayfish *Astacus leptodactylus*. Juvenile crayfish were fed *artemia* enriched with commercial emulsions (red pepper and olio ω3) and un-enriched artemia (control). The highest eicosapentaenoic acid (EPA) level was found in *artemia* enriched with olio ω3 (3.17 %) and the highest docosahexaenoic acid (DHA) level was found in *artemia* enriched with red pepper (3.56 %). The weight gain, specific growth, and survival rates of juvenile crayfish increased with increasing amount of EPA and DHA in dietary *artemia* respectively (0.04%, 2.32%). Finally, the juveniles fed with *artemia* enriched with olio ω3 and red pepper had a better weight gain, specific growth rate, and survival than those fed with un-enriched *artemia* (p<0.05).

Keywords: Enrichment *artemia*, *Astacus leptodactylus*, Growth, Survival

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Introduction

Intensification in culture of astacid crayfish so far posed serious problems such as low survival and growth rate during first months of independent life (Gonzalez *et al*., 2010). The researchers studied different feeds on astacid crayfish (Mason, 1979; D'Abramo *et al*., 1985; Celada *et al*., 1989, 1993; Blake *et al*., 1994; Ackefors *et al*. 1989, 1992, 1995; Sa´ez-Royuela *et al*., 1995, 1996). In all of these studies, after exceeding the critical period of first 2–3 months and leaving aside high mortalities sometimes recorded, there are generally low survival rates (50%) and growth values. The low survivals rates and growth values were mainly due to nutritional deficiency (Gonzalez *et al*., 2010). This nutritional deficiency is related to unknown essential factors that can be provided by zooplanktonic live feed (Sa´ez-Royuela *et al*., 2007; Gonza´lez *et al*., 2008).

Artemia nauplii and rotifers are main food sources for larval forms of crustaceans (Immanuel *et al*., 2007). However, rotifers and artemia are naturally low in fatty acids, so enrichment of live foods with essential fatty acids (EFA) is necessary to achieve beter larval growth and survival (Rainuzzo *et al*., 1994). Venero *et al*. (2008) indicated that linolenic (18:3n-3), linoleic (18:2n-6) acids, EPA, DHA, ARA, and unsaturated fatty acids are considered essential in crustacean diets. There are studies on enrichment of live food with EFA in shrimp culture (Rees *et al*.,1994; Alam *et al*., 1995; Immanuel *et al*., 2004; Das *et al*., 2007). There is only one study in lobsters (Chakraborty *et al*., 2010). However, there is no puplished study on freshwater crayfish regarding this subject.

In the present study, the effects of *artemia* enriched with commercial emulsions on growth and survival of juvenile freshwater crayfish (*A. leptodactylus* Esch, 1823) is investigated.

Materials and methods

Crayfish

The juvenile crayfish (*A. leptodactylus* Esch, 1823) were obtained from broodstock captured from Egirdir Lake, Turkey. The initial weight and total length of juvenile crayfish were 0.039±0.00g and 11.60±0.07mm, respectively.

Rearing conditions

The juvenile crayfish were stocked into 9 aquaria (40 x 70 cm bottom area). Pipes (2cm diameter and 4 cm length) were placed in each aquarium to provide refuge. The juvenile crayfish were stocked at aquariums (107 juveniles/ m^2). The three treatments (un-enriched *artemia*, *artemia* enriched with red pepper, and *artemia* enriched with olio ω3 group) were carried out in triplicates. The light and dark regime was 12h on and 12h off. Aeration was provided through the application of a blower motor. The water quality parameters were monitored and provided as optimum. Feed remains and feces were siphoned and 20% of water was exchanged daily. Juvenile crayfish were

fed *ad libitum*. The crayfish were measured to the nearest 1 mm from the tip of the rostrum to the end of telson to give the total body length. The animals were weighed to the nearest 0.1 mg after removing excess water.

Artemia culture

About 0.5-1.0 g of encapsulated *Artemia* cysts (INVE Aquaculture, Izmir, Turkey) were hydrated, disinfected in 0.4% aqueous sodium hypochlorite solution (w/v) for 2h following Lavens and Sorgeloos (2000), and hatched (Chakraborty *et al*., 2007). Freshly hatched nauplii were harvested after 18 h.

Enrichment of artemia

Two commercial emulsions (red pepper and olio ω3) were purchased for *artemia* enrichment. The fatty acid composition of red pepper and olio ω3 emulsion are given in Table 1.

Table 1: Fatty acid profile of the emulsions (%).

	Red pepper	**Olio ω3**	**α**
C12:0	0.11 ±0.01	0.10± 0.01	ns
C14:0	5.57 ±0.46	5.94 ±0.18	ns
C14:1	0.18 ±0.00	0.15± 0.00	*
C15:0	-	0.07 ±0.00	-
C16:0	34.90 ±1.77	14.44± 0.25	**
C16:1	1.87± 0.03	9.45 ±0.17	*
C17:0	0.07 ±0.01	0.26 ±0.00	*
C17:1	0.10 ±0.01	0.25 ±0.01	*
C18:0	0.88 ±0.05	2.10± 0.04	*
C18:1 n9	2.45 ± 0.26	2.89± 0.00	ns
C18:1n7	-	6.75± 0.09	-
C18:2 n6	1.48 ±0.01	3.83 ±0.00	**
C18:3 n3	0.41 ±0.01	3.81± 0.05	**
C20:0	0.17 ±0.02	0.17± 0.01	ns
C20:1	0.21 ±0.01	0.11± 0.01	*
C20:2	2.24 ±0.11	1.05± 0.01	*
C20:3n6	0.09 ± 0.03	0.12±0.00	ns
C20:4 n6	0.54 ±0.09	1.03± 0.03	*
C20:5 n3	2.12 ±0.13	19.13± 0.18	**
C22:1n9	0.55 ±0.14	0.61± 0.01	ns
C22:2	0.32 ±0.02	0.03 ±0.00	*
C23:0	-	0.06 ±0.00	-
C24:0	0.41 ± 0.04	1.69 ±0.05	*
C22:6 n3	27.59 ±0.92	13.30 ±0.21	*
SFA	42.10 ±1.19	24.82± 0.41	*
MUFA	5.35± 0.17	20.19± 0.24	**
PUFA	34.76 ±1.26	42.29± 0.38	*
HUFA	32.88±1.24	34.65±0.42	ns
Total lipid	20.14±0.30	19.94±0.35	ns

Level of statistical significance (α): ns = $p>0.05$, *$p<0.05$, **$p<0.01$

Artemia were enrichment in plastic cups (5l) and stocked 252 nauplii/ml. Olio ω3 and red pepper were added 0.42g/l and 1.26 g/l respectively to the plastic cups. The freshly hatched *artemia* nauplii were enriched for 24h. An aeration was provided to maintain the O_2 level at 5 ppm. The *artemia* were removed from enriched media and washed thoroughly with tap water and stored at –20°C for fatty acids analyses.

Biochemical analyses of enriched artemia

Biochemical analyses of *artemia* were determined according to Standard AOAC methods (AOAC, 1995). Total lipids were determined by the chloroform-methanol extraction method (Bligh and Dyer, 1959).

Fatty acid analysis of enriched artemia

The operating conditions of the GC-MS: Column was SGE (60 m x 0.25 mm ID. BPX5. 0.25 μm USA), the oven temperature was maintained at 60°C for 10 min and increased to 220°C at a rate of 4°C/min. The oven temperature was maintained at 220°C for 10 min, then increased to 250°C at a rate of 4°C/min and maintained at 250°C for 10 min, the Carrier gas was Helium (1.5 mL/min), and Injector temperature was 240°C, Split ratio was 0, Mass spectra was 70 eV, and Mass range was 35-425 m/z

Growth parameters

The growth parameters were calculated by the following formulas (Felix and Sudharsan, 2004; Venkat et al., 2004).

Survival rate(%)=(Final crayfish number/Initial crayfish number) x 100

Weight gain (g)=final weight-initial weight

Specific Growth Rate (SGR)=(lnWf-lnWi) /t x100

Statistical analyses

The results were examined with a one-way analysis of variance (ANOVA) using the SPSS 13.0 computer program (SPSS Inc., Chicago, USA). Mean comparisons were tested using Duncan's test ($p<0.05$). Fatty acid profile results of emulsions are presented as mean±SE and subjected to independent-samples t-test for determining significant differences between treatment means.

Results

In the present study, juvenile crayfish fed with *artemia* enriched with commercial emulsions (red pepper and olio ω3) had higher weight gain, specific growth and survival rate compared to un-enriched group ($p<0.05$, Table 2). In addition, weight gain, specific growth and survival rate of juvenile crayfish increased with increasing amount of EPA (3.17 % in *artemia* group enriched with olio ω3) and DHA (3.56 % in *artemia* group enriched with red pepper, Table 3).

Table 2: Parameters of growth of *A. leptodactylus* juveniles.

	Un-enriched	Red pepper	Olio ω3
Final weight (g)	0.052±0.00[b]	0.078±0.01[a]	0.080±0.01[a]
Carapace (mm)	6.94±0.11[b]	7.51±0.11[a]	7.50±0.10[a]
Total length (mm)	12.82±0.18[b]	14.48±0.19[a]	14.13±0.15[a]
Weight gain (g)	0.01±0.00[b]	0.04±0.01[a]	0.04±0.00[a]
Specific growth rate (%)	0.98±0.14[b]	2.32±0.54[a]	2.32±0.16[a]
Survival rate (%)	40.00±1.92[b]	72.22±4.84[a]	78.88±2.22[a]

All values are given in mean± SE. Values with differing letters were significantly diferent (p<0.05) from others in the same line.

Table 3: Fatty acid profile of *artemia* of un-enriched and enriched groups (%).

	Un-enriched	Red pepper	Olio ω3
C12:0	1.85±0.12	1.51±0.18	1.33±0.01
C14:0	1.11±0.00[b]	1.48±0.04[a]	1.01±0.01[b]
C14:1	0.48±0.00[a]	0.10±0.01[c]	0.44±0.01[b]
C15:0	0.16±0.01[b]	0.17±0.00[a]	0.18±0.00[a]
C15:1	0.53±0.02	0.49±0.00	0.49±0.01
C16:0	10.16±0.17[b]	13.06±0.29[a]	10.69±0.04[b]
C16:1	1.84±0.01[b]	1.81±0.02[b]	2.98±0.26[a]
C16:2	0.88±0.05[a]	0.37±0.02[b]	0.47±0.08[b]
C16:3	1.29±0.13	0.87±0.12	0.93±0.05
C17:0	0.28±0.05[a]	0.18±0.00[ab]	0.13±0.00[b]
C18:0	5.68±0.40[a]	3.86±0.09[b]	3.78±0.01[b]
C18:1 n-9	19.26±0.18	17.67±0.29	18.76±0.54
C18:1n-7	8.97±0.13[a]	6.17±0.08[b]	6.01±0.05[b]
C18:2 n-6	6.34±0.16	6.02±0.01	6.40±0.05
C18:3 n-3	26.61±0.62	25.21±0.54	25.11±0.29
C20:4 n-6 (ARA)	0.74±0.02	0.75±0.02	0.80±0.01
C20:5 n-3 (EPA)	1.56±0.11[b]	1.46±0.04[b]	3.17±0.00[a]
C22:6 n-3 (DHA)	0.28±0.07[c]	3.56±0.15[a]	1.39±0.01[b]
SFA	19.22±0.64[a]	20.25±0.24[a]	17.12±0.04[b]
MUFA	31.07±0.33[a]	27.13 ±0.20[c]	28.67 ±0.25[b]
PUFA	37.69±0.93	38.23±0.33	38.26±0.22
Total HUFA	2.58±0.03[c]	5.77±0.09[a]	5.36±0.00[b]

All values are given in mean± SE. Values with differing letters were significantly diferent (p<0.05) from others in the same line.

When profile of fatty acids of the emulsions are examined, it is observed that the differences among fatty acids; C16:0, C16:1, C18:0, C18:2n6, C18:3n3, C20:5n3 and C22:6n3 are statistically significant (p<0.05). Especially in emulsions, it is seen that DHA content of red pepper was high (27.59 %) and EPA content (19.13 %) of olio ω3 was higher comparing to other groups. From the point of view of HUFA and total lipid contents, there was no significant difference between the two emulsions (p>0.05, Table 1).

The *artemia* enriched with red pepper (20.25%) and un-enriched *artemia* (19.22%) groups had higher SFA (saturated fatty acid) content than *artemia* enriched with olio ω3 (17.12%) group, (p<0.05). MUFA content of un-enriched *artemia* (31.07 %) group was higher than olio ω3 (28.67 %) and red pepper (27.13%) groups (p<0.05). PUFA (polyunsaturated fatty acids) and ARA (arachidonic acid; 20: 4n – 6) contents were similar in all groups of this study (p>0.05). The *artemia* enriched with olio ω3 group (3.17%) had

the highest EPA (20: 5*n* – 3) content than those of the un-enriched group (1.56%) and enriched with red pepper (1.46%) group (p<0.05). The highest DHA (docosapentaenoic acid; 22:6*n*–3) content was found in *artemia* enriched with red pepper (3.56%) group (p<0.05).

According to the results of the analyses of juvenile crayfish fatty acids, there was no significant difference between SFA and MUFA values. Besides, there were significant differences between PUFA and HUFA values between the groups fed with enriched feed and the control group (p<0.05). While the highest EPA value was found at the group fed with *artemia* enriched with olio ω3, the highest DHA value was found at the group fed with *artemia* enriched with red pepper (p<0.05).

The highest DHA/EPA rate was found in *artemia* enriched with red pepper (2.43%). The highest EPA /DHA rate was found in un-enriched *artemia* (5.57%). The *artemia* enriched with red pepper showed higher protein content (53.08 %) than olio ω3 (51.00 %) and un-enriched (49.06%) groups. The total lipid content in *artemia* ranged from 12.82 to 28.70 % in this study. Total lipid content was found higher in *artemia* enriched with red pepper (28.70%) and olio ω3 (24.76%) than un-enriched *artemia* (12.82%) group (p<0.05). The highest protein/lipid rate was determined in un-enriched *artemia* (3.84%) compared to other groups (p<0.05, Table 4).

Table 4: Protein, lipid, Protein/lipid, DHA, EPA, DHA/EPA and EPA /DHA rates of enriched *artemia*.

	Un-enriched	Redpepper	Olio ω3
Protein (%)	49.06±0.20^{a}	53.08±0.51^{b}	51.00±1.07^{ab}
Lipid (%)	12.82±0.86^{a}	28.70±1.41^{b}	24.76±0.11^{b}
Protein/Lipid	3.84±0.24^{a}	1.85±0.07^{b}	2.06±0.05^{b}
C22:6 n-3 (DHA, %)	0.28±0.07^{c}	3.56±0.15^{a}	1.39±0.01^{b}
C20:5 n-3 (EPA, %)	1.56±0.11^{b}	1.46±0.04^{b}	3.17±0.00^{a}
DHA/EPA	0.18±0.05^{b}	2.44±0.17^{a}	0.44±0.00^{b}
EPA /DHA	5.57±1.84^{a}	0.41±0.03^{b}	2.28±0.01^{ab}

All values are given in mean± SE. Values with differing letters were significantly diferent (p<0.05) from others in the same line.

Discussion

There are several studies on enrichment of live food with EFA in shrimp (Rees, *et al*.,1994; Alam *et al*., 1995; Immanuel *et al*., 2001; Immanuel *et al*., 2004; Das *et al*., 2007). There is also one study on lobsters (Chakraborty *et al*., 2010). However, there is no published study on freshwater crayfish. These studies indicated that survival rate and growth increased in shrimps and lobsters fed with *artemia* nauplii enriched with HUFA (Millamena *et al*., 1988; Abelin, 1991; Citarasu *et al*.,1998; Immanunuel *et al*., 2001; Immanunuel *et al*., 2004; Chakraborty *et al*., 2010). The results of these studies are similar to those of the present study, juvenile crayfish fed wiht *artemia* enriched with high HUFA (in *artemia* enriched with

red pepper, 5.77% ; in *artemia* enriched with olio ω3, 5.36 %) displayed the highest survival rate and growth. However, Rees *et al*. (1994) indicated that *n*-3HUFA (8.10 %) in enriched *artemia* caused increased growth and survival rate of *P. monodon* postlarvae. But the high levels of *n*-3HUFA (16.60 %) did not show any impact on growth. Romdhane *et al*. (1995) indicated that freshwater prawn (*Macrobranchium rosenbergii*) require a minimum n-3 HUFA of 35mg/g concentration in its diet. Nghia *et al*. (2007) reported that an emulsion containing total HUFA of 30% showed the best growth in larval development of the mud crab *Scylla paramamosain*.

In the present study, the highest EPA content (3.17%) was found in the group enriched with olio ω3 compared to the un-enriched *artemia* and enriched *artemia* with red pepper groups (p<0.05). The highest DHA content (3.56 %) was found in *artemia* enriched with red pepper (p<0.05). Growth, specific growth rate and survival rate of juvenile crayfish are increased with increased amount of EPA and DHA in the diet. Similarly, Das *et al*. (2007) indicated that the growth and survival rates of *M. rosenbergii* larvea fed with *Moina micrura* enriched with sunflower oil, cod liver oil and maxi EPA capsules (a capsule with a product name) were increased with increased amounts of EPA and DHA in the diet. Immanuel *et al*. (2001; 2004) reported that EPA and DHA rates were increased from un-enriched *artemia* to *artemia* enriched with 3 % *O. niger* liver oil. However, the growth reduced with decreasing EPA and DHA rates in *artemia* enriched with 4 % *O. niger* liver oil. Rees *et al*. (1994) determined that EPA and DHA were increased from un-enriched *artemia* to the enriched group in 400ppm 12h SELCO but, growth of *P. monodon* larvea reduced after fed with enriched *artemia* with 300ppm 12h SELCO.

In this study, the highest EPA/DHA rate (5.57) was determined in un-enriched *artemia* and high EPA/DHA rate was effected as negative on growth of crayfish. The highest DHA/EPA rate was determined in enriched *artemia* with red pepper and high DHA/EPA rate had positive effect on growth of the crayfish. Results of the present study are in agreement with those of Rees *et al*. (1994). EPA/DHA rate was decreased from 7.25 in un-enriched *artemia* group to 2.03 in enriched *artemia*. The growth increased with decreasing of EPA/DHA rates (Rees *et al*., 1994). However, Wouters *et al*. (1997) indicated that DHA/EPA rates increased in *artemia* enriched with coconut oil including 4 different HUFA rates but significant difference was not observed in growth and survival of *Penaeus vannamei* larvae. Sui *et al*. (2007) indicated that optimal DHA/EPA in *artemia* should be 0.56 for growth and survival of the crab larvae (*E. sinensis*). Nghia *et al*. (2007) recommended that DHA/EPA ratio in live food should be 4 for larval development of mud crab.

In the present study, linoleic and linolenic acids contens in between un-

enriched or enriched *artemia* groups were not significant (p>0.05). Therefore, we do not consider, linoleic and linolenic acids contens effective on growth of juvenile crayfish. Similarly, the nutritional trails on *P. japonicus* demonstrated that n-3 PUFAs such as EPA and DHA were more effective in promoting growth than linoleic (18:2n-6) and linolenic (18:3n-3) acids (Kanazawa *et al*., 1979; Kayama *et al*., 1980). However, Immanuel *et al*. (2001; 2004) indicated that concentration of linolenic acid (18:3n-3) was increased from un-enriched to 3% lipid diet and low concentration resulted in poor performance in terms of survival and development in shrimp.

In the present study, PUFA contents differences were not statistically significant among all groups (p>0.05). However, Immanuel *et al*. (2001, 2004) indicated that PUFA content increased from un-enriched *artemia* to 3% lipid diet group. They observed that growth increased until the 3% lipid diet group.

In the present study, SFA and MUFA contents showed variation in all groups. Similary, Immanuel *et al*. (2001, 2004) determined that SFA and MUFA contents of enriched *artemia* and un-enriched showed variation. Decreasing MUFA content of enriched *artemia* groups did not show any adverse effect on growth of juvenile crayfish.

In the present study, protein/lipit rates were found in enriched *artemia* with red pepper (1.85) and in enriched *artemia* with olio ω3 (2.06) to show better growth than those fed with un-enriched *artemia*. The highest protein/lipit rate was obtained from un-enriched group (3.84) and this group showed the worst growth (p<0.05). Cervantes-Santiago *et al*. (2010) indicated that the best growth rate was observed in crayfish juneniles of *P. acanthophorus* fed with 2.08 (protein/lipid) level diet. Carmona-Osalde *et al*. (2005) recommended using of diet with 1.7 (protein/lipid) for growth and diet, and with 2.5 (protein/lipid) for maturation of crayfish.

In the present study, better growth values were obtained in crayfish fed with *artemia* enriched with red pepper and olio ω3. These groups (28.70 %, 24.76 %) contain higher lipid than un-enriched *artemia* (12.82 %) group (p<0.05). Rees *et al*. (1994) pointed that high lipid rates was obtained from enriched *artemia* groups while the lowest lipid rates obtained from un-enriched *artemia*. The best growth values were obtained from shrimps fed enriched *artemia* containing 21 % and 23 % lipid.

In this study, while EPA content (12.78 %) at the bodies of crayfish fed with *artemia* enriched with olio w3 was found high, DHA value (10.04 %) at the bodies of crayfish fed with *artemia* enriched with red pepper was found high (p<0.05). When looked at other studies on crustacean species, it is not seen as high DHA and EPA values as these at the analyses made on the body of the crustacean (Rees *et al*., 1994; Immanuel *et al*., 2001; Immanuel *et al*., 2004; Das *et. al*., 2007).

Besides, Harlioglu *et al*., (2012) results were compatible with the present study results, DHA values are found

7.77 % and 6.02 % respectively in the study looked at the content of EFA (*A. leptodactylus*) of the wild and captive crayfish. These values are similar to the control group and the group fed with *artemia* enriched with olio w3 in the present study (6.17 %, 7.42%).

Also compatible with Harlioglu *et al*. (2012) findings of high EPA rates (6.11 %, 12.785) is the present study results. Both of the two studies showed that *A. leptodactylus is* a species that needs DHA and EPA in high rates.

In conclusion, emulsions containing high HUFA such as EPA and DHA increased growth and survival rates of juvenile crayfish (*A. leptodactylus*). It was shown that juvenile crayfish require high HUFA (especially EPA and DHA) in their diets. There is a need to determine required HUFA, especially EPA, DHA and DHA/EPA rates for juvenile crayfish in future studies. Also, we can recommend combined uses of emulsions containing high EPA and DHA.

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