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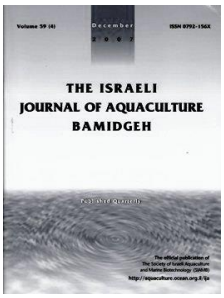

Growth, Survival and Fatty Acid Composition of Freshwater Crayfish (*Astacus leptodactylus*) Juveniles Fed Enriched *Daphnia magna* as an Alternative to *Artemia*

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Key words: enrichment; *Daphnia magna*; anchovy oil emulsion; *Astacus leptodactylus;* growth; survival

Abstract

This experiment was conducted to investigate the effects *Daphnia magna* enriched with lipid emulsions as an alternative to *Artemia*, on growth, survival rate, and fatty acid composition of freshwater crayfish (*Astacus leptodactylus* Esch. 1823). The four treatment groups were (a) unenriched *Artemia* (UEA), (b) unenriched *D. magna* (UED), (c) *D. magna* enriched with redpepper emulsion (DER), and (d) *D. magna* enriched with anchovy oil emulsion (DEA). All tests were carried out in triplicate for 30 days. The crayfish (mean weight 0.12g) were fed ad libitum once daily. At the end of experiment, the highest eicosapentaenoic acid, 20:5n-3 (EPA) level was found in the DEA group (5.77%). The highest DHA (docosahexaenoic acid, 22:6n-3) level was found in the DER group (2.73%) which was statistically similar to the DEA group. In addition, high n-3 HUFA (high unsaturated fatty acid) levels were detected in enriched *D. magna* groups with emulsions. However, high EPA levels in enriched *D. magna* groups with emulsions were not reflected in crayfish tissues, but DHA level was reflected in crayfish tissues fed with anchovy oil emulsion. The crayfish fed with *D. magna* showed similar growth to that of the *Artemia* fed groups. The growth of the enriched *D. magna* groups did not differ.

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Introduction

Astacus leptodactylus is a native crayfish species in Turkey (Köksal, 1988). There is no crayfish aquaculture in Turkey and all production is obtained from wild harvests (Harlıoglu et al, 2012). Turkey was the largest supplier of *A. leptodactylus* to Western Europe from 1970 until 1986 but harvests were severely reduced in most wild populations due to infection by crayfish plague, (*Aphanomyces astaci)* after 1985 (Köksal, 1988; Ackefors, 2000). The harvest in 2012 was only 492 tons (Anonymous, 2014). To replenish native stocks of A. *leptodactylus,* exogenous production of juvenile crayfish is needed. However, the nutritional requirements of this species in culture are unknown.

Artemia nauplii are a main food source for larval forms of crustaceans (Immanuel et al., 2007). However, these are expensive and scarce (Das et al., 2007). Crayfish in nature feed mainly on rotifers and benthonic crustaceans (cladocerans and copepods) and, later on larvae of aquatic insects (Hessen, 1989). Cladocerans can possibly substitute for *Artemia* in cultured freshwater crustaceans (Alam, 1995; Das et al. 2007). Commercial emulsions are expensive, therefore, both *Artemia* and commercial emulsions are not practical in freshwater crayfish culture.

In shrimp fed cladoceran enriched with highly unsaturated fatty acids, (HUFA), growth and survival rates increased (Das, et al., 2007). HUFA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), have been recognized as very important nutrients for the growth of crustaceans (Alam, 1995; Das et al., 2007). However, there is no literature on crayfish fed with enriched cladocerans.

In the present study, the effects of enriched *Daphnia magna* with lipid emulsions as an alternative to *Artemia* on growth, survival rate, and fatty acid profile in *A. leptodactylus,* were investigated.

Materials and Methods

Fatty acid profile of anchovy oil and ingredient of anchovy oil emulsion. The ingredients of anchovy oil emulsion, and the fatty acid profile of anchovy oil for emulsion, are given in Tables 1 & 2.

Table 1. Ingredients of anchovy oil emulsion

Ingredients	*Amount*
Water (ml)	100
Anchovy oil (ml)	50
Egg yolk (ml)	20
Vitamin mix(g)	0.50

Vitamin mix (Rovimix BCK for 100g): 500mg vitamin B1; 1000mg vitamin B2; 500mg vitamin B6; 3mg vitamin B12; 10000mg vitamin C; 5000mg niacin; 300mg vitamin K3; 10mg D-Biotin; 1500mg Cal.D. Pantothenate; folic acid 150mg.

Table 2. Fatty acid profile of anchovy oil as percentage of total lipid

Fatty acid	*Anchovy oil*
12:0	0.06±0.00
14:0	6.03±0.00
15:0	0.13±0.00
16:0	15.69±0.18
17:0	0.35±0.01
18:0	2.91±0.03
20:0	0.26±0.01
23:0	0.11±0.01
24:0	1.02±0.02
14:1	0.25±0.01
16:1	9.11±0.40
17:1	0.63±0.01
20:1	0.29±0.01
18:1 n9	13.00±0.15
18:1n7	3.04±0.06
22:1n9	0.17±0.02
18:2 n6	1.91±0.01
20:3n6	0.11±0.00
20:4 n6	0.54±0.01
18:3 n3	1.22±0.01
20:5 n3 (EPA)	11.29±0.09
22:6 n3 (DHA)	18.43±0.24
20:2	1.07±0.00
22:2	0.17±0.02
SFA	26.53±0.23
MUFA	26.48±0.29
PUFA	34.72±0.31
HUFA	31.60±0.30

Table3. Fatty acid profile of redpepper and anchovy oil emulsions as percentage of total lipids.

Fatty Acid	*Redpepper emulsion*	*Anchovy oil emulsion*	*α*
12:0	0.11±0.01	0.06±0.00	*
14:0	5.57±0.46	5.66±0.16	ns
14:1	0.18±0.01	0.25±0.01	*
15:0		0.12±0.00	-
16:0	34.90±1.77	15.61±0.08	**
16:1	1.87±0.03	8.90±0.28	**
17:0	0.07±0.01	0.33±0.01	**
17:1	0.10±0.01	0.59±0.00	**
18:0	0.88±0.05	2.87±0.03	**
18:1 n9	2.45±0.26	17.46±0.19	**
18:1n7		0.18±0.01	-
18:2 n6	1.48±0.01	2.78±0.03	**
18:3 n3	0.40±0.02	1.20±0.01	**
20:0	0.17±0.02	0.30±0.02	*
20:1	0.21±0.01	0.31±0.02	*
20:2	2.24±0.11	1.06±0.00	**
20:3n6	0.09±0.03	0.10±0.01	ns
20:4 n6	0.54±0.09	0.28±0.02	ns
20:5 n3 (EPA)	2.12±0.13	10.82±0.10	**
22:1n9	0.55±0.14	0.20±0.06	ns
22:2	0.32±0.02	0.28±0.03	ns
24:0	0.41±0.04	0.96±0.00	**
22:6 n3 (DHA)	27.59±0.92	18.10±0.12	**
SFA	42.10±1.19	25.92±0.28	**
MUFA	5.35±0.17	27.87±0.14	**
PUFA	34.76±1.26	34.61±0.02	ns
HUFA	32.88±1.24	30.64±0.03	ns

Enrichment and culture of D. magna. Two concrete ponds (20 tons capacity) were prepared for mass culture of *D. magna* on a rotation basis. Inorganic fertilizers containing nitrogen and phosphate were used for culture of *D. magna*. The water quality parameters for the rearing of *D. magna* were temperature, 22-24°C; pH, 7.0-7.5; and dissolved oxygen, 5.2-6.7 mg/L throughout the culture period. Each separate *D. magna* group was kept in 10 L capacity plastic buckets containing 4 L of water. The enrichment was conducted in emulsions containing anchovy oil (1.25g/L) and redpepper (0.75 g/L) (Akuamaks, Ankara/TURKEY**)** in two parts for a time period of 24 h with mild aeration. Fatty acid profiles of emulsions are given in Table 3.

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

Artemia culture. 0.5-1.0g of encapsulated *Artemia salina* cysts (INVE Aquaculture, Izmir/TURKEY) were hatched and harvested according to Sorgeloos et al., 1986.

Rearing conditions of crayfish. The crayfish were obtained from broodstock captured from Egirdir Lake, Turkey. A 30 day feeding trial was conducted with crayfish (initial weight 0.12g). The crayfish were stocked into 12 aquaria (40 x 70 cm base area) at a density of 35 individuals /m^2. Pipes (2cm diameter and 4 cm length) were placed in each aquarium to provide shelter. The physicochemical water conditions were: temperature 18.3±0.07°C, dissolved oxygen 6.2±0.09 mg/L, ammonium 0.01±0.07 mg/L, pH 7.5-8.5, calcium 64.8 mg/L, magnesium 11.2 mg/L, hardness 77.1 mg/L, photoperiod 12/12 which were all kept constant throughout the experiment. Feed remains and feces were siphoned daily and 20% of water was exchanged. Four treatments UEA, UED, DER, and DEA, were applied in triplicate. The crayfish were fed ad libitum once daily (Calvo et al.,

2013). They were measured to the nearest 1 mm from the tip of the rostrum to the end of telson, and weighed to the nearest 0.1 mg after removing the excess water.

Growth parameters. The growth parameters were calculated at the end of the feeding experiment:

Weight gain (g) = final weight - initial weight

Specific Growth Rate (SGR) % = (ln final weight - ln initial weight) / days x100

Survival rate (%) = (Final crayfish number/Initial crayfish number) x100

Fatty acid analyses. The live food, crayfish tissue, and emulsions were stored until further analysis at -80°C. Fatty acid analyses of live food, crayfish tissue, and emulsions, were analyzed by GC Clarus 500 with autosampler (Perkin Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE (scientific glass engineering) column (30 mX 0.32 mm ID X 0.25 µm BP20 0.25 UM, USA). The oven temperature was 140°C, held for 5 min, raised to 200°C at rate 4°C/min and held at 220°C at rate 1°C/min, while the injector and the detector temperature were set at 220°C and 280°C, respectively. The sample size was 1 µl and the carrier gas was controlled at 16 ps. The split used was 1:50. Fatty acids were identified by comparing the retention times of FAME with the standard 37 component FAME mixture. Two replicate *gas chromatography* (GC) analyses were performed and the results expressed in GC area % as a mean value and ± standard deviation.

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

Fatty acid profile of emulsions. The highest EPA content (10.82%) was found in anchovy oil emulsions while the highest DHA content was found in redpepper emulsions ($P<0.01$). There was no significant difference in PUFA and HUFA contents between the different emulsions. The highest SFA content (42.10 %) was found in redpepper emulsions while the highest MUFA content was found in anchovy oil emulsions ($P<0.01$).

Growth and survival. Final weight, carapace length, total length, weight gain, and SGR showed no significant differences among the groups ($P>0.05$). However, enriched groups showed a trend towards increased final weight, weight gain, and SGR. UED and DER groups showed higher survival rate ($P<0.05$) than UEA and DEA groups (Table 4).

Table 4 Survival and growth performance of *A. leptodactylus*

	UEA	*UED*	*DEA*	*DER*
Initial weight (g)	0.12±0.02	0.12±0.01	0.12±0.02	0.12±0.02
Final weight (g)	0.28±0.03	0.29±0.02	0.30±0.03	0.30±0.02
Weight gain (g)	0.16±0.01	0.17±0.02	0.18±0.01	0.18±0.01
Final carapax (mm)	11.26±0.36	11.42±0.29	11.74±0.33	11.56±0.29
Final total length (mm)	21.96±0.77	22.52±0.57	22.97±0.62	22.47±0.59
Survival rate (%)	60.00±1.92^{b}	90.00±3.85^{a}	70.00±1.92^{b}	88.88±2.94^{a}
SGR (%)	2.88±0.45	2.96±0.29	3.11±0.45	3.03±0.51

All the values are given in mean± SE. Values with different superscript letters were significantly different ($P<0.05$) from others in the same row.

Fatty acid profile in tissues of D. magna and Artemia. A total of sixteen acids were detected and the fatty acid profiles in tissues of *D. magna* and *Artemia* were recorded (Table 5). The enrichment process affected the fatty acid profile of *D. magna*. The amount of EPA and DHA increased in enriched *D. magna* groups. DEA group showed the highest level (5.77 %) of EPA ($P<0.05$). This value was considerably higher than in the other groups. The highest level of DHA (2.73 %) was found in DER group ($P<0.05$). Contents of n-3 HUFA were higher in enriched *D. magna* tissues than in unenriched *D. magna* and *Artemia* ($P<0.05$). The highest n-6 HUFA content was found in the *Artemia* tissues ($P<0.05$). The n-6/n-3 ratio ranged from 0.29 to 0.96.

Table 5. Fatty acid profile in tissues of *D. magna* and *Artemia*

Fatty Acid	*UEA*	*UED*	*DEA*	*DER*
Saturated fatty acids				
14:0	0.69±0.02^{b}	3.16±0.03^{a}	3.51±0.27^{a}	3.13±0.14^{a}
15:0	-	0.19±0.02	0.29±0.12	0.20±0.01
16:0	7.21±0.22^{d}	19.55±0.04^{a}	16.40±0.17^{c}	18.12±0.66^{b}
17:0	0.63±0.02^{a}	0.62±0.02^{a}	0.35±0.01^{b}	0.23±0.01^{c}
18:0	4.48±0.05^{a}	2.24±0.07^{c}	3.25±0.01^{b}	1.94±0.43^{c}
20:0	0.51±0.05^{a}	0.09±0.02^{b}	0.17±0.01^{b}	0.19±0.04^{b}
Total SFA	13.52±0.25^{c}	25.85±0.08^{a}	23.95±0.21^{b}	23.80±0.39^{b}
Monounsaturated fatty acids				
18:1n-7	-	3.82±0.42^{c}	8.35±0.55^{a}	6.25±0.15^{b}
18:1n-9	12.40±0.10^{ab}	16.42±0.38^{a}	12.31±0.32^{ab}	9.36±2.56^{b}
20:1	-	0.18±0.02	0.34±0.03	0.61±0.33
Total MUFA	12.40±0.10^{b}	20.41±0.05^{a}	21.01±0.84^{a}	16.22±3.01^{ab}
Polyunsaturated fatty acids				
18:2n-6	4.99±0.03^{c}	10.76±0.23^{a}	4.81±0.06^{c}	6.14±1.94^{b}
20:3n-6	1.22±0.06^{a}	0.07±0.00^{c}	0.34±0.00^{b}	0.15±0.09^{bc}
20:4n-6	1.70±0.21^{a}	0.27±0.03^{b}	0.33±0.02^{b}	0.33±0.14^{b}
18:3n-3	24.71±0.77^{a}	10.53±0.10^{b}	3.21±0.20^{c}	3.83±1.23 ^{c}
20:5n-3 (EPA)	1.84±0.13^{b}	0.17±0.06^{c}	5.77±0.77^{a}	3.25±0.10^{b}
22:6n-3 (DHA)	0.35±0.10^{c}	0.89±0.12^{cb}	1.99±0.52^{ab}	2.73±0.18^{a}
20:2	0.36±0.03^{d}	0.93±0.05^{c}	1.72±0.03^{b}	1.99±0.12^{a}
Total PUFA	35.15±1.17^{a}	23.61±0.08^{b}	18.15±0.03^{c}	18.41±1.50^{c}
Total n-6	7.91±0.13^{b}	11.10±0.20^{a}	5.48±0.05^{c}	6.61±0.66^{bc}
Total n-3	26.89±1.27^{a}	11.58±0.07^{b}	10.96±0.05^{b}	9.81±0.95^{b}
n-6/n-3	0.29±0.02^{d}	0.96±0.02^{a}	0.50±0.01^{c}	0.67±0.00^{b}
Total n-6 HUFA	2.92±0.15^{a}	0.34±0.03^{b}	0.67±0.02^{b}	0.48±0.23^{b}
Total n-3 HUFA	2.18±0.03^{c}	1.05±0.17^{d}	7.76±0.25^{a}	5.98±0.28^{b}

All the values are given in mean± SE. Values with different superscript letters were significantly different (P<0.05) from the others in the same row.

Fatty acid composition of A. leptodactylus. Fatty acid profile in tissues of crayfish is given in Table 6. The highest DHA content in tissue was found in crayfish fed DEA (9.07 %). This was similar to the DER group. The high DHA content in enriched *D.magna* groups was reflected in crayfish tissues. The highest EPA level (5.77%) was found in the *D. magna* enriched with anchovy oil emulsion, EPA levels in crayfish tissues were similar in all groups (P>0.05). n-3 HUFA contents were similar in all groups (P<0.05).

Table 6. Fatty acid composition in tissues of *A. leptodactylus*

Fatty Acid	*UEA*	*UED*	*DEA*	*DER*
Saturated fatty acids				
14:0	2.36±0.23^{b}	5.13±0.57^{a}	5.37±0.07^{a}	4.96±0.74^{a}
15:0	0.29±0.08^{a}	0.10±0.00^{b}	0.07±0.01^{b}	0.08±0.00^{b}
16:0	14.68±0.89^{b}	17.25±0.02^{ab}	18.23±0.04^{a}	17.72±0.58^{a}
18:0	4.16±0.22	4.38±0.24	3.84±0.04	4.46±0.04
20:0	0.26±0.03	0.28±0.06	0.21±0.02	0.34±0.13
Total SFA	21.74±0.80^{b}	27.14±0.63^{a}	27.71±0.03^{a}	27.56±0.25^{a}
Monounsaturated fatty acids				
15:1	0.25±0.06^{d}	0.58±0.01^{c}	0.78±0.02^{b}	1.36±0.05^{a}
16:1	3.66±0.18^{b}	4.43±0.08^{a}	4.54±0.10^{a}	3.84±0.14^{b}
18:1n-9	22.70±1.07^{ab}	24.66±0.29^{a}	23.55±0.13^{ab}	22.37±0.02^{b}
22:1n-9	0.05±0.01^{c}	-	0.26±0.02^{a}	0.18±0.01^{b}
Total MUFA	26.65±0.95^{b}	29.66±0.20^{a}	29.12±0.03^{a}	27.75±0.13^{ab}
Polyunsaturated fatty acids				
18:2n-6	6.88±0.60	7.69±0.21	7.83±0.05	6.51±0.28
20:3n-6	1.05±0.03^{a}	0.22±0.01^{b}	0.18±0.00^{b}	0.22±0.00^{b}
20:4n-6	0.79±0.08^{a}	0.31±0.02^{b}	0.35±0.05^{b}	0.29±0.02^{b}
18:3n-3	1.14±0.11^{c}	1.84±0.01^{a}	1.67±0.05^{ab}	1.58±0.03^{b}
20:5n-3 (EPA)	6.16±0.17	5.35±0.45	5.72±0.55	6.01±1.07
22:6n-3 (DHA)	6.67±0.44^{c}	7.54±0.40^{bc}	9.07±0.08^{a}	7.99±0.01^{ab}
Total PUFA	22.67±0.33^{ab}	22.93±0.23^{ab}	24.81±0.67^{a}	22.59±0.76^{b}
Total n-6	8.72±0.70^{a}	8.21±0.18^{ab}	8.36±0.10^{ab}	7.02±0.30^{b}
Total n-3	13.96±0.38	14.73±0.05	16.46±0.58	15.58±1.06
n-6/n-3	0.63±0.07^{a}	0.56±0.01^{ab}	0.51±0.01^{ab}	0.45±0.05^{b}
Total n-6 HUFA	1.85±0.11^{a}	0.53±0.03^{b}	0.53±0.05^{b}	0.51±0.06^{b}
Total n-3 HUFA	12.82±0.27	12.89±0.06	14.79±0.62	14.00±1.08

All the values are given in mean± SE values with different superscript letters were significantly different (P<0.05) from the others in the same row

Discussion

To our knowledge there are no published studies on the effects of *Daphnia magna* enriched with lipid emulsions used as an alternative to *Artemia,* on growth and fatty acid composition of crustaceans.

In this study, *D. magna* and *Artemia* fed groups showed similar growth performance. The enriched *D. magna* groups did not show differences in final weight, weight gain, and SGR. Growth rate and survival increased in shrimp fed enriched cladoceran with HUFA (Alam, 1995; Das et al., 2007). These positive effects were also observed on growth and survival rate in crustacea fed enriched *Artemia* (Millamena et al., 1988; Abelin 1991; Romdhane et al., 1995; Citarasu et al., 1998; Immanuel et al., 2001; Immanunuel et al. 2004; Chakraborty et al. 2010). High levels of n-3 HUFA in diets had no growth promoting effects in shrimps (Rees et al. 1994). In the present study, n-3 HUFA content increased in enriched *D. magna* tissues (DEA and DER groups) compared with unenriched groups (UEA and UED groups). The growth and survival rates did not increase with increasing n-3 HUFA levels. The optimum HUFA levels in *A. leptodactylus* still need investigation.

Growth performance of shrimp increased with increasing EPA and DHA rates in enriched *Artemia* (Immanuel et al., 2001; 2004; Rees et al., 1994). Growth and survival rates of *Macrobrachium rosenbergii* larvae fed enriched *Moina micrura* increased with increasing amount of EPA and DHA in the diets (Das et al. 2007). Increasing the level of both EPA and DHA above a certain inclusion level resulted in a decrease in growth of *Penaeus monodon* (Glencross & Smith, 2001). In the present study, the highest EPA content was found in *D. magna* enriched with anchovy oil while the highest DHA content was found in *D. magna* enriched with redpepper. These enrichments did not result in enhanced growth.

Increasing levels of EPA and DHA affected the n-6:n-3 balance and adversely affected growth (Glencross & Smith 2001), however, n-6/n-3 in the diet ratios showed a decline in enriched *Moina micrura* and improved growth of *M. rosenbergii* post larvae fed the lowest n-6/n-3 ratio (Das et al. 2007). In the present study, n-6/n-3 ratio was lower in the enriched groups of *D. magna* than in the unenriched ones. The lowest n-6/n-3 ratio was determined in the *Artemia* fed group. This may be because the C18:3 n3 level in *Artemia* was much higher than in the *D. magna* groups.

EPA and DHA levels of *Moina micrura* increased with enrichment (Das et al. 2007). Similarly, in the current study, EPA and DHA contents showed significant increase with enrichment of *D. magna*. Although EPA and DHA levels of *D. magna* enriched with emulsions were the highest we believe that these were not sufficient to enhance growth. DHA and EPA contents in tissues of wild and captive adult *A. leptodactylus* ranged between 7.77%-23.10% and 6.02%-13.51% respectively (Harlioglu et al., 2012). In the current study, DHA levels were higher or equal while EPA levels were lower and therefore need to be enhanced.

Post larvae fed higher dietary n-3 fatty acids showed higher n-3 fatty acids in tissues (Das et al. 2007). In contrast, the highest n-3 fatty acids were seen in the *Artemia* diet in the current study, but n-3 fatty acids in crayfish tissues were similar in all groups.

Crayfish groups fed with *D. magna* showed similar growth to the *Artemia* group however growth of crayfish groups fed enriched *D. magna* with emulsions was not affected. DHA levels were high in crayfish tissues of groups fed anchovy oil emulsion yet we believe that HUFA levels of emulsions were not sufficient in the enriched groups and that the HUFA requirements of *A. leptodactylus* still need to be determined.

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