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Enrichment of *Daphnia magna* with Canola Oil and its Effects on the Growth, Survival and Stress Resistance of the Caspian Kutum (*Rutilus frisii kutum*) Larvae

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Abstract

In freshwater larviculture, *Daphnia* species have received considerable attention as live food. This study examined the use of canola oil to enrich *Daphnia magna* as a function of enrichment duration. The experiment compared four enrichment durations (3, 6, 9, and 12 h) with a non-enriched control group. An enrichment time up to 6 h resulted in the highest amounts of polyunsaturated fatty acids (PUFAs) in the fatty acid profile of *D. magna*. Afterward, *D. magna* enriched within 6 h was fed to *Rutilus frisii kutum* larvae (initial weight of 52.62 mg) for 14 days in order to compare performance of these larvae with those fed non-enriched *D. magna* and also with larvae fed a commercial dry feed (Biomar). Specific growth rates (SGR) showed significant differences ($P \leq 0.05$) between larvae fed enriched *D. magna* and Biomar-fed fish. Larvae fed enriched and non-enriched *D. magna* presented total weight and length (173.1 mg and 31.3 mm) and (150.1 mg and 27.5 mm, respectively), and the lowest values (138.4 mg and 25.8 mm) were recorded in larvae fed Biomar. Survival rates during the larval culture period and resistance of larvae against salinity (25‰) stress were not statistically different ($P \geq 0.05$) among the treatments. However, a salinity stress of 13‰ led to significant differences ($P \leq 0.05$) with highest survival (93.33%) in fish fed 6-h-enriched *D. magna*. Enrichment of *D. magna* with canola oil can be applied as a useful method to increase the stock enhancement of such valuable species as *R. frisii kutum*.

Keywords: Enrichment, *Daphnia magna*, Canola oil, *Rutilus frisii kutum*, Essential fatty acids (EFAs), Specific growth rate.

Introduction

The relative dependence of farmed fish larvae upon a variety of live feed during early feeding stages is considered to be inevitable for larviculture of most fish species (De Pauw *et al*., 1981; Lavens and Sorgeloos, 1996; Han *et al*., 2001). Lipids provide energy, and EFAs (Essential fatty acids) function as precursors in defense mechanisms as well as important components of cell membrane (Vance and Vance, 1985; Izquierdo, 1996; Sargent *et al*., 1999). Freshwater animals are not able to synthesize three fatty acids including linoleic, linolenic, and oleic acids; hence, one or more fatty acids must be included in their diets (Sargent *et al*., 1999). If these are not supplied through the diets, enrichment can be employed as an effective live feed complement technique (Sundbom and Vrede, 1997; Von Elert, 2002). Various techniques are applied for the enrichment of live feed, among which fatty acid emulsions, microencapsulated, and enriched algae with polyunsaturated fatty acids (PUFAs) are mostly used in aquaculture (Sundbom and Vrede, 1997; Weers and Gulati, 1997).

Daphnia magna is one of the most important live feed organisms for feeding freshwater fish larvae, which, in spite of having favorable protein sources for larvae development, contains a broad spectrum of digestive enzymes such as proteinases, peptidases, amylases, lipases and even cellulase (Lavens and Sorgeloos, 1996; Macedo and Pinto-Coelho, 2001). This species biosynthesizes only a very low portion (< 5%) of total fatty acid content and alternatively acquires its most required lipid from consumed foodstuffs (Goulden and Place, 1990). *D. magna* reared in cultivation ponds contained only 12% oleic acid, and the contents of EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) were reported to be 10 and 0.2%, respectively (Lavens and Sorgeloos, 1996). Ravet *et al.* (2003) enriched *D. magna* using three complements of Cyanophytes without PUFAs, a mixture of Cyanophytes plus Cryptophytes, and Cyanophytes with PUFAs. They observed the highest values for growth rate and fatty acid content in *D.*

magna enriched with Cyanophytes plus PUFAs and concluded that artificially supplemented diets are vital for enhanced performance and nutritive quality of this species. Also, Becker and Boersma (2005) showed that increased dietary concentrations of EFAs (*e.g.*, EPA and arachidonic acid) resulted in elevated deposits of these compounds in *D. magna*.

Lipids have been reported to promote the growth of Cladoceran species; high zooplankton growth rates could be attainable when direct dietary sources of HUFAs are available for fast-growing zooplankton (Müller-Navarra, 1995). Besides, inadequate lipid content in cultured fish diets can adversely affect the performance of larvae during the grow-out stage, since larvae often have low energy reserves and require substantial energy sources for their high somatic growth rates and development of their bodies (Fraser *et al*., 1988). Nowadays, animal lipids sources have been replaced by plant ones as a consequence of limited access and high costs of the former. Canola (*Brassica napus*), for instance, is a major oil-producing crop, and the global production of rapeseed oil was over 22 million tons during 2009 to 2010, which is considered to be the third largest source of the vegetable oil supply (Ahmadi and Ardekani, 2006). Canola oil is of high nutritional value with high concentrations of unsaturated C_{18} fatty acids (>60%), and is known to contain high quantities of oleic, linoleic, and α-linolenic acids as well as vitamins E and K, which produces no peroxides up to 200°C (Ahmadi and Ardekani 2006). These features have rendered canola oil as a suitable complement for diverse live feeds.

Limited studies have so far investigated on the enrichment of *D. magna* using vegetable oils and most examinations have used such animal products as cod oil for enrichment of *Daphnia*. Therefore, in the present study, *Daphnia magna* was enriched with canola oil at different durations to investigate possible changes in fatty acid profile of the animal as a function of time. Afterwards, the enriched *D. magna* was fed to Caspian kutum (*Rutilus frisii kutum*) larvae to study specific growth rate, survival, and resistance against salinity stress of these larvae compared to those fed non-enriched *D. magna* and Biomar (a commercial dry feed)-fed larvae.

Materials and Methods

Daphnia magna was caught by the use of zooplankton nets (100 µm) from warm-water fish ponds located at Shahid Rajaee Propagation Facilities (Sari, Iran). The samples were then purified and reared in ten 120-L tanks with an initial density of 20-100 L^{-1} individuals. A mixture of organic fertilizers and microalgae (*Chlorella* sp.) were fed to the stock cultures. The algal density was set as 10^5-10^6 cells ml^{-1} in order to have a final *D. magna* density of 5000 L^{-1} (Ghazy *et al*., 2009; Gholami, 2010). The water quality parameters for rearing of *D. magna* were contained temperature, 24°C; pH, 7.1-7.6; total hardness, 265 mg L^{-1} $CaCO_3$; and dissolved oxygen, 5.5-6.2 mg L^{-1} throughout the culture period.

The enrichment solution with canola oil, *i.e*., polysorbate (Tween 80, Merck) and freshwater were prepared as described in Ako *et al.* (1994). To do this, polysorbate (5 ml) was added to freshwater (50 ml), mixed, and then canola oil (50 ml) was included and merged. Afterward, a volume of 0.3-0.5 ml L^{-1} of the final solution as the enrichment material was added to enrichment containers (water temperature, 20°C) stocked with *D. magna* at a density of 1000 L^{-1} (Ako *et al*., 1994; Von Elert, 2002). There were four enrichment containers (each in three replicate, *n*=3) at durations of 3, 6, 9, and 12 h, being aerated via conditioning equipment. To conserve the quality of the enrichment solutions and protect them from decay, all solutions were prepared and consumed daily. The samples were washed by freshwater (to eliminate emulsified particles), then collected and oven-dried for 24 h at 60°C.

The dried *D. magna* were transferred to a laboratory at Artemia and Aquatic Animal Research Center (Urmia University, Urmia, Iran) to determine fatty acid composition. All samples were immediately frozen at -80°C prior to analysis. Fatty acid analysis of *D. magna* before and after enrichment was conducted using the direct methyl-esterification method (Folch *et al*., 1957).

Following this stage, appropriate timings for the enrichment process were determined and the best fatty acid-enriched *D. magna* were selected for feeding the Caspian kutum (*R. frisii kutum*) larvae. The kutum larvae (12 days old; initial weight, 52.62 mg) were obtained from Shahid Rajaee Propagation Facilities (Sari, Iran) and transferred to laboratory conditions. After the larvae acclimatized to the new conditions, they were distributed randomly in three 20-L tanks, each containing 15 L holding 10 fish L^{-1}. Each aquarium allocated to treatments as fish fed enriched *D. magna* with canola oil, fish fed a commercial dry feed (Biomar), and a control group fed non-enriched *D. magna* (each in three replicate, *n*=3). Biomar (a commercial dry feed) is a special diet often used at larval, fingerling and grow-out stages for trout and salmon aquaculture industry. According to the feed label Biomar (300 µ feed size) includes: crude protein 58%, crude fat 15 %, carbohydrate 8%, fiber 0.1%, ash 11.5%, phosphorus 0.7%, gross energy 21.1 MJ kg^{-1}, vitamin A 7500 U.I. Kg^{-1}, vitamin D_3 1500 U.I. kg^{-1}, vitamin E 400 mg kg^{-1}, and vitamin C 1000 mg kg^{-1}.

The larvae were fed four times a day (7:00, 11:00, 15:00 and 19:00) with *D. magna* at a density of 1.5-2 individuals ml^{-1} (Kolkovski *et al*., 2000). The larvae were fed daily at an amount of 10% body dry weight. Daily feeding was calculated based on dry weights of both the *Daphnia* and Biomar (Cauchie *et al*., 2000). The food residue was siphoned before each feeding time to preserve water quality. On each

sampling day, 5 larvae (15 per treatment) were randomly sampled for morphometric measurements from each experimental tank on 1, 5, 9, and 14 days. The growth and survival parameters, such as specific growth rate (SGR), survival rate, condition factor, and percentage body weight index were determined by the following formulae:

$$SGR\ (\%\ /\ day) = (LnW_t - LnW_i) / T \times 100$$

Survival rate (%) = No. of live animals / No. of animals initially introduced × 100

$$\%\ BWI = (BW_f - BW_i) / BW_i \times 100$$

$$\text{Condition factor } (C_f) = W_t \times L_t^{-3} \times 100$$

where W_t mean final weight, W_i mean initial weight, L_t mean final length, BW_f mean final weight in each tank, BW_i mean initial weight in each tank, and T total experimental days.

At the completion of the experimental period (day 14), the larvae were exposed to salinity stress test. To do this, three 20-L tanks, each containing 10 L with two salinities of 13 and 25 ‰ were prepared having three replicates for each salinity level (Kolkovski *et al*., 2000). Thirty larvae were randomly allocated to the salinity treatments and after 15 min, the survived fish were counted (Hung *et al*., 1989).

Statistical Method

One-way ANOVA with the Duncan's multiple comparison tests were applied to compare means differences in growth and survival of larvae and lipid class, and fatty acid composition of *D. magna* between treatments. Differences were considered significant at P≤0.05 level. The experimental data were processed by SPSS software (Version 17).

Results

Lipid Content of *D. magna*

Lipid content was significantly different (P≤0.05) between enriched and non-enriched *D. magna* (Figure 1). The lipid level was a function of enrichment duration showing an ascending trend with longer timings. Animals supplemented with canola oil for 12 h contained the highest amount (17.36 %), and lowest amount (2.24 %) recorded in the non-enriched group.

Fatty Acid Composition of *D. magna*

Table 1 displays percentage fluctuations in diverse essential fatty acid composition of enriched *D. magna* with canola oil depending on enrichment durations. Oleic acid (18:1n9) level elevated with increasing enrichment time being significantly (P≤0.05) higher in 12-h-enriched (66.73%) as opposed to the control group (31.66 %). Linoleic acid (18:2n6) level increased as a result of canola oil supplementation but it was not statistically higher than other enriched treatments (P≥0.05); it was, however, different from un-enriched group (P≤0.05). Linolenic acid (18:3n3) significantly (P≤0.05) rose in 3 and 6 h enriched treatments, which decreased in 9 and 12 h durations. It was the highest (3.37 %) in *D. magna* enriched for 6 h and the lowest (2.21 %) in 12 h-enriched group. EPA and DHA contents were different each other with increasing enrichment duration. DHA level (0.35 %) increased in 6 h enriched treatment, which a decreasing trend was obtained in 9 and 12 h durations. EPA content rose with increasing enrichment periods (P≥0.05).

Figure 2 illustrates amounts of SFA, MUFA, PUFA, and n-3 HUFA fatty acids in *D. magna* depending on enrichment durations. MUFA significantly (P≤0.05) elevated in 12 h treatment

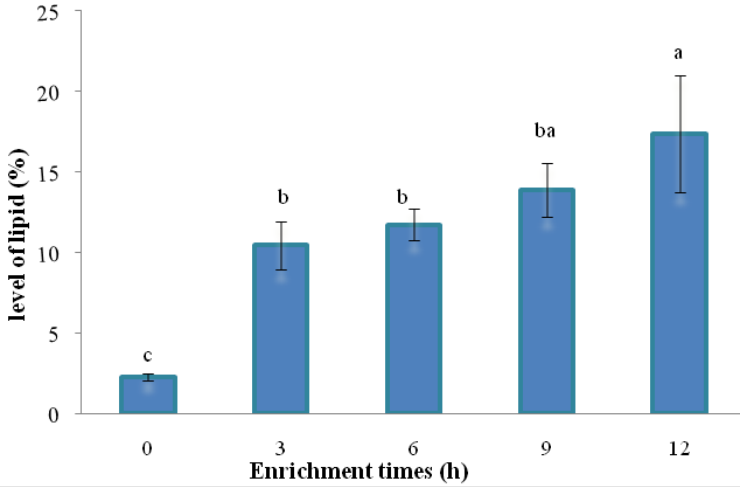

Figure 1. Lipid content of *D. magna* enriched with canola oil at durations of 3, 6, 9, and 12 h versus non-enriched (control) group (0 h). Error bars show standard deviations with letters denoting level of significance.

Table 1. Fatty acids profiles (as percentages of total fatty acids) (± SD) of *D. magna* before and after enrichment durations (3, 6, 9, and 12 h) using canola oil

Fatty acids	Non-enriched *D. magna*	Enrichment times (h) for *D. magna*				Canola Oil	Biomar
		3	6	9	12		
14:0	2.68±0.28^{a}	0.61±0.03^{b}	0.69±0.02^{b}	0.68±0.01^{b}	0.33±0.01^{b}	0.09	5.74
16:0	14.97±0.24^{a}	7.41±1.45^{b}	7.74±0.92^{b}	7.78±0.57^{b}	6.54±0.81^{b}	5.09	19.6
18:0	4.34±0.06^{a}	2.81±0.12^{a}	3.4±1.27^{a}	3.47±0.02^{a}	3.23±0.52^{a}	2.73	3.1
20:0	0.45±0.19^{a}	0.61±0.05^{a}	0.35±0.07^{a}	0.52±0.05^{a}	0.52±0.04^{a}	0.06	1.1
22:0	1.25±1.59^{a}	0.79±0.56^{a}	0.05±0.02^{a}	0.05±0.01^{a}	0.29±0.25^{a}	0.02	N.D.
∑SFA	23.69±1.6^{a}	12.23±0.18^{b}	12.23±3.09^{b}	12.5±1.2^{b}	10.91±0.7^{b}	7.99	29.54
14:1n5	0.54±0.04^{a}	0.09±0.04^{b}	0.14±0.09^{b}	0.15±0.04^{b}	0.07±00^{b}	N.D.	1.7
16:1n7	0.42±0.14^{c}	1.19±0.04^{b}	2.24±0.7^{a}	1.87±0.24^{ab}	0.88±0.46^{b}	0.2	13.88
18:1n9	31.66±0.64^{a}	58.41±2.99^{b}	58.94±4.72^{b}	59.31±5.48^{b}	66.73±2.07^{b}	63.48	22.6
20:1n9	0.19±0.04^{c}	1.07±0.07^{b}	1.18±0.19^{b}	1.13±0.11^{b}	1.48±0.09^{a}	1.26	16.91
∑MUFA	32.59±3.5^{b}	60.76±2.4^{a}	62.49±4^{a}	62.46±5.3^{a}	69.16±2.4^{a}	64.94	55.09
18:2n6	6.78±0.15^{b}	11.09±0.83^{a}	10.22±0.69^{a}	9.6±1.11^{a}	9±1.41^{ab}	16.92	4.7
18:3n6	0.21±0.04^{c}	0.65±0.05^{b}	0.76±0.09^{b}	0.68±0.07^{b}	1.02±0.14^{a}	0.82	6.47
20:3n6	2.49±1.74^{a}	0.39±0.05^{b}	1.72±0.91^{ab}	0.93±0.19^{b}	0.38±0.02^{b}	0.27	0.5
20:4n6	0.07±00^{a}	0.11±00^{a}	0.14±0.04^{a}	0.17±0.06^{a}	0.19±0.04^{a}	0.01	0.19
22:5n6	0.11±0.02^{a}	0.14±0.01^{a}	0.24±0.03^{a}	0.4±0.02^{a}	0.32±0.31^{a}	0.18	0.01
∑n-6	9.66±0.04	12.38±0.02	13.08±0.04	11.78±0.04	10.91±0.03	18.2	11.87
18:3n3	3.1±0.48^{a}	3.3±0.09^{a}	3.37±0.03^{a}	2.58±00^{a}	2.21±0.01^{a}	5.73	2.5
18:4n3	0.14±0.01^{b}	0.33±0.28^{ab}	0.79±0.41^{a}	0.46±0.02^{ab}	0.14±0.06^{b}	0.06	0.3
20:3n3	1.2±0.08^{a}	1.29±0.82^{a}	1.85±0.23^{a}	0.84±00^{a}	0.59±00^{a}	0.36	0.22
20:5n3	0.07±0.01^{b}	0.35±0.01^{a}	0.42±0.06^{a}	0.56±0.08^{a}	0.57±0.19^{a}	0.15	0.27
22:5n3	0.14±0.01^{a}	0.12±0.01^{a}	0.13±0.04^{a}	0.14±0.06^{a}	0.14±0.02^{a}	0.16	0.2
22:6n3	0.03±0.01^{c}	0.19±0.03^{b}	0.35±0.19^{a}	0.09±0.07^{c}	0.06±00^{c}	0.68	0.12
∑n-3	4.68±0.01	5.58±0.05	6.91±1.02	4.67±0.02	3.71±0.03	7.14	3.61
∑PUFA	14.34±1.1^{a}	17.96±0.39^{a}	19.99±1.2^{a}	16.45±3.7^{a}	14.62±2.5^{a}	24.1	14.29
∑n-3 HUFA	0.24±0.00^{c}	0.66±0.01^{b}	0.9±0.3^{a}	0.79±0.08^{ab}	0.77±0.16^{b}	1.05	0.89
n-3/n-6	0.48	0.45	0.53	0.40	0.34	0.39	0.30
DHA/EPA	0.43	0.54	0.83	0.16	0.11	4.53	0.44

Values are expressed as the mean ± SD from three replicate. Σ SFA: Total saturated fatty acid; Σ MUFA: Total monounsaturated fatty acid; Σ PUFA: Total polyunsaturated fatty acid; Σ(n-3) HUFA: Total n-3 highly unsaturated fatty acid; N.D.: Not detected.

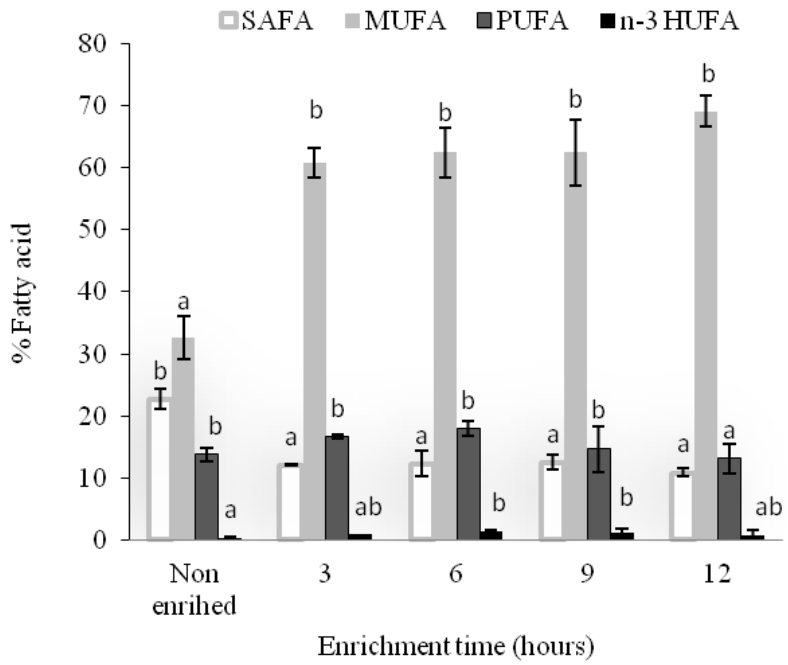

Figure 2. The amounts of EFAs are expressed as percentages of total fatty acids. Means with similar superscript letters denote insignificant differences (P≥0.05) and those with similar superscript letters signify differences (P≤0.05) between the experimental groups. SFA: Total saturated fatty acids; MUFA: Total monounsaturated fatty acids; PUFA: Total polyunsaturated fatty acids; n-3 HUFA: Sum of EPA, DHA and 22:5n-3.

(69.16 %) compared to other groups while the control contained the smallest amount (32.59 %). PUFA reached maximum content (19.99 %) in 6 h treatment but this was not significant (P≥0.05) compared to other treatments. The level of n-3 HUFAs exhibited a significant (P≤0.05) rise in 6 h treatment (0.9 %) with the least amount (0.24 %) in the control group. Altogether, enrichment time of 6 h yielded greatest amounts of PUFAs in enriched *D. magna*. Accordingly, this group was selected for feeding the Caspian kutum larvae at the 2nd part of the current study.

Effects of Feeding of the Caspian Kutum Larvae with Enriched *d. Magna*

Weight gain percentage was significantly highest (≈ 229 %; P≤0.05)) in larvae fed enriched *D. magna*; and the larvae fed Biomar showed the lowest values (163 %) (Table 2). Comparison of SGR also revealed significant difference (P≤0.05) between the enriched and Biomar-fed larvae. No considerable differences (P≥0.05) were observed with respect to fish survival rate among the experimental groups. Nonetheless, survival of all kutum larvae was fairly high (94.3-96.8 %), for which maximum and minimum rates recorded in fish fed enriched *D. magna* and those fed Biomar, respectively (Table 2).

The resistance of larvae at all treatments against salinity of 25‰ was not statistically different (P≥0.05), the consequence of which was also rather high fish mortality (75-80%). On the other hand, salinity of 13 ‰ (the same salinity level as the Caspian Sea) revealed significantly greater resistance (P≤0.05) in larvae fed enriched *D. magna* than the other two treatments being minimal in fish fed Biomar (Table 2).

Discussion

Effect of Canola Oil Enrichment on Lipid Content and Fatty Acid Profile of *d. Magna*

Live feed enrichment has been applied through different approaches conducted previously (Ako *et al*., 1994, Coutteau and Sorgeloos, 1997; Weers and Gulati, 1997; Von Elert, 2002; Ravet *et al*., 2003; Jalali *et al*., 2008; Hafezieh *et al*., 2009; Loh, 2011). In the current study, the enriched and non-enriched *D. magna* were markedly different in lipid contents. Our results showed that the *D. magna* enriched with canola oil for 12 h showed maximum average lipid accumulation of 17.36 % dry weight (Figure 1). Accordingly, the lipid content increased as the enrichment time rose. This is consistent with Das *et al.* (2007) who observed maximum lipid content in 24-h-enriched *Moina micrura* using a variety of emulsified oils compared to shorter durations. They resulted in increases of 19.43, 20.03, and 18.21 % caused by sunflower, cod liver, and Max EPA (a commercial diet), respectively, compared to a lipid level of 10.82 % in the control *M. micrura* (Das *et al*., 2007). Moreover, Macedo and Pinto-Coelho (2001) reported elevated levels of lipid content in both in *M. micrura* (from 11.4 to 19.9 %) and *Daphnia laevis* (from 1.11 to 22.2 %) fed a mixture of *Scenedesmus quadricauda* and *Ankistrodesmus gracilis*. Hafezieh *et al.* (2009) obtained an increasing trend in the initial total lipid of 16.72 mg g^{-1} DW (dry weight) to 19.45 mg g^{-1} DW in nauplii of *Artemia urmiana* enriched with linseed oil for 24 h.

Goulden and Place (1993) stated that zooplankton including Cladoceran can accumulate considerable amount of lipid (≥ 20% dry weight) in the body. However, some previous findings revealed total lipids of 1.09% (Ghazy *et al*., 2009) and 0.91% (Habashy, 1998) in *D. magna*; whereas, the total lipids of 7.29-7.73 % in *D. carinata* (Kibria *et al*., 1999) and 13% in *D. magna* (Watanabe *et al*., 1983) were also reported. Such discrepancies in total body lipids of *Daphnia* species can be attributed to the consumed feeds, culture conditions, species differentiation and/or the enrichment methods.

Oleic acid, with a level of 63.48 % in canola oil, ranged between 58-66 % in the enriched animals at different times. Also, linoleic and linolenic acids presented similar trends. Because of rather high levels of linoleic and linolenic acids (16.92 and 5.73%,

Table 2. Average growth characteristics, survival rates, and salinity resistance (means ± SD) of the *Caspian kutum* larvae fed *D. magna* enriched with canola oil, non-enriched *D. magna* (control), and Biomar during 14 days

Treatments Parameters	Biomar-fed larvae	Non-enriched larvae fed *Daphnia*	Enriched larvae fed *Daphnia*
Initial total length (mm)	21.8±0.33	21.8±0.33	21.8±0.33
Final total length (mm)	25.8±0.2 ^{c}	27.5±0.3 ^{b}	31.3±0.1 ^{a}
Initial weight (mg)	52.62±2.1	52.62±2.1	52.62±2.1
Final weight (mg)	138.4±1.5 ^{c}	150.1±3.1 ^{b}	173.1±2.3 ^{a}
Body weight increase (%)	163.01±3.1 ^{c}	185.25±2.1 ^{b}	228.96±1.2 ^{a}
SGR (%)	6.2±0.08 ^{c}	7.5±0.17 ^{b}	8.5±00 ^{a}
Condition factor (%)	0.82±0.05	0.71±0.03	0.58±0.02
Survival rate (%)	94.3±4.2 ^{a}	96.1±2.1 ^{a}	96.8±2.2 ^{a}
Survival rate (%) in 13 ‰	73.3±0.2 ^{c}	76.6±0.2 ^{b}	93.3±0.1 ^{a}
Survival rate (%) in 25 ‰	17.3±0.2 ^{a}	17.5±0.1 ^{a}	20.1±0.3 ^{a}

respectively) in canola oil, greater amounts of C_{18} PUFAs were accumulated in *D. magna* during complementation. Canola oil like other vegetable oils lacks adequate levels of DHA, EPA, 22:5n3, and ARA (arachidonic acid).

The present research found traces of these fatty acids in the canola oil and in the canola-enriched *D. magna* over different times; accordingly, the *n*-3 HUFAs index cannot be an acceptable enrichment criterion. The enrichment process, therefore, mostly affected amounts of MUFA and C_{18} PUFAs in *D. magna*. Therefore, C_{18} PUFAs and MUFA indices can be more suitable criteria to be considered for the duration of canola oil enrichment in *D. magna*. Because the total indices of PUFAs were not dissimilar at different times, and also better yields of MUFA and *n*-3 HUFAs up to 6 h, it is possible to suggest a maximum time of 6 h as an appropriate enrichment time for *D. magna* using canola oil. Other investigations concerning live feed enrichment have often used such animal oils as cod oil, which contains high levels of *n*-3 HUFAs. Nazari *et al.* (2008), for instance, enriched *D. magna* with cod oil and found a rise in DHA only at 6 h, while the current study detected increasing DHA up to 6 h. Our results revealed that monounsaturated fatty acids (MUFAs) of *D. magna* were positively affected by the canola oil enrichment. This may indicate that this zooplankter preferably uses MUFAs for metabolism (Dalsgaard *et al*., 2003). When *Daphnia* consumes MUFA-poor cryptophytes, it accumulates about twice the proportion of these FA as in their diets (Brett *et al*., 2006; Müller-Navarra, 2006). Thus, consumers can selectively use fatty acids changing them into other forms. For example, *Daphnia* spp. that consumes SFA-rich cyanobacteria tends to have only about half as much of these FA as their diet. A *Daphnia* species fed cryptophyte, cyanophyte, and chlorophyte algae contained lesser SFAs and more arachidonic acid (ARA) levels (Brett *et al*., 2006). Brett *et al.* (2006) stated that *Daphnia* is capable of using SFA and MUFA as likely energy sources for metabolism and growth.

Growth, Survival and Stress Resistance of the Caspian Kutum Larvae

Kutum larvae fed enriched *D. magna* gained greater weights and higher SGR values as opposed to other groups. Environment and/or trophic levels are major factors, with freshwater/diadromous species generally requiring C_{18} polyunsaturated fatty acids (PUFA); whereas marine fish have a strict requirement for long-chain PUFA (EPA, DHA and ARA) (Tocher, 2010). Increased growth and SGR of fish larvae fed enriched live feed using animal oils are generally attributed to the enhancements in such components as *n*-3 HUFA. Many observations reported in Iranian sturgeon, *Acipenser persicus* larvae fed *Daphnia* and/or *Artemia* supplemented HUFA-rich cod oil and exhibited considerable growth improvement compared to larvae fed non-enriched live feed (Nazari *et al*., 2008; Hafezieh *et al*., 2009). However, such a trend could not be very acceptable for live feed enriched with vegetable oils including canola because of limited *n*-3 HUFA sources. The experimental larvae fed enriched *D. magna* revealed marked differences in weight gain; therefore, it appears that C_{18} PUFA contributed effectively to the promoted growth of the kutum larvae. In other studies, the need for linoleic, linolenic, and C_{18} PUFA for enhanced growth was reported in *Oncorhynchus mykiss* (Takeuchi and Watanable, 1977) and *Tilapia zilli* (Kanazawa, 1980). Likewise, Wirth *et al.* (1997) concluded that *O. mykiss* larvae required α-linoleic acid and DHA in the diet for optimum growth, and that their deficiencies would lead to diminished larvae growth.

The present study recorded high survival rates of the larvae, which revealed no statistical differences among the treatments; hence, canola oil enrichment showed no effects on survival rates. Nazari *et al.* (2008) also detected no significant differences in survival rates of *Acipenser persicus* larvae fed *D. magna* enriched with cod oil. However, enrichment of rotifer with HUFA for newly hatched yellowtail flounder (*Limanda ferruginea*) larvae resulted in improved survival of the larvae compared to those fed non-enriched rotifer, which signifies the importance of *n*-3 HUFA in elevating endurance of marine fish species. It seems that kutum larvae, similar to marine fish larvae, need to *n-3* HUFA for considerable growth improvement and health (Ouraji *et al*., 2011); and accordingly, it would be better to use live feeds that can transfer greater levels of *n-3* HUFA to the larvae naturally and/or when enriched at larviculture stage in future.

Stress resistance of the larvae is affected by levels of salinity, temperature, environment and nutrition (Jalali *et al*., 2008; Gholami, 2010). Feeding the larvae with enriched *D. magna* in this research may account for the high fish durability in salinity stress condition. Jalali *et al.* (2008) reported that the promoted sturdiness of the sturgeon *Huso huso* larvae may be attributed to using HUFA-bearing feed under salinity stress condition. *D. magna* enriched with canola oil can probably transfer considerable amounts of PUFAs to the larvae. PUFAs contribute to cell membranes and osmosis regulation leading to physiological promotion of the kutum larvae (Ouraji *et al*., 2011; Ako *et al*., 1994). Furthermore, findings of Gapasin *et al.* (1998) demonstrated that supplying fish with dietary live feed containing lots of long-chain fatty acids reinforces the larvae against stressful conditions of the cultivation water.

Conclusion

In this study, canola oil was applied for the enrichment of *Daphnia magna* to be used as a live

feed for *R. frisii kutum* larviculture. The results of *D. magna* enrichment and feeding kutum larvae with the enriched zooplankton were mostly promising to be exploited in larviculture industry. Fatty acid profile of *D. magna* was considerably elevated as a result of enrichment with canola oil emulsion. Canola oil, therefore, offers an advantage through enhancing different fatty acid supply as well as increasing MUFA and C_{18} PUFAs of the live feed zooplankter making the oil a viable alternative for *Daphnia* enrichment. This investigation showed that enrichment duration up of 6 h yielded greatest amounts of C_{18} PUFAs in *D. magna* compared to shorter and longer times. The data on feeding 6-h-enriched *D. magna* to kutum larvae point out that weight gain, SGR, and resistance to salinity stress all markedly improved in the treated larvae. Enriching of *Daphnia* by the use of canola oil, therefore, maximizes its potential as live feed for culture of larval fish and crustacean species.

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