

Enrichment of *Artemia* (Leach) Nauplii with Canola Oil: Effect on *Heros severus* (Heckel) Larvae Performance and Environmental Stress

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Abstract

The effect of canola oil enriched *Artemia* (Leach) nauplii on growth, survival, resistance to environmental stresses of temperature and oxygen deficiency and fatty acid composition in severum fish, *Heros severus* (Heckel), larvae were investigated. The larvae (average weight 3 mg ± 0.83) were fed 4 times daily starting at the onset of exogenous feeding for 18 days. Triplicate groups of fish were offered one of two treatments: 1) newly hatched *Artemia* (Leach) nauplii (unenriched) and 2) canola oil enriched *Artemia* (Leach) nauplii. Then all groups of fish were switched to the commercial diet for an additional period of 18 days. Statistical analysis of growth after 18 days and at the end of the experiment (36 days), showed that the highest specific growth rates (9.65% ± 0.3), (17.44% ± 0.31) the average weight (17 mg ± 0.95), (65.2 mg ± 0.53) were observed in treatment 2 respectively, but there was no significant difference in survival rate between treatments. The best result of resistance to oxygen deficiency (5 min) was observed in larvae reared on treatment 2 with 75.67% ± 0.66 after 36 days. Result of temperature stress showed no significant difference between treatments. The larvae were also found to convert n-3 fatty acids to EPA and DHA.

Keywords

Canola Oil, *Heros severus*, Growth, Survival, EPA, DHA

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1. Introduction

Successful rearing of larval fish is the most critical stage in the production cycle for many species. *Heros severus* (Heckel) is a freshwater tropical cichlid native to the Amazon region in South America and Singapore in Southeast Asia [1]. Since no artificial feed formulation is yet available to completely substitute for *Artemia* (Leach), feeding live prey to young fish larvae still remains essential in commercial hatchery operations [2].

The use of *Artemia* (Leach) nauplii is well established due to its many advantages: year-round availability as on-the shelf cysts; good nutritional value for some fish; and relatively easy improvement through simple enrichment techniques [3]. Nutritional deficiencies have been another concern when using brine shrimp. Some stocks of *Artemia* (Leach) nauplii have shown a deficiency in eicosapentaenoic acid (EPA; 20:5n-3) and doccosahexanoic acid (DHA; 22:6n-3) [4]. The essential fatty acids (EFA) for fish are broadly recognized to comprise polyunsaturated fatty acids (PUFA) with carbon chain lengths of 18 and HUFA with carbon chain lengths of 20 and 22, of both the n-3 and n-6 series. Hence, these fatty acids must be provided in the diet to meet the fish's requirements. Several studies have demonstrated the positive effect of enriched live food on the growth, survival performance of various aquaculture species [5]-[7]. Also use of *Artemia* (Leach) enriched with long-chain unsaturated fatty acids for larvae and fry develops non-specific mechanisms of fish immunity and increases their resistance to diseases and environmental stresses. Gapasin *et al.* [5] studied effect of live food enriched with fatty acid on fish (*Chanos chanos* (Forsskål)) and their role in increasing the stress resistance of larvae and Ashraf *et al.* [8] considered effect of enriched diet with fatty acids on the survival rate and salinity stress on the fish side Silverstein (*Menidia beryllina* (Cope)). Also in Iran, Noori *et al.* [7] studied the effect of enriched *Artemia* (Leach) with fatty acids on the resistance to salinity stress in Iranian sturgeon fish, *Acipenser persicus*, (Borodin). Also, Sorgent *et al.* [9] reported that in freshwater fish, linolenic acid and linoleic acid are more than saltwater fish [9].

The name "canola" was chosen by the board of the Rapeseed Association of Canada in the 1970s. The "can" part stands for Canada and "ola" refers to oil. Canola was developed through conventional plant breeding from rapeseed. Rapeseed is the highest-producing oil seed crop in the United States. The major customers of canola seed are Japan, Mexico, China, and Pakistan, while the bulk of canola oil and meal goes to the United States, with smaller amounts shipped to Mexico, China, and Europe [10].

The objective of this study was to investigate canola oil in enhancing severum larval growth, survival and resistance to environmental stresses of temperature and oxygen deficiency and fatty acid composition temperature stress (up 24°C).

2. Material and Methods

2.1. Fish

In July 2011, severum, *Heros severus* (Heckel), larvae at the first feeding stage (swim up) were purchased from Spanta Co., Mahyana Ryapars, Iran, and transferred in the Fisheries Laboratory, Department of Natural Resources, Isfahan University of Technology. 480 uniformly sized yolk-sac larvae ($3 \text{ mg} \pm 0.83$) were randomly divided into 6 groups (two treatments, three replicates) of 80 individuals. Fishes of each group were transferred in to a 35 liter tank. Aeration was applied through a number of narrow pipes terminating to bubblers. Culture tanks were cleaned daily, and physic-chemical parameters were measured every morning prior to feeding. Water quality was maintained within optimum range: temperature ($27.1^\circ\text{C} \pm 1^\circ\text{C}$), dissolved oxygen ($5.76 \pm 0.96 \text{ mg/L}$), pH (8.43 ± 0.59), total ammonia (0.02 mg/L), total hardness ($165 \pm 8 \text{ mg/L CaCO}_3$) and the photoperiod was set at 12L: 12D cycle (light period from 8 - 20 hours) and light intensity was kept at 40 lux at the tank surface. Dead larvae were removed twice daily and counted.

2.2. Hatching and Enrichment of *Artemia* (Leach) Nauplii

Artemia cysts (Urmia Lake, Iran) were hatched following standard procedures [11] [12]. Newly hatched *Artemia* (Instar I) nauplii (200,000 nauplii/L) were divided in batches in 5 L plexiglass tanks. The enrichment protocol followed the method of Clawson and Lovell [13]. 0.5 g of lecithin was dissolved in 100 ml 50°C water and then 5 g canola oil was mixed it. It was homogenized using a blender and stored in the refrigerator for 1 week. 0.5 mL of the enrichment suspension (assuming a density of 200 *Artemia* per mL) was added per liter to the incubation water at the onset of the enrichment period. Another 0.5 ml/L of the enrichment diet was added 12 hours before harvesting and nauplii were harvested after 24 hours [14]. Newly hatched *Artemia* nauplii served as the

control [3].

2.3. Treatments

After 7 days of acclimation to the condition, fish were divided in two treatments (in a completely randomized design with 3 replicates per treatment) were: 1) larvae fed newly hatched *Artemia* nauplii and 2) larvae fed canola oil enriched *Artemia* nauplii. The fish larvae in all treatments were fed 4 times per day for 18 days. Then all groups of fish were switched to the commercial diet for an additional period of 18 days.

2.4. Sampling

Feeding was stopped six hours prior to sampling fish for chemical analysis, survival and growth measurement on days 18 and 36. All fish from each replicate were harvested at weekly intervals, bulk-weighted and the total length (TL) was taken. The amount of feed given per group was recorded and used to calculate feed conversion ratios (FCR) [15]. Ninety fish per treatment (30 fish per replicate tank) and 3 replicate of enriched and unenriched *Artemia* nauplii (200 thousand *Artemia* nauplii for each replicate) were randomly collected on days 7 and 28. Samples were oven-dried at 60°C for 24 h then stored at -20°C. These dried samples were later analyzed for fatty acid methyl esters [16], using gas chromatography (GC) (Agilent 6890N). Results were expressed as % total body dry weight. Also the number of surviving fish was recorded and used for calculating mortality. At the end of feeding trial all fish each tank were taken and their weights and lengths were measured. Specific growth rates (SGR), feed conversion ratios (FCR) and survival rate were calculated as following:

$$\text{SGR} = 100 \times (\text{Ln final weight} - \text{Ln initial weight})/\text{day} [17]$$

$$\text{FCR} = \text{feed intake (g)}/\text{weight gain (g)} [15]$$

$$\text{Survival} = 100 \times (\text{initial fish number} - \text{dead fish number})/(\text{initial fish number})$$

2.5. Resistance to Environmental Stresses

Thirty severum larvae were subjected to temperature stress test following the method described by [18] [19]. The test involved immersing fish, 10 fish larvae/replicate in 34°C (as high temperature) and 16°C (as low temperature) for a period of one hour. Sixty severum larvae (20 larvae/replicate) of each treatment were exposed for 2 and 5 min under low oxygen tension [18] [19]. The mortality was recorded at every 1 h interval up to 24 h.

2.6. Statistical Analysis

At the end of the experiment the number of surviving fish was recorded and used for calculating mortality. Diet effects on total length, SGR, FCR, survival, weight, and environmental stress were analyzed using independent T test at confidence level 5% ($P = 0.05$) (SPSS version 9).

3. Results

Fatty acid contents of newly hatched and enriched *Artemia* are shown in **Table 1**. The individual fatty acid levels of linolenic (w_3) and linoleic (w_6) acid were consistently higher in the canola oil-enriched nauplii than in the newly hatched nauplii. The HUFA and EPA levels (2.15% and $2.15\% \pm 0.07$) were highest in newly *Artemia*-nauplii.

Fatty acid content of severum larvae is shown in **Table 2**. The EPA ($3.37\% \pm 0.07\%$) level in treatment 1 was generally high compared to treatment 2 ($2.8\% \pm 0.25\%$). The PUFA level was significantly different in all treatments ($P < 0.05$), with highest PUFA observed in treatment 2 ($P < 0.05$). The HUFA level was high in treatment 1 (**Table 2**).

Fatty acid content of canola oil is shown in **Table 3**. The linoleic (18:2n-6) and linolenic acid (18:3n-3) levels in canola oil were generally high compared to EPA and DHA. The HUFA level was zero in canola oil.

Severum fed with *Artemia* enriched with canola oil (treatment 2) exhibited significantly higher ($P < 0.05$) growth compared to treatment 1 (fed newly *Artemia* nauplii) after 18 and 36 days of culture (**Table 4**). After 36 days of culture, survival significantly differed among the treatments ($P < 0.05$). The highest survival was observed in treatment 1 (**Table 4**).

When 36-day-old severum were subjected to temperature stress, mortality rate of the severum fed with *Artemia*

Table 1. Certain fatty acids (%) of newly *Artemia nauplii* (A), *Artemia* enriched with canola oil (B).

| Fatty acid | A | B |
|-----------------|---------------------------|----------------------------|
| 14:0 | tr | tr |
| 14:1n-5 | tr | tr |
| 16:0 | 0.01 ^a ± 19.5 | 0.005 ^b ± 21.3 |
| 16:1n-7 | 0.01 ^b ± 3.49 | 0.007 ^a ± 16 |
| 18:0 | 0.03 ^a ± 6.9 | 0.007 ^b ± 11.35 |
| 18:1n-9 | 0.03 ^b ± 18.73 | 0.005 ^a ± 15.62 |
| 18:2n-6 | 0.14 ^a ± 5.1 | 0.03 ^b ± 6.5 |
| 18:3n-3 | 0.49 ^a ± 29.52 | ^b 0.07 ± 41.72 |
| 20:0 | tr | tr |
| 20:1n-9 | tr | tr |
| 20:2n-6 | tr | tr |
| 20:3n-3 | tr | tr |
| 20:4n-6 | tr | tr |
| (20:5n-3) (EPA) | 0.07 ^b ± 2.15 | 0 ^a |
| (22:6n-3) (DHA) | 0 | tr |
| SFA Σ | 26.4 ^a | 65 ^b .32 |
| USFA Σ | 22.19 ^a | 17.22 ^b |
| PUFA Σ | 34.62 ^a | 48.22 ^b |
| HUFA Σ | 2.15 ^b | 0 ^a |

Values in each row with different superscripts are significantly different ($P < 0.05$). Data are mean \pm SD ($n = 3$); SFA = saturated fatty acid; USFA = unsaturated fatty acid; HUFA = highly unsaturated fatty acid and PUFA = poly unsaturated acid; tr = trace.

Table 2. Whole-body fatty acid composition (%) of 18-day old severum larvae fed of different diets.

| Fatty acid | Treatment 1 | Treatment 2 |
|-----------------|-------------------------------|--------------------------------|
| 14:0 | tr | tr |
| 14:1n-5 | tr | tr |
| 16:0 | 26.29 \pm 0.43 ^b | 25.20 \pm 0.012 ^a |
| 16:1n-7 | 2.29 \pm 0.01 ^a | 2.50 \pm 0.007 ^a |
| 18:0 | 21.07 \pm 0.19 ^b | 17.80 \pm 0.23 ^a |
| 18:1n-9 | 22.23 \pm 0.04 ^a | 23.04 \pm 0.04 ^b |
| 18:2n-6 | 4.78 \pm 0.01 ^a | 5.93 \pm 0.06 ^b |
| 18:3n-3 | 3.61 \pm 0.007 ^a | 5.98 \pm 0.01 ^b |
| 20:0 | tr | tr |
| 20:1n-9 | tr | tr |
| 20:2n-6 | tr | tr |
| 20:3n-3 | tr | tr |
| 20:4n-6 | tr | tr |
| (20:5n-3) (EPA) | 3.37 \pm 0.075 ^b | 2.80 \pm 0.25 ^a |
| (22:6n-3) (DHA) | 16.33 \pm 0.03 ^a | 16.53 \pm 0.01 ^a |
| SFA Σ | 47.36 ^b | 43 ^a |
| USFA Σ | 24.52 ^a | 25.54 ^b |
| PUFA Σ | 8.39 ^a | 11.91 ^b |
| HUFA Σ | 19.7 ^b | 19.33 ^a |

Values in each row with different superscripts are significantly different ($P < 0.05$). Treatment 1: severum larvae fed with newly *Artemianauplii*; Treatment 2: severum larvae fed with *Artemia* enriched with canola oil; Tr = trace.

Table 3. Certain fatty acids (%) of canola oil.

| Fatty acid | Canola oil |
|-------------------|------------|
| 14:0 | 0 |
| 16:0 | 5.77 |
| 18:0 | 1.27 |
| Other SFA | 4.76 |
| SFA Σ | 11.80 |
| 18: 1n-9 | 77.33 |
| Other MUFA | 0.58 |
| MUFA Σ | 77.91 |
| 18: 2n-6 | 1.01 |
| 18: 3n-6 | 0.38 |
| 20: 3n-6 | tr |
| 20:4n-6 | tr |
| Other PUFA n-6 | 0.14 |
| PUFA n-6 Σ | 1.53 |
| 18: 3n-3 | 7.05 |
| 18: 5n-3 | tr |
| 22: 5n-3 | tr |
| 22: 6n-3 | tr |
| Other PUFA n-3 | 1.71 |
| Σ PUFA n-3 | 8.76 |

Table 4. Average total weight and length^a, Specific growth rate (SGR), food conversion ratio (FCR) and percent survival of fish fed various dietary treatments. Values are mean \pm standard deviation (n = 10).

| Treatment | Time (day) | Average weight (mg) | Average total length (mm) | FCR | SGR (%) | Survival (%) |
|-----------|------------|-------------------------------|-------------------------------|------------------------------|------------------------------|-------------------------------|
| 1 | 18 | 13.5 \pm 0.75 ^a | 7.42 \pm 0.63 ^a | - | 8.31 \pm 0.33 ^a | 81.87 \pm 0.29 ^a |
| 2 | 18 | 17.1 \pm 0.95 ^b | 9.34 \pm 0.44 ^b | - | 9.65 \pm 0.30 ^b | 80.04 \pm 2.24 ^a |
| 1 | 36 | 37.50 \pm 0.37 ^a | 13.84 \pm 1.09 ^a | 4.18 \pm 0.2 ^b | 5.37 \pm 0.47 ^b | 96 \pm 1.09 ^a |
| 2 | 36 | 65.20 \pm 5.70 ^b | 14.17 \pm 0.55 ^b | 3.27 \pm 0.15 ^a | 7.44 \pm .53 ^a | 100 ^b |

Within columns values with different superscripts are significantly different ($P < 0.05$). ^aInitial weights and lengths of severum larvae 3 (mg) \pm 0.83 SD and 6.82 (mm) \pm 0.83 SD respectively.

enriched with canola oil (treatment 2) and newly *Artemia* nauplii (treatment 1) was no significant difference ($P > 0.05$) in temperature 16°C and 34°C (**Table 5**). The highest mortality was observed in treatment 1. When 36-days-old severum were exposed to oxygen deficiency (5 min), the survival rate was significantly high ($P < 0.05$) in treatment 2 (75.62% \pm 0.66%) compared to treatment 1 (47.5% \pm 1.42%) after 24 h (**Table 5**).

4. Discussion

Several studies have demonstrated the positive effect of enriched live food on the growth performance of various species. HUFA-enriched *Artemia* nauplii fed to *Fenneropenaeus indicus* (H. Milne-Edwards), [20], *Sepia*

Table 5. Results of temperature stress (34°C and 16°C) and oxygen deficiency (2 and 5 min) on mortality rates of different treatments in 36-day-old severum.

| Treatment | Survival% | | | |
|-----------|----------------------|----------------------|-------------------------|---------------------------|
| | Temperature 16°C | Temperature 34°C | Oxygen deficiency 2 min | Oxygen deficiency 5 min |
| 1 | 100 ± 0 ^a | 100 ± 0 ^a | 100 ± 0 ^a | 47.5 ± 1.42 ^a |
| 2 | 100 ± 0 ^a | 100 ± 0 ^a | 100 ± 0 ^a | 75.63 ± 0.66 ^b |

Within columns values with different superscripts are significantly different ($P < 0.05$).

officinalis (Linnaeus) [17], and *Chanos chanos* (Forsskål) [5] exhibited better growth and survival. Gilthead sea bream larvae also grow better if fed rotifers enriched with highly unsaturated n-3 HUFA [21]. Similar to the finding of Tamaru *et al.* and Hosseinpour *et al.* [22] in the present study significant differences were found in the growth of severum larvae fed different diets, larvae fed *Artemia* enriched with canola oil (treatment 2) exhibited significantly higher growth than larvae fed unenriched nauplii (treatment 1) after 36 days of culture (Table 4). Tamaru (1998) used *Artemia* nauplii enriched with different oils for the production of ornamental fish and significant influences obtained on the growth and survival ($P < 0.05$). Smith *et al.* [23] with research on essential fatty acids in the diet *Bidyanus bidyanus* reported that linolenic acid in the diet increased fish growth rate.

On the other hand, survival of 36 days-old severum fed various diets was significantly different (Table 4). Mortality of the canola oil treated fish was significantly lower than unenriched nauplii fed fish supporting the results of *Limanda ferruginea* (D. H. Storer) [24], *Peterophylum scalare* (Schultze) [25] and *Oncorhynchus mykiss* (Walbaum) [26]. Akbary *et al.* [26] concluded that survival rate of rainbow trout larvae fed with enriched *Artemia* (HUFA + vitamin C) during 29 days of testing (96%) is higher than larvae fed unenriched nauplii (84%) and larvae were fed with commercial food (67%). In the current investigation, the larvae fed by canola oil for 18 days exhibited more resistance to oxygen deficiency (Table 5), compared to treatment 1. When subjected to temperature stress test, 36 days old *Heros severus* larvae fed canola oil *Artemia* exhibited no significant difference with larvae fed unenriched nauplii. The mortality in larvae fed enriched *Artemia* was lower than control. Due to the high levels of linolenic acid in treatment 2 supporting the results of Smith *et al.* [23], Kiron *et al.* [4]. [4] reported that n-3 fatty acids are important precursors in the synthesis eicozanoids that is an important mediator in inflammatory reactions and immune responses. When dietary have a deficiency of essential fatty acid n-3, antibacterial activity of macrophage cells is reduced. If macrophages receive linolenic acid, their bactericidal ability will rise. Van Stappen [27] reported that freshwater species would need to the fatty acids linolenic and linoleic and Marine fish species would need to EPA and DHA.

Milkfish larvae given *Artemia* enriched with HUFA + vitamin C showed better growth and higher survival after a stress test [5]. Ako *et al.* [18], Gapasin *et al.* [5] observed no or few mortalities among fish fed *Artemia* enriched with menhaden oil (high DHA:EPA ratio) compared to high mortalities among fish fed unenriched *Artemia*. Red sea bream, *Pagrus major*, (Temminck & Schlegel) and marble sole *Euryglossa orientalis*, (Bloch & Schneider) larvae given diets containing DHA and lecithin tolerated temperature and salinity changes, low oxygen and air exposure better than the larvae given DHA and lecithin-free diets [28]. Furuita *et al.* [29] reported that yellowtail larvae and red sea bream juvenile fed *Artemia* enriched with DHA exhibited higher survival in the stress test than those fed *Artemia* enriched with EPA. In the present study, severum larvae fed *Artemia* enriched with canola oil (Treatment 2) showed better growth and increased resistance to oxygen deficiency than those given unenriched diet (treatment 1). This result is similar to *Chanos chanos* [5] *Sepia officinalis* [17].

The preceding studies attest the importance of EFA and in fish growth and development. Severum fed with canola oil exhibited significantly ($P < 0.05$) higher growth than those given unenriched live food after 36 days of culture. When subjected to oxygen deficiency, mortality of the canola oil treated fish was significantly lower ($P < 0.05$) among the treatment groups. Optimum requirements of these nutrients in severum, however, are not yet known, using of *Artemia* enriched with long chain polyunsaturated fatty acids in proliferation centers of ornamental fish can increase fish resistance against stress-induced changes in environmental conditions.

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