Potential of plant oils as alternative to fish oil for live food enrichment: effects on growth, survival, body compositions and resistance against environmental stresses in rainbow trout, *Oncorhynchus mykiss*

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Abstract

Enrichment of live foods by essential nutrients such as highly unsaturated fatty acids is an important tool for improvement of larval fish quality. In this study, nutritional effects of *Artemia urmiana* enriched by fish and plant oils on growth, survival rate, body compositions and resistance against thermal, salinity and hypoxic stresses in rainbow trout, *Oncorhynchus mykiss* were examined. Six food treatments comprising a commercial feed, non-enriched *Artemia* nauplii and the nauplii enriched by either of fish, sunflower, canola and soybean oils were used in triplicates. The fish fed with *Artemia* enriched by fish, sunflower and canola oils had significantly higher (p<0.05) survival rate, total length, wet and dry weights, specific growth rate and lower food conversion ratio than those fed the commercial feed. However, there were no significant differences in growth indices between the fish fed fish oil-enriched and plant oil-enriched *Artemia*. Minimum eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were reported in the fish fed with the commercial feed, while maximum EPA and DHA were in the fish fed canola oil-enriched and nonenriched *Artemia*, respectively. The fish fed canola oil enriched *Artemia* had significantly higher (p<0.05) resistance against environmental stresses compared to fish fed the commercial diet. Results showed that the plant oils, mainly canola and sunflower oils can be used for *Artemia* enrichment as a suitable substitute for the more expensive and rare fish oil for improving growth and resistance to environmental stresses in rainbow trout larvae.

Keywords: Rainbow trout, *Artemia urmiana*, Enrichment, Plant oils, Growth indices, Resistance against environmental stresses

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Introduction

The limited availability of fish oil and fish meal, together with the increase in aquaculture activities calls for the identification of alternative lipid and protein sources for the development of sustainable fish farming practices (Santigosa *et al*., 2011). Plant oils rich in C18 polyunsaturated fatty acids (PUFA) are potential candidates for this replacement (Al-Owafeir *et al*., 1996; Huanng *et al*., 2008; Hafezieh *et al*., 2010). The past decade has seen an increase in the use of terrestrial plant oils, such as canola, soybean, flax, and palm oils, to replace fish oil in aquafeeds (Naylora *et al*., 2009). On the other hand, world production of oils from plant seeds has increased in recent years resulting in higher availability and lower or unchanged costs (USDA, 2007; Chhetri *et al*, 2008). These together with having renewable and reliable sources are major advantages of the oils over fish oil as ingredients in aquafeeds (Miller *et al*., 2008). However, plant oils cannot meet the fatty acid requirement of all fish species (Kamarudin *et al*., 2011). Success in a hatchery production ssystem largely depends on the availability of suitable live feed organisms for feeding the sensitive fry and fingerling stages of fish (Arulvasu and Munuswamy, 2009). Considering the pre-requisites of n3 and n6 highly unsaturated fatty acids (HUFA) in live feeds, various studies have been conducted to improve their availability through enrichment methods (Immanuel *et al*., 2007). Among all the live foods used in aquatic larviculture, the brine shrimp, *Artemia* sp., is the most widely used (Narciso *et al*., 1999). *Artemia* enriched with several components such as PUFAs, amino acids, vitamins, drugs, vaccines, hormones and edible colors can serve as a carrier of these materials to fish (Lepage and Roy, 1984). This will increase quality, survival rate and resistance of the fish larvae against environmental stresses (Bell *et al*., 2002) Lipids provide the main source of metabolic energy and essential fatty acids (EFA) in aquafeeds for many carnivorous fish, particularly salmonids (Miller *et al*., 2008).

They also play an important role in fish as substantial elements for the formation and maintaining the structural and functional integrity of cell and tissue membranes (Narciso *et al*., 1999; Sargent *et al*., 1999). In the last decades, a large amount of research effort has been directed towards the development of lipid enrichment products, in order to raise the EFA content, particularly of the n-3 HUFA in the nutritionally deficient live prey used in larviculture of fish and shellfish (Morais *et al*., 2006). Given that *Artemia* sp. is a non-selective filter feeder, it is possible to modify its nutritional profile through the conjugation of different enrichment products or emulsions, and enrichment periods to achieve the required concentrations of different EFAs (Narciso *et al*., 1999). Using EFA-enriched *Artemia* resulted in significantly higher growth and survival rates in Indian white shrimp, *Penaeus indicus* (Immanuel *et al*., 2001), Senegalese sole, *Solea senegalensis* (Morais *et al*., 2006), Persian sturgeon, *Acipenser persicus* (Hafezieh *et al*., 2010) and rainbow trout, *Oncorhynchus mykiss* (Mirzakhani, 2004; Akbary *et al*., 2011) larvae. Freshwater teleosts including rainbow trout have a natural ability to convert C18 PUFAs especially linolenic acid to longer-chain HUFAs, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Sargent *et al*., 1999; Kamarudin *et al*., 2011). This capability allows the aquaculturist to substitute cheaper plant oils containing linolenic acid for expensive less available marine fish oil in live food enrichment protocols (Huanng *et al*., 2008). A number of fish species have shown substantial growth and survival rates and resistance against environmental stressors when fed with *Artemia* enriched with plant oils (Bell *et al*., 2001; Piedecausa *et al*., 2007; Kamarudin *et al*., 2011). Nevertheless, replacement of fish oil with plant oils in fish nutrition is still a new and ongoing field of research. In this study effects of using selected plants oils for enrichment of *A. urmiana* on growth, survival, body composition and resistance against thermal, salinity and hypoxic stresses in rainbow trout have been assessed.

Materials and methods

Fish and cultural conditions

Rainbow trout, *O. mykiss*, (mean weight 100 mg) were supplied from a local fish farm (Urmia, Iran). The fish were acclimated to culture conditions for two weeks, and then randomly assigned to the feeding treatments. The treatments were set up in triplicate with 1000 fish per each 100 L polyethylene tank containing 75 L of well water. Water had a flow rate of 20 L/min, temperature of 14.5±0.6 °C, dissolved oxygen of 8±0.5 mg/L and pH of 7.57±0.3.

Enrichment of Artemia

A. urmiana cysts were provided from the cyst bank of *Artemia* and Aquatic Animals Research Institute of Urmia University. The cysts had a hatching percent of 85% and were decapsulated and hatched according to the standard methods (Lavens and Sorgeloos, 1996). Enrichment media were prepared by the addition of 1g lecithin and 10g each of the fish or sunflower, soybean and canola oils to 100 ml water at 40 °C and mixed by an electrical stirrer for 10 min. Oil particles in the suspensions were measured using a microscope equipped with an ocular micrometer to ensure that the particles have a size of less than 30 µm. Newly-hatched *Artemia* nauplii were divided into five batches (200,000 nauplii/conical container); one left without enrichment and the remaining four batches were enriched using the standard enrichment protocol of Le´ger *et al.* (1987). using each enrichment medium. Enrichment was done in conical containers containing 1 L Sea water (33 ppt), using

3 replicates for each treatment. A single dose of 2 mL enrichment solution was added to each conical vessel and enrichment was continued for 12 h. Nauplii were harvested after 12 h and stored in an incubator at 4°C and utilized during the next 24h. Water temperature in enrichment cones was maintained at 28°C, pH at 8.5 and with constant aeration.

Feeding trial

Six feeding treatments comprising a commercial rainbow trout feed manufactured by Chineh, Iran (control), non-enriched newly hatched *Artemia*, and *Artemia* nauplii enriched by fish oil or either of sunflower, soybean or canola oils were used to feed the fish for a culture period of 10 days. Daily supplements of the commercial feed were 12.5% and 12% of the fish biomass during the first 5 days and onward, respectively. Fish of the remaining feed treatments were fed with *Artemia* nauplii at a rate of 6% of their biomass (equivalent dry weight) throughout the experiment. Total daily feed was divided into five equal portions and fed at 8 and 11am and 14, 17 and 20 pm. Water flow was turned off at each feeding time. Daily mortality was recorded and the dead larvae were removed prior to the first daily feeding.

Estimation of growth indices

On day 11, a total of 20 growing larvae were randomly taken from each tank and their weight and total length were measured. Specific growth rate (SGR), food conversion ratio (FCR), condition factor (CF), fish body water content (WC) and ash content were calculated by the following formulas:

$$SGR = (\ln Wf - \ln Wi) \times 100 / t$$

$$FCR = F / (Wf - Wi)$$

$$CF = W / L^3 \times 100$$

Where, Wi and Wf are the weight in g at the beginning and the end of each period between samplings; t is the duration of culture period (in days); F is amount of feed used; W is average final weight and L is average final length of the fish.

$$WC = (G1 - G2) \times 100 / (G1 - C)$$

Where, G1 and G2 are wet and dry weights of the fish, respectively and C is weight of the container

$$Ash = (E - B) \times 100 / (D - B)$$

Where, E is weight of container plus ash; B is weight of container and D is weight of container with dry sample. The initial mean weight of the larvae was determined by weighing 60 randomly selected larvae before the start of the experiment.

Fatty acids profiles

Fatty acid composition of the commercial feed, enriched and non-enriched *Artemia* and fish larvae samples was determined by gas chromatography. Fatty acid methyl esters (FAME) were prepared via a modified procedure of Lepage and Roy (1984). This method implicates a direct acid catalyzed transesterification without prior extraction of total fat. To each sample 10% of an internal standard (20:2n-6) was added prior to the reaction. FAME was extracted with hexane. After evaporation of the solvent the FAME was prepared for injection by dissolving them in iso-octane (2 mg mL)1). Quantitative determination of fatty acids was done by DANI 1000 gas chromatograph, equipped with an auto sampler and a temperature programmable on-column injector (Lepage and Roy, 1984).

Stress resistance tests

For each of the stress trials, 30 fish per treatment (10 per tank) were randomly chosen. Fish of each replicate were treated separately. For hypoxic test, the fish were transferred to the water containing dissolved oxygen at concentration of 5mg/L, for osmotic stress at salinities of 10, 15 and 20ppt and for thermal stress to temperatures of 20, 25 and 30°C. Behavior and mortality of the fish were monitored every 3 h (for hypoxia) and 6 h (for salinity and thermal stresses) on the first day, and at 12 h intervals from the second day up to the fourth day.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) and Duncan's test using SPSS, ver.15 software. Differences were considered to be significant at $p<0.05$.

Results

Growth and survival

The fish fed with the commercial feed had minimum and significantly lower wet weight (0.16±0.01 g), dry weight (0.02±0.00 g) and total length (2.65±0.06 cm) than those fed with sunflower and canola-enriched *Artemia* ($p<0.05$) (Table 1). Minimum (4.8±0.98%/day) and maximum (6.23±0.48) SGR belonged to the control group and to the fish fed with *Artemia* enriched by canola oil, respectively. These extremes had significantly different SGR from those of the other feeding groups ($p<0.05$). Minimum FCR (0.67±0.08) was obtained for the fish fed with *Artemia* enriched with fish oil, while maximum FCR (1.88±0.58) was for the fish fed with the commercial feed. There were significant differences in the FCR between the control group and the other feeding treatments ($p<0.05$). However, there was no significant difference in CF between the treatments ($p>0.05$). Maximum (99.33±0.46%) and minimum (95.67±0.65%) survival rates belonged to the fish fed with *Artemia* enriched with sunflower oil and to those fed the commercial feed, respectively. There was significant difference in survival rate between the latter and the remnant treatments ($p<0.05$) (Table 1).

Table 1: Mean±standard deviation of the estimated parameters of the fish of different feeding treatments.

Parameter	Commercial feed	*Artemia* enriched with fish oil	*Artemia* enriched with sunflower oil	*Artemia* enriched with canola oil	*Artemia* enriched with soybean oil	Non-enriched *Artemia*
WW (g)	0.16±0.01[a]	0.19±0.01[b]	0.19±0.01[b]	0.18±0.01[ab]	0.18±0.01[ab]	0.19±0.01[b]
DW (g)	0.02±0.00[a]	0.02±0.01[ab]	0.03±0.01[b]	0.02±0.00[ab]	0.03±0.00[ab]	0.03±0.01[b]
Length(cm)	2.65±0.06[a]	2.84±0.06[b]	2.84±0.05[b]	2.76±0.02[ab]	2.80±0.08[b]	2.84±0.08[b]
SGR	4.80±0.97[a]	6.20±0.69[b]	6.22±0.51[b]	6.11±0.71[b]	5.98±0.72[ab]	6.23±0.48[b]
FCR	1.88±0.58[b]	0.67±0.08[a]	0.72±0.06[a]	0.70±0.12[a]	0.75±0.06[a]	0.73±0.55[a]
WC (%)	85.44±1.14[a]	84.24±0.55[a]	83.39±0.20[a]	83.91±0.54[a]	83.60±1.88[a]	80.03±2.31[a]
CF	0.86±0.06[a]	0.82±0.04[a]	0.81±0.02[a]	0.87±0.04[a]	0.82±0.02[a]	0.81±0.04[a]
Ash (%)	9.63±2.10[a]	12.01±1.26[a]	15.30±1.70[a]	10.25±0.49[a]	10.63±0.72[a]	15.10±1.25[a]
SR (%)	95.67±0.65[a]	98.24±1.00[b]	99.33±0.46[b]	98.09±0.10[b]	97.99±0.87[b]	98.45±1.33[b]

WW=wet weight; DW=dry weight; SGR=Specific growth rate; FCR=food conversion ratio; WC= water contents of fish body; CF=condition factor; SR=survival rate; In each row data having different alphabetic letters are significantly different ($p<0.05$)

Fatty acids profile

Values of the fatty acids in the feed items and carcass of the studied fish are shown in Tables 3 and 4. Oleic, palmitoleic, linoleic, eicosapentaenoic and stearic acids had the highest levels in the treated feeds, while in all the treatments the maximum fatty acid levels of the carcasses belonged to oleic, linoleic, linolenic, stearic and docosahexaenoic acids. The commercial feed had significantly lower EPA (0.73±0.06%) but higher DHA (1.02±0.01%) than the other examined feeds ($p<0.05$). Maximum EPA was in the *Artemia* enriched with fish oil (0.88±0.09%) and this was significantly higher than EPA levels of the other feeds ($p<0.05$). DHA was nil in both non-enriched and enriched *Artemia* (Table 3). The fish fed with the commercial feed had minimum EPA (0.58±0.10%) and DHA (2.25±0.44 %), while maximum EPA (1.07±0.07%) and DHA (2.73±0.29%) were in the fish fed with canola oil-enriched and non-enriched *Artemia*, respectively. There was no significant difference in DHA levels among the fish of all treatments ($p> 0.05$) (Table 4).

Table 2: Content of fatty acids (mean±standard deviation) in the used feeds. Data are in percent.

Fatty acid	Commercial feed	*Artemia* enriched by fish oil	*Artemia* enriched by sunflower oil	*Artemia* enriched by canola oil	*Artemia* enriched by soybean oil	Non-enriched *Artemia*
C14:0	6.39±0.11[bc]	6.64±0.89[c]	5.11±0.80[ab]	6.19±0.04[bc]	5.59±0.55[bc]	4.20±1.49[a]
C14:1n5	0.16±0.04[a]	1.88±0.49[b]	1.88±0.65[b]	2.78±0.09[c]	2.02±0.07[bc]	1.37±0.88[b]
C16:0	17.52±0.06[b]	16.24±3.58[ab]	15.40±1.32[ab]	15.86±0.78[ab]	15.97±0.97[ab]	14.33±1.43[a]
C16:1n7	2.23±0.04[a]	10.84±2.14[b]	9.78±1.18[b]	12.14±1.60[b]	10.28±1.21[b]	9.68±1.49[b]
C18:0	2.33±0.03[b]	4.55±0.22[cd]	4.74±0.61[cd]	4.04±0.10[c]	4.11±0.14[c]	4.91±0.72[d]
C18:1n9	16.87±0.02[a]	17.30±0.22[ab]	18.06±1.67[ab]	17.84±1.06[ab]	20.70±1.87[c]	18.27±1.03[ab]
C18:1n7	0.00±0.00[a]	10.89±1.78[bc]	10.59±2.45[bc]	9.16±1.20[b]	10.76±0.33[bc]	12.78±1.40[c]
C18:2n6	26.61±0.02[d]	4.38±1.91[a]	9.93±0.91[c]	9.09±0.52[c]	8.45±0.77[c]	6.21±0.05[b]
C18:3n6	0.00±0.00[a]	0.39±0.34[b]	0.59±0.05[b]	0.52±0.02[b]	0.41±0.09[b]	0.65±0.14[b]
C18:3n3	3.93±0.06[cd]	2.24±1.11[a]	3.20±0.30[bc]	2.77±0.04[ab]	3.49±0.24[bcd]	3.08±0.10[bc]
C20:0	1.46±0.06[c]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
C20:1n9	0.00±0.00[a]	3.96±0.43[c]	3.53±0.51[bc]	2.72±0.27[b]	2.81±0.34[b]	5.42±1.09[d]
C20:4n6	0.00±0.00[a]	1.40±0.19[bc]	1.42±0.20[bc]	1.26±0.02[b]	1.36±0.14[bc]	1.56±0.14[c]
C20:5n3 (EPA)	0.73±0.06[a]	6.77±1.04[c]	5.56±0.54[b]	4.88±0.16[b]	4.84±0.24[b]	6.68±0.40[c]
C22:6n3 (DHA)	1.02±0.01[c]	0.65±0.11[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

In each row data having different alphabetic letters are significantly different ($p<0.05$)

Table 3: Content of fatty acids (mean±standard deviation) in the carcass of the fish of different feed treatments. Data are in percent.

Fatty acid	Commercial feed	*Artemia* enriched by fish oil	*Artemia* enriched by sunflower oil	*Artemia* enriched by canola oil	*Artemia* enriched by soybean oil	Non-enriched *Artemia*
C18:0	5.18±0.64[a]	5.96±0.34[a]	5.81±0.52[a]	4.93±1.97[a]	5.72±0.41[a]	5.82±0.13[a]
C18:1n9	19.59±2.02[a]	19.66±2.20[a]	21.84±0.90[a]	28.90±1.35[b]	20.40±1.54[a]	21.89±1.59[a]
C18:2n6	16.61±2.18[d]	7.07±1.44[a]	9.55±0.40[bc]	10.88±0.78[c]	8.56±0.58[ab]	8.26±0.51[ab]
C18:3n3	2.10±0.19[a]	3.30±0.41[b]	3.99±0.54[bc]	4.40±0.24[c]	3.53±0.35[b]	3.71±0.45[bc]
C20:4n6	0.94±0.20[a]	1.13±0.22[ab]	1.18±0.17[ab]	1.81±0.16[c]	1.32±0.11[b]	1.35±0.07[b]
C20:5n3 (EPA)	0.58±0.10[a]	0.93±0.07[cd]	0.77±0.12[b]	1.07±0.07[d]	0.83±0.06[bc]	0.90±0.03[bc]
C22:6n3 (DHA)	2.25±0.44[a]	2.64±0.13[a]	2.27±0.24[a]	2.62±0.56[a]	2.39±0.19[a]	2.73±0.29[a]

In each row data having different alphabetic letters are significantly different ($p<0.05$)

Resistance against stresses

Maximum (99.67±1.34%) and minimum (91.67±10.85%) survival rates at temperature of 20 °C were for the fish fed with *Artemia* enriched with canola and soybean oils, respectively (Table 2). The fish fed *Artemia* enriched with sunflower and canola oils had significantly higher resistance to thermal stress at 20°C ($p<0.05$). Feeding canola oil-enriched *Artemia* resulted in highest fish resistance at 25°C (survival rate 87.02±15.74 %) and this was significantly different from the other treatments except from those fed *Artemia* enriched with fish oil. Fish of all the treatments could only resist 30 °C for less than one hour.

Maximum resistance (i.e., 100% survival rate) at salinity of 10 ppt was observed in the fish fed *Artemia* enriched with fish and soybean oils, while the control fish had minimum survival rate (79.69±19.41%) at this salinity. There were significant differences in survival rate between the control group and all the other treatments (p<0.05). The fish fed *Artemia* enriched with canola oil had maximum survival rate (97.79±3.65%) at 15 ppt which was significantly different from those of the other treatments except in the fish fed non-enriched *Artemia* and *Artemia* enriched with soybean oil. Minimum survival rate at 15 ppt was in the fish fed the commercial feed (76.07±21.81%). Maximum (51.69±30.77%) and minimum (19.80±30.44%) survival rates at 20 ppt were observed in the fish fed canola oil-enriched *Artemia* and the commercial feed, respectively. Significant differences in tolerance at 20ppt were observed between the fish fed *Artemia* enriched with canola oil and those fed the commercial feed and *Artemia* enriched with sunflower oil (p<0.05). With a survival rate of 99.03±2.05%, the fish fed *Artemia* enriched with canola oil showed maximum resistance against hypoxic stress and this was significantly different from survival rates of the fish fed the commercial feed and non-enriched *Artemia* (p<0.05).

Table 4: Survival rate (mean±standard deviation) of the fish of different feeding treatments under environmental stresses.

Stress condition	Commercial feed	*Artemia* enriched with fish oil	*Artemia* enriched with sunflower oil	*Artemia* enriched with canola oil	*Artemia* enriched with soybean oil	None-nriched *Artemia*
Temperature (20°C)	94.77±5.29[b]	92.35±8.43[b]	99.44±1.84[a]	99.67±1.34[a]	91.67±10.85[b]	93.44±5.74[b]
Temperature (25°C)	17.01±3.46[c]	82.02±5.93[a]	63.68±10.68[b]	87.02±5.74[a]	17.01±3.98[c]	17.68±4.64[c]
Salinity (10 ppt)	79.69±9.41[b]	100±0.00[a]	98.01±7.38[a]	99.04±2.67[a]	100±0.00[a]	99.67±1.32[a]
Salinity (15 ppt)	76.07±6.81[c]	86.77±8.39[b]	88.44±8.73[b]	97.79±3.65[a]	93.44±8.49[ab]	93.33±6.24[ab]
Salinity (20 ppt)	19.80±3.14[b]	44.40±1.78[a]	32.11±11.22[b]	51.69±7.77[a]	41.35±7.44[a]	43.22±8.58[a]
Hypoxia (5 ppm)	91.62±5.01[b]	95.92±4.37[ab]	97.59±3.05[a]	99.03±2.05[a]	97.71±1.59[a]	90.20±15.20[b]

In each row data having different alphabetic letters are significantly different (p< 0.05)

Discussion

The important role of lipids in fish growth has been discovered and a number of lipid source of animal and plant origin have been used in fish feed formulation. In general, adequate fatty acids in fish diet will guarantee fish growth (Legendre *et al*., 1995). However, none of the fish species or *Artemia* can synthesize the essential fatty acids such as EPA, DHA and arachidonic acid (Arulvasu and Munuswamy, 2009). Fatty acids such as oleic, linoleic and DHA were high in the fish of all feeding treatments, and there was no significant difference in arachidonic acid and EPA levels among the treatments. DHA, EPA and arachidonic acid are three main fatty acids which are essential for normal growth and development of fish (Sargent *et al*., 1999). Results of this study showed that using fish oil for enrichment of *Artemia* resulted in lower DHA levels in the fish, whereas feeding *Artemia* enriched with canola and sunflower oils enhanced DHA levels in the fish. This indicates the suitability of these plant oils to be readily used as a substitute for fish oil in the enrichment of *Artemia*. This can ultimately carry essential fatty acids to the fish. The high concentrations of 18:3n-3 provide a significant n-3/n-6 ratio in plant oils, while animal oils always present EPA and DHA at different levels (Narciso *et al*., 1999). Enrichment of *Artemia* with canola and sunflower oils has resulted in maximum survival rates in the fish, while feeding *Artemia* enriched with cotton seed oil led to significantly lower survival rate in Persian sturgeon as compared with the fish fed *Artemia* enriched with fish oil (Hafezieh *et al*., 2010). Thus, it can be assumed that canola and sunflower oils may have nutritional advantages over some other plant oils such as cotton seed oil. Bell *et al*. (2001) did not observe significant differences in survival rate of Atlantic salmon (*Salmo salar*) following replacement of fish oil with coconut oil in its diet. However, n-3 fatty acids content was significantly lower in the fish fed with 50 and 100% coconut oil. Similarly, in this study no significant differences were observed in survival rates of the fish following total replacement of fish oil with the plant oils. However, there were higher n-3 fatty acids levels in the fish fed *Artemia* enriched with canola and sunflower oils. Using corn oil as the sole lipid source in brown trout (*S. trutta*) resulted in lower growth and elevated mortality (Phillips *et al*., 1963). Though this study was performed in the most critical stage of the fish growth, mortality was notably low by using the plant oils. In this study feeding the fish with *Artemia* resulted in significantly higher growth rate as compared with feeding the commercial feed. This was in agreement with previous studies on rainbow trout (Boye *et al*., 1997; Kolkovoski *et al*., 1997).

Furthermore, fish growth rates did not differ when fed *Artemia* enriched with either fish or plant oil. Similarly, replacement of fish oil with soybean and coconut oils in the diet did not affect growth and FCR of Pacu, *Colossoma macropomum* (Viegas and Contreras, 1994) and Nile tilapia, *Oreochromis niloticus* (Al-Owafeir and Belal, 1996). Growth of African catfish, *Heterobranchus longifilis*, was higher after feeding a diet containing coconut oil as compared with those fed peanut, cotton seed and fish liver oils (Legendre *et al*., 1995). In contrast, Yildiz and Sener (1997) after using fish, sunflower and soybean oils in the diet of seabass (*Dicentrarchus labrax*) found that maximum weight gain and minimum FCR in the fish fed fish oil. Feeding

Senegalese sole (*Solea senegalensis*) larvae with *Artemia* enriched with a soybean oil emulsion, when compared to non-enriched *Artemia*, resulted in slower amino acid absorption and reduced food intake (Morais *et al*., 2006). Santigosa *et al*. (2011) noted that although inclusion of plant oil in the diet of a carnivorous fish (*Sparus aurata*) had led to growth rates similar to those obtained under fish oil diets, this may affect physiological processes of the fish such as digestive enzyme activity. Possible effect of lipid source on enzymatic activities and consequently the fish growth has been addressed by Morais *et al*. (2006).

Results of this study showed that fatty acid profile of the fish was notably dependent on that of their consumed food. Higher HUFA levels are translated to higher fatty acids allocated to cellular metabolism and as a result, to fish growth. Furthermore, higher monounsaturated fatty acids (MUFA) will mean that there are more fatty acids for synthesizing of longer chain fatty acids that can consequently convert to EPA (Halver and Hardy, 2002). The n-3 fatty acids are essential for a number of physiological functions including normal growth and development of most fish species (Narciso *et al*., 1999). However, enrichment of *Artemia* with fatty acids did not result in any significant difference in the growth of the Caspian Kutum, *Rutilus frisii kutum* (Imanpour, 2005). In the study of Javaheri (2006) no significant difference was observed in the dry weight and survival rate of the Caspian trout (*S. trutta caspius*) between the fish fed with non-enriched *Artemia* and *Artemia* enriched with HUFA. Narciso *et al*. (1999) compared the nutritional quality of *Artemia* sp. enriched with various oils of plant and animal origins and found that HUFA content and DHA: EPA ratio were much lower in *Artemia* enriched with plant oils. Reinitz and Yu (1981) and Sargent *et al*. (1989) suggested that some plant oils such as cotton seed oil which are rich in alpha-linoleic acid and can be involved in the synthesis of HUFA, may serve as suitable substitutes for fish oil in fish diets. Caballero *et al*. (2002) after replacing fish oil with other lipid sources including soybean, canola and olive oils in the diet of rainbow trout observed that feeding soybean oil with 25% linoleic acid increased DHA in the fish. This was in accordance with the study of Skonberg *et al*. (1994) on Coho salmon (*O. kisutch*) in which partial replacement of fish oil with sunflower oil resulted in increased DHA level in the fish tissues. Using *Artemia* enriched withby plant oils enhanced resistance against the environmental stresses in the studied fish and they have much higher survival rates than the fish fed with commercial feed and almost comparable resistance with those fed fish oil-enriched *Artemia*. Our results are in agreement with those in the study of Akbary *et al*. (2011) in which rainbow trout fed with *A. urmiana* enriched with HUFA and vitamin C showed significantly higher survival rates under hyperthermal and hypoxic conditions. Gapasin *et al*. (1998) and Lim *et al*. (2002) found that high levels of fatty acids in the diet of fish can promote its resistance and immune response against stress conditions. It was also reported that supplementation of HUFA- enriched *A. franciscana* improved survival, growth, disease and environmental stress resistance in shrimp (Immanuel *et al*., 2007). Therefore, in the current work the increased resistance to stresses in the fish fed enriched *Artemia* is likely due to the existence of adequate n-3 HUFAs in the fish larvae which improve their physiological functions. However, it is not possible to find which fatty acid plays the main role in stress resistance, as the level of almost all of them increases following the enrichment process. Ako *et al*. (1994) named DHA to be the main factor inducing resistance against stresses. Azari Takami *et al*. (2004) found significantly higher survival rates against stresses of high temperature, hypoxia and high density in rainbow trout fed with *A. urmiana* enriched with vitamin C. Piedecausa *et al*. (2007) found that sharpsnout seabream, *Diplodus puntazzo*, fed a diet containing linseed oil were more susceptible to stress conditions than those fed fish or soybean oils. Mirzakhani (2004) observed that using HUFA- enriched *Artemia* increased resistance against thermal and pH stresses in rainbow trout. Enrichment of *Artemia* with vitamin C increased survival of African catfish, *Clarias gariepinus* under salinity stress (Merchie *et al*., 1997).

In general, different fish species may have various responses differentially to feeding diets containing plant oils. However, no previous study has addressed effects of replacing the costly fish oil with plant oils especially canola and sunflower oils in the enrichment of *Artemia in* feeding rainbow trout larvae. The larvae of this fish are not usually fed live food but the commercial feeds used are high in fish oil (Naylora *et al*., 2009). Considering the elevated HUFA levels and consequently, higher growth and survival rates in the fish fed *Artemia* enriched with the plant oils compared to those fed commercial feed, replacement of formulated feed with *Artemia* nauplii enriched with oils from plant sources may be recommended. A further advantage is that less time duration is needed for enrichment of *Artemia* with plant oils and this can help with supplying high-quality and less expensive feed within a shorter time. Furthermore, using plant-oil-based feeds such as that containing canola oil can lessen susceptibility of the fish in varying environmental conditions which may occur in fish farms. In comparison with the previous studies using various plant oil sources, it seems that canola and sunflower oils are more suitable substitutes for fish oil in trout feeding. Considering that studies on the effects of using plant oils in fish diet on its growth and fatty acids profile are limited, results of this study can serve as basic guidelines for fish feed formulation.

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References

Akbary, P., Hosseini, S.A. and Imanpoor, M.R., 2011. Enrichment of *Artemia* nauplii with essential fatty acids and vitamin C: effect on rainbow trout (*Oncorhynchus mykiss*) larvae performance. *Iranian Journal of Fisheries Sciences*, 10(**4**), 557–569.

Ako, H., Tamani, C.S., Bass, P. and Lee, C.S., 1994. Enhancing the resistance to physical stress in larvae of *Mugical cephalus* by the feeding of enriched *Artemia* nauplii. *Aquaculture*, 122, 81–90.

Al-Owafeir, M.A. and Belal, I.E.H., 1996. Replacing palm oil for soybean oil in tilapia, *Oreochromis niloticus* (L.), feed. *Aquaculture Research*, 27, 221–224.

Arulvasu, C. and Munuswamy, N., 2009. Survival, growth and composition of *Poecilia latipinna* fry fed enriched *Artemia* naupli. *Current Science*, 96, 114-119

Azari Takami, G., Meshkini, S., Rasouli, A. and Amini, F., 2004. Effects of feeding *Artemi urmiana* nauplii enriched by vitamin C on growth, survival and resitance against environmental stresses. *Pajohesh Va Sazandegi*, 66, 25–32. (In Persian).

Bell, G.J., Mcevoy, J., Tocher, D.R., Mcghee, F., Campell, P.J. and Sargent, J.R., 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid composition and hepatocyte fatty acid metabolism. *Journal of Nutrition*, 131, 1535–1543.

Bell, G.J., Henderson, R.J., Tocker, D.R., Ghee, F.M., Dick, J.R., Porter, A., Smullen, R.P. and Sargent, J.R., 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon *Salmo salar*) affect muscle fatty acid composition and hepatic fatty acid metabolism. *Journal of Nutrition*, 132, 222–230.

Boye, J.I., Ma, C.Y. and Hardwalkar, V.R., 1997. Thermal denaturation and coagulation of proteins. In: S. Damodaran and A. Paraf (Eds), Food proteins and their Applications. Marcel Dekker Inc, New York. pp. 255–260.

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. and Izquierdo, M.S., 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 214, 253–271.

Chhetri, A.B., Tango, M.S., Budge, S.M., Watts, K.C. and Islam, M.R., 2008. Non- dibble plant oils as new sources for biodiesel production. *International Journal of Molecular Science*, 9(**2**), 169–180.

Gapasin, R.S.J., Bombeo, R., Lavens, P., Sorgeloos, P. and Nelis, H.J., 1998. Enrichment of live food with essential fatty acids and vitamin C: effects on milkfish (*Chanos chanos*) larval performance. *Aquaculture*, 162, 269–285.

Hafezieh, M., Kamarudin, M.S., Bin Saad, C.R., Kamal Abd Sattar, M., Agh, N., Valinasab, T., Sharifian, M. and Hosseinpour, H., 2010. Effect of enriched *Artemia urmiana* on growth, survival and fatty acid composition of the Persian sturgeon larvae. *Iranian Journal of Fisheries Sciences*, 9(**1**), 61–72.

Halver, J.E. and Hardy, R.., 2002. Fish nutrition. Academic Press, New York. pp. 182–246.

Huanng, S.S.Y., Fu, C.H.L., Higgs, D. A., Balfry, S.K., Schulte, P.M. and Brauner, C.J., 2008. Effects of dietary canola oil level on growth performance, fatty acid composition and immuonoregulatory development of spring Chinook salmon parr, *Oncorhynchus tshawytscha*. *Aquaculture*, 274, 109–117.

Imanpour, M.R., 2005. Effects of photoperiod and feeding with enriched *Artemia* on growth and osmoregulation of the Caspian whitefish, *Rutilus frisii kutum*. PhD Thesis. Gorgan University of Agriculture and Natural Resources. 110P (In Persian).

Immanuel, G., Citarasu, T., Sivaram, V., Shankar, V.S. and Palavesam, A., 2007. Bioencapsulation strategy and highly unsaturated fatty acids (HUFA) enrichment in *Artemia franciscana* nauplii by using marine trash fish *Odonus niger* liver oil. *African Journal of Biotechnology*, 6(**17**), 2043–2053.

Javaheri, M., 2006. Study on the effects of feeding *Artemia* enriched with highly unsaturated fatty acids as starter food on Caspian Sea salmon (*Salmo salar caspius*). *Journal of Enviromental Sciences*, 11, 55–64.

Kamarudin, M.S., Ramezani-Fard, E., Saad, C.R. and Harmin, S.A., 2011. Effects of dietary fish oil replacement by various vegetable oils on growth performance, body composition and fatty acid profile of juvenile Malaysian mahseer, *Tor tambroides*. *Aquaculture Nutrition*, 18(5), 532–543.
doi: 10.1111/ j. 13652095. 2011. 00907.x

Kolkovoski, S., Arieli, A. and Tandler, A., 1997. Visual and chemical cues stimulate microdiet ingestion in sea bream larvae. *Aquaculture International*, 5, 527–536.

Lavens, P. and Sorgeloos, P., 1996. Manual on the production and use of live food for aquaculture FAO Fisheries Technical Paper. No. 361. Rome, FAO. 295P.

Legendre, M., Kerdchuen, N., Corraze, G. and Bergot, P., 1995. Larval rearing of African catfish, *Heterobranchus longifilus* (Teleostei, Clariidae): effect of dietary lipids on growth, survival and fatty acid composition of fry. *Aquatic Living Resources*, 8, 355–363.

Leger, P. Bengston, D.A. and Sorgeloos, P., 1987. The nutritional value of Artemia. *Artemia Research and Its Application*, 24,521-623

Lepage, G. and Roy, C.C., 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *Journal of Lipid Research*, 25, 1391–1396.

Lim, L.C., Dhert, P., Chew, W.Y., Dermaus, V., Nelis, H. and Sorgeloos, P., 2002. Enhancement of stress resistance of goppy *Poecillia reticulate* through feeding with vitamin C supplement. *Journal of the World Aquaculture Society*, 33, 32–40.

Merchie, G., Lavens, P., Verreth, J., Ollevier, F., Nelis, H., DeLeenheer, A., Storch, V. and Sorgeloos, P., 1997. The effect of supplemental ascorbic acid in enriched live food *Clarias gariepinus* larvae at start feeding. *Aquaculture*, 151, 245-258.

Miller, M.R., Nichols, P.D. and Carter, C.G., 2008. *n*-3 Oil sources for use in aquaculture – alternatives to the unsustainable harvest of wild fish. *Nutrition Research Reviews*, 21, 85–96.

Mirzakhani, M., 2004. Effects of using non-enriched *Artemia* and *Artemia* enriched by highly unsaturated fatty acids on growth and survival rate of rainbow trout, *Oncorhynchus mykiss*. Masters Disertation. Tarbiat Modarres University, Tehran, Iran. 52P (In Persian).

Morais, S., Caballero, M.J., Conceição, L.E.C., Izquierdo, M.S. and Dinis, M.T., 2006. Dietary neutral lipid level and source in Senegalese sole (*Solea senegalensis*) larvae: Effect on growth, lipid metabolism and digestive capacity. *Comparative Biochemistry and Physiology, Part B*, 144, 57–69.

Narciso, L., Pousão-Ferreira, P., Passos, A. and Luís, O., 1999. HUFA content and DHA/EPA improvements of *Artemia* sp. with commercial oils during different enrichment periods. *Aquaculture Research*, 30, 21–24.

Naylora, R.L., Hardy, R.W., Bureauc, D.P., Chiua, A., Elliottd, M., Farrelle, A.P., Forstere, I., Gatlin, D.M., Goldburgh, R.J., Huac, K. and Nicholsi, P.D., 2009. Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences*, 106(**36**), 15103–15110.

Phillips Jr, M., Livingston, D.L., Poston, H.A. and Brooke, H.A., 1963. The effect of diet mixture and calorie source on growth, mortality, conversion and chemical composition of brook trout. *The Progressive Fish Culturists*, 25(**1**), 8-14. DOI: 10.1577/1548-8659 (1963) 25 [8:TEODMA] 2.0.CO;2

Piedecausa, M.A, Mazon, M.J., Garcia, B. and Hernandez, M.D., 2007. Effect of total replacement of fish oil by vegetable oils in the diets of sharpsnout seabream (*Diplodus puntazzo*). *Aquaculture*, 263, 211– 219.

Reinitz, G.L. and Yu, T.C., 1981. Effects of dietary lipids on growth and fatty acids composition of rainbow trout (*Salmo gairdneri*). *Aquaculture*, 35, 19–27.

Santigosa, E., García-Meilań, I., Valentín, M.V., Navarro, I, Pérez-Sańchez, J. and Gallardo, M.A., 2011. Plant oils' inclusion in high fish meal-substituted diets: effect on digestion and nutrient absorption in gilthead sea bream (*Sparus aurata* L.). *Aquaculture Research*, 42, 962–974.

Sargent, J., Bell, G., McEvoy, L., Tocher, D. and Estevez A., 1999. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, 177, 191–199.

Sargent, J., Henderson, R.J. and Tocher, D.R., 1989. The lipids, In: Halver, J. E. (Ed.), Fish Nutrition. pp. 154–209.

Skonberg, D.I., Rasco, B.A. and Dong, F.M., 1994. Fatty acid composition of salmonid muscle changes in response to a high oleic acid diet. *Journal of Nutrition*, 124, 1628–1638.

USDA (United States Department of Agriculture), 2007. http://www.fas.usda.gov/psdonline.

Viegas, E.M.M. and Contreras, E.S.G., 1994. Effect of dietary crude palm oil and a deodorization distillate of soybean oil on growth of tambaqui (*Colossoma macropomum*). *Aquaculture,* 124, 127–131.

Yildiz, M. and Sener, E., 1997. Effect of dietary supplementation with soybean oil, sunflower oil or fish oil on the growth of seabass (*Dicentrarchus labrax* L.). Workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean, 24-26 June 1996, Zaragoza, Spain, 9P.