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[Research]

Effects of Using *Artemia urmiana* Enriched with N-3 HUFA in First Feeding of Rainbow Trout (*Oncorhynchus mykiss*) Larvae.

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ABSTRACT
Effects of using n-3 HUFA-enriched Artemia and non-enriched Artemia as the starter food for growth and survival of rainbow trout larvae compared to commercial concentrated food were investigated in this study. The larvae with average weight of 92.9 mg were fed for 20 days with four food treatments included of commercial concentrated food, newly hatched Artemia, enriched Artemia, and mixture of enriched Artemia and concentrated food. At the end of experiment, the highest weight gain percentage was 104.4% in those larvae fed with enriched Artemia ($p< 0.05$). The highest survival rate was 96.5 % observed in larvae fed with mixture of enriched Artemia and commercial concentrated food ($P<0.05$). Larvae of third treatment that were fed with enriched Artemia with a survival rate of 98.9 %±1.9 under stress condition of low pH and survival rate of 66.5%±3.7 under stress condition of higher pH in rearing environment, were the most resistant larvae against stress resulting from pH fluctuation ($P<0.05$). Meanwhile the highest resistance against stress, resulting from temperature changes, was seen in larvae of the third and fourth treatment with survival rate of 77.8 % at 24°C respectively.

Keywords: Enriched Artemia, Growth, *Onchorhynchus Mykiss,* Stress, Survival, Unsaturated Fatty Acid.

INTRODUCTION

Successful rearing of larval fish is the most critical stage in the production cycle for many species. The primary problem in rearing larval fish is that of food supply (Leger *et al*., 1986; Abi-Ayad and Kestemont, 1994); a readily available diet which has a high nutritional quality and is easily accepted and digested by the larval fish must be used (Kim *et al*., 1996). Live prey organisms, especially zooplanktons, are used as food larvae for certain species of fishes. Among preys, Artemia nauplii is mostly used due to having advantages like easy availabil-ity during the year, high nutritional value, and possibility of improvement of its value through enrichment techniques. (Leger *et al*., 1986).

Artemia nauplii has widely been used for rearing many species of marine and freshwater fish (Tuncer and Harrell 1992), but due to shortage of necessary fatty acids, particularly n-3 HUFA, Artemia can not supply all the nutrients required for growth of fish larvae, reason that, Artemia in most cases is first enriched before using (Lemm and Lemarie, 1991).

Salmonids have a fully developed digestive system at the first feeding stage and they are normally fed prepared diets from first feeding. But the quantity of feed used in early rearing is small, and using an expensive starter feed should not substantially increase the cost of production, providing that any growth advantage obtained early in rearing cycle is sustained after the fish are switched to regular prepared feeds (Kim *et al*., 1996). The authors hypothesized that if Artemia enriched had been fed, growth rates would likely have been higher for the fish fed nauplii than for those fed the pelleted feeds used at that time.

Stress resistance experiments are conducted based on exposing larvae to unbalanced

physical, chemical or biological conditions for short periods of time (Ako *et al*., 1994). In this study, effects of foods and subsequently quality of produced larvae are determined through a fast and simple experiment by measuring larvae's resistance and reviewing their survival while facing generated stress.

High larvae mortality these days can be seen, in most hatcheries due to first feeding of these larvae. For this reason and considering need of Rainbow trout to n-3 HUFA (Takeuchi and Watanabe 1982, Tacon, 1990), Artemia and enriched Artemia were used in this research to compare their influences on increase of growth and survival and resistance of larvae against environmental stress with artificial feed.

MATERIALS AND METHODS

Larvae Rearing

In this study, trays with dimensions of 42.5 cm x 42.5 cm x 20 cm special for incubation of Rainbow trout eggs were used for rearing of larvae. In the first step, every tray was divided into two parts using a plastic wall, then two trays were placed in one trough, and each trough was used for running one treatment with three replicates. A water flow with 10-15 liter discharge per minute was directed in each trough. Water source was a mixture of spring water and river water at 9°C, with 8 mg/l of dissolved oxygen and PH= 7.8. Two days before starting of treatments, (the larvae produced in hatchery that $^2/_3$ yolk sack of which had been absorbed were counted about 330 larvae and then transferred to each part of tray). At this time, larval density was about 25 larvae per liter (Kim *et al*., 1996).

Preparation and Enrichment of Artemia

The cyst of Artemia used for this study obtained from Urmia Lake. Hatching and collecting of nauplii was carried out according to Lavens and Sorgeloos (1996).

Enrichment solution used in this experiment contained Cod Liver Oil (Seven Seas), Polysorbat (Tween 80, Merck) and freshwater. First, 5 ml of polysorbat was added to 50 ml of water and mixed by stirring. Then 50 ml of the oil was added to the compound and stirring was continued till the solution homogenized. For each liter of water, 0.3 ml to 0.5 ml of this solution was added to incubator of Artemia nauplii for enrichment. Artemia nauplii were enriched at instar II for 9 hours with a density of 300-500 thousand of nauplii per liter of incubator environment (Ako *et al*., 1994). After enrichment, to preserve nutrition value and decrease metabolism and consumption of fatty acids, enriched Artemia were preserved at 5°C and in the water with salinity of 25- 30 ppt (Leger *et al*., 1987).

Food Treatments

In this study, effects of four foods on Rainbow trout larvae were examined in terms of growth, survival, and resistance against stress.

First Treatment: commercial concentrated food special for Rainbow trout larva supplied from Chineh Co. Second Treatment: Newly-hatched Naoplius of Artemia (Instar I) 3rd Treatment: Enriched Artemia. 4th Treatment: A compound of 50% commercial concentrated food and 50% enriched Artemia.

Amount of daily food for each group of larvae was calculated based on nutrition tables considering temperature of water and average larvae weight, and were fed to the larvae 8 times (Tacon, 1990). Required cyst for culture in each day, were calculate based on dry weight of individual nauplii of *Artemia urmiana* which is about 2.7 micro grams and hatching efficiency of Artemia cyst.

Growth and Survival

To determine growth and survival, dead larvae were collected and calculated in each treatment on a daily basis. To determine growth and survival, larvae weight index was measured by randomly sampling of 25 larvae of each treatment on 10^{th} and 20^{th} days. Some formula for calculating of fish growth is fallow (Tacon, 1990).

Weight gain (%) = (Wt (final) – Wt (initial) / Wt (initial)) x 100

SGR= [LN final wt – LN initial wt / No. of days] x 100

pH Stress Resistance Experiment

After 20 days period for rearing of larvae, to determine larvae's resistance, an experiment was conducted using different pHs including pH= 3.5 and 5.8 as low pH level, and pH= 9.8 as high pH level. To do such experiment, first some larvae rearing

Table 1. Average content of fatty acid in concentrated food and Artemia urmiana before and after enrichment and preservation at low temperature (in mg per dry gram of Artemia)

Fatty acids	Commercial food	Artemia instar I	Enriched Artemia	Enriched Artemia (after 6 h preservation)	Enriched Artemia (after 12 h preservation)
14:0	1.8±0.11	1.5±0.04	1.1±0.05	1.1±0.12	1.6±0.17
16:0	21.9±1.2	18.2±0.08	15.4±0.37	16.8±0.57	19.4±0.42
16:1(n-7)	3.6±0.81	4.9±0.12	4.6±0.25	4.6±0.62	7±0.07
18:0	3.7±0.36	3.9±0.18	4.6±0.14	4.6±0.4	4.5±0.07
18:1(n-9)	5.4±0.63	20.4±1.31	18.4±0.42	20.4±0.13	18.6±0.11
18:2(n-6)	32.7±0.3	9.8±0.32	11±0.07	8.5±0.14	10±0.23
18:3(n-3)	4.7±0.06	23.7±0.54	26.8±0.37	24.2±0.83	20.7±0.18
EPA 20:5(n-3)	-	2.6±0.11	8.5±0.67	3.8±0.64	3.5±0.54
DHA 22:6(n-3)	-	Trace*	1.4±0.12	0.49±0.03	0.4±0.08
(∑UFA)	66.4±0.28	61.4±2.18	68.7±2.75	62±1.05	60.2±0.58
∑n-3PUFA	4.7±0.06	26.3±0.43	36.7±0.18	28.7±0.1	24.7±0.64
∑n-3HUFA	-	2.6±0.11	9.9±0.55	4.3±0.67	3.9±0.46

* Trace= very small
∑UFA= total unsaturated fatty acid
∑n-3PUFA= total n-3 poly unsaturated fatty acid
∑n-3HUFA= total n-3 highly unsaturated fatty acid

environment water was poured into an aquarium and 12 little baskets were placed inside the aquarium. To decrease pH normal HCl (0.1 %) and to increase pH, NaOH was used. To examine larvae's resistance in the nominated pHs, 30 larvae were taken randomly from each replicate and then transferred to the little baskets inside the aquarium. Larvae survival was examined during the 60 minutes with counting of dead larvae.

Temperature Stress Resistance Experiment

To examine larvae's resistance against stress resulting from temperature fluctuations, stress experiment was conducted at 18-24°C as high temperature stress and at 1°C as low temperature stress. 30 larvae were taken accidentally from each replicate and transferred to the little baskets inside the aquarium at pre-determined temperature. Larvae's survival during one hour was checked by gathering and counting of death larvae.

Determining Fatty Acid Content in Foods and Body of Rainbow Trout Larvae

Gas chromatography equipment was used to determine fatty acid profile in concentrated food, enriched and non-enriched Artemia and Rainbow trout larvae. The samples were dried in a vessel at 60 for 24 hours (AOAC, 1990) and then preserved closed containers and in freezer until fatty acid we extracted.

Statistical Analysis

To analyze statistical results, one-way ANOVA was applied and the mean compression was done through DMRT at reliability level of 5%. Data analysis was done in SPSS soft ware.

RESULTS

Enrichment of Artemia

Results of examination of fatty acid profile of newly-hatched Artemia, enriched Artemia and Artemia preserved in the fridge are shown in Table 1. As we can see, EPA content and n-3 HUFA in newly-hatched Artemia is 2.6 mg per dry gram of Artemia, and DHA content is very small. After enrichment of *Artemia urmiana* with Cod Liver Oil, EPA, DHA, and n-3 HUFA contents increased which were 8.5, 1.4 and 9.9 mg respectively per 1 dry gram of Artemia. The content of n-3 HUFA was decreased after preservation of enriched Artemia in the fridge for 6 hours and 12 hours, nevertheless, the amount of these compounds were higher than newly-hatched nauplii of Artemia. There is no n-3 HUFA in commercial concentrated food (Table 1).

Fatty Acids in Rainbow Trout Larvae

Results of examination of fatty acid composition in Rainbow trout larvae after feeding with different food are shown in Table 2. EPA and DHA were respectively 2.07 and 0.37 mg per dry gram of larva only in third treatment that fed with enriched

Table 2. Average fatty acid content in Rainbow trout larvae at the end of experiment (in mg per dry gram)[1]

Fatty acids	Treatment 1	Treatment 2	Treatment 3	Treatment 4
14:0	2.05±0.08	0.73±0.04	0.98±0.04	2.57±0.15
16:0	23.17±1.09	22.15±0.12	19.33±0.07	27.98±0.46
16:1(n-7)	3.59±0.19	3.48±0.03	3.34±0.19	3.88±0.11
18:0	7.69±0.12	5.26±0.08	5.68±0.13	4.31±0.05
18:1(n-9)	15.04±0.06	14.88±0.1	17.14±0.25	15.42±0.4
18:2(n-6)	17.97±0.07	13.12±0.11	12.01±0.14	18.72±0.14
18:3(n-3)	1.36±0.05	3.37±0.07	5.04±0.14	2.09±0.18
EPA 20:5(n-3)	-	-	2.07±0.01	-
DHA 22:6(n-3)	-	-	0.37±0.01	-
(∑UFA)	38.84±0.58 ab	34.84±0.1 a	39.45±0.28 ab	40.1±0.54 ab
∑n-3PUFA	1.36±0.05 a	3.37±0.07 c	7.48±0.12 d	2.09±0.18 ab
∑n-3HUFA	-	-	2.44±0.02	-

[1] Values in rows with different letters have significant differences (P<0.05).
* Trace= very small

Artemia, and no n-3 HUFA was seen in other treatments. Meanwhile, Linolenic acid (18:3n-3) that is necessary for growth of Rainbow trout in third treatment larvae was higher than in larvae of other treatments with 5.04 mg per dry gram of larva. Linolenic acid in first treatment larvae was also less than that in concentrated food.

Growth Results

Results of examination of body weight of rainbow trout larvae on first day (before starting of feeding), 10th and 20th days in post experiment are shown in fig 1. As result showed, growth was better in treatments 3 and 4, fallowed with treatments 1 and 2 respectively. In 10th and 20th days of experiment, weight gain percentage of third treatment larvae was 70.1 % and 104.4 % respectively, which was the highest weight gain compared to larvae in other treatments (p<0.05). SGR in 10th day significantly was better in third treatment than other treatments but after 20 days there was no significant difference between treatments (Table 3).

Survival of Rainbow Trout Larvae

Results of survival examination of Rainbow trout larvae up to 20 days of experiment are shown in Table 3. The highest percentage of survival up to 20th day was 96.5 % that was seen in fourth treatment larvae fed with mixture of concentrated food and enriched Artemia. Difference between average survivals rates of those larvae were fed with daily food containing Artemia was considerably different with first treatment larvae which were fed with concentrated food.

Resistance of Rainbow Trout Larvae Against pH Stress

To examine effect of pH on each of four group of rainbow trout larvae resistance against stress conditions resulting from environment pH fluctuations, first pH= 5.8 was considered as low pH stress (as larvae rearing environment pH was 7.8). After one hour, no mortality was seen in any treatment. Then 30 larvae were placed in an environment with pH=3.5. Results of larvae survival under stress of pH= 3.5 are shown in Table 4. The difference among the treatments was not considerable, but third treatment larvae (fed with enriched Artemia) showed the highest survival percentage.

To examine larvae resistance under stress conditions with pH higher than that in breeding environment, 30 larvae of each replicate were placed in an pH= 9.8. Larvae

Table 3. Average weight gain percentage, SGR and survival in Rainbow trout larvae fed different foods[1]

Treatment	WG (%) (Day 10)	WG (%) (Day 20)	SGR (Day 10)	SGR (Day 20)	SV (%) (Day1- 10)	SV (%) (Day1- 20)
1	49.35±8.87 a	93.6±8.4 a	3.96±0.59 a	3.3±0.21 a	96.7±1.56 a	90.7±2.95 a
2	61.12±3.39ab	89.47± 1.2 a	4.77±0.21 ab	3.19±0.03 a	97.1±0.19 a	95.4±1.37 b
3	70.1±6 b	104.42±9.47b	5.31±0.35 b	3.57±0.23 a	97.9±0.3 a	95.6±0.91 b
4	58.93±7.47ab	99.52±11.2ab	4.63±0.47 ab	3.45±0.28 a	98.1±1.43 a	96.5±1.69 b

[1] Values in columns with different letters have significant differences (P<0.05).
WG= weight gain, SGR= Specific Growth Rate, SV= Survival Rate

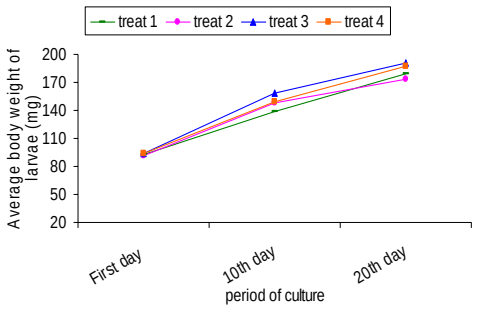

Fig 1. Average weight of rainbow trout larvae fed different foods in the culture periods

survival results under this condition are shown in Table 4.

As it is seen, difference among treatments is significant (P<0.05). In high pH, larvae fed with enriched Artemia showed the highest resistance.

Resistance of Rainbow trout larvae against temperature stress

To examine effect of temperature on each of four group of rainbow Trout larvae resistance against stress conditions, resulting from environment temperature fluctuations, first 18°C and 1°C temperatures were used as high and low temperature stresses respectively (considering temperature 9°C as larva rearing environment). After one hour, no difference was found in larvae from different treatments between these two temperatures. (The temperature was increased to 24°C and 30 larvae of each replicate were placed in this environment). Results of larvae resistance under stress condition at 24°C are shown in Table 4. As it is seen, larvae of fourth and third treatments with survival rate of 77.8% showed the highest resistance and larvae of first treatment fed with concentrated food showed the lowest resistance of 33.3%. Survival rate was significant among treatment (P<0.05).

DISSCUSION

In this study, it was observed that feeding Rainbow trout larvae with HUFA-enriched Artemia resulted in growth improvement and increase of survival rate compared to concentrated food and non-enriched Artemia. It is maybe due to quality of enriched Artemia in comparison to artificial feed and non-enriched Artemia. This correlation has been reported in some other species of fishes (Dhert *et al.*, 1990; Mouriente *et al.*, 1993; Gapasin *et al.*, 1998). Results of this study are in accordance with, previous findings as to importance of HUFA in feeding Rainbow trout larvae. Takeuchi and Watanabe (1982) showed that adding 1% of DHA or 1% of HUFA to daily food result in growth improvement and decline in food conversion ratio. It was proved in their findings that Rainbow trout has better growth improvement with DHA and HUFA compared to linolenic acid (18:3n-3).

Additionally the period of intake after feed distribution may have been longer for Artemia than for pellets because their movement may have elicited a longer feeding response. As a result, Artemia consumption and thus growth might have been higher. In both Rainbow trout (Ware, 1973) and Atlantic salmon (Rimmer and Power, 1978; Holm and Moller, 1984), prey motion has been shown to be of major importance in triggering feeding response.

Enrichment method of Artemia used in this study considerably boosted up fatty acids in Artemia, results which is relatively similar to that of other studies conducted with different oil sources for enrichment of Artemia (Ako *et al.*, 1994).

Triplication of EPA and emergence of DHA is worth stating that both of these acids play a critical role in growth and evolution of fish larvae specially sea fishes (Dhert *et al.*, 1990).

Results of profile of fatty acids existing in larvae indicate that third treatment of the experiment fed with enriched Artemia had a better growth than those in other treatments. N-3 HUFA content is higher in them and proves importance of this group of fatty acids in growth of Rainbow trout. In body composition of Rainbow trout larvae, fatty acids of linolenic acid were higher value

Table 4. Average Survival Rate (%) of Rainbow trout larvae against pH and Temperature stress condition

Treat.	pH=3.5	pH=9.8	Temp. (24 ℃)
1	92.2±6.9 ^{a}	40±14.6 ^{a}	33.3±6.6 ^{a}
2	94.4±1.9 ^{a}	46.7±3.4 ^{ab}	62.2±3.9 ^{b}
3	98.9±1.9 ^{a}	65.5±3.7 ^{b}	77.8±6.9 ^{c}
4	97.9±1.9 ^{a}	58.9±16.4 ^{ab}	77.8±11.7 ^{c}

Values in columns with different letters have significant differences (P<0.05).

compared to linoleic acid (Sedwick, 1990). Additionally, the need of Rainbow trout and other salmon fishes for n-3 HUFA have been proved to be ineffective growth. (Takeuchi and Watanabe, 1982). Nevertheless, Rainbow trout larvae are not able to convert fatty acids of linoleic family to HUFA like EPA and DHA (Sedwick, 1990), therefore, adding HUFA to food dosage of these fishes particularly during larval period, seems to be very critical and necessary.

Results of this study proves that feeding Rainbow trout with live food containing high n-3 HUFA content increases larvae resistance in stress conditions, resulting from fluctuations of pH and water temperature. Relationship between feeding of larvae by HUFA-enriched Artemia and enhancing resistance against environmental stress has been reported in other species of fishes (Dhert *et al*., 1990, Ako *et al*, 1994), but nothing has been reported as to increase in Rainbow trout larvae resistance by using HUFA-containing Artemia.

Examination of Rainbow trout larvae resistance in this study shows that feeding *Oncorhynchus Mykiss* larvae with instar I (non-enriched) compared to concentrated food increases resistance against environmental stress to some extent. However using HUFA in daily food that was seen in third and fourth treatments increases larvae resistance to greater extent and the reason is possibly availability and interference of n-3 HUFA in larvae cellular membranes that physiologically improves their condition.

No one can state which fatty acid plays the main and most important role in resistance against stress, since content of all such acids increased due to enrichment (Lavens and Sorgeloos, 1996). Nevertheless, in findings of other studies, DHA was reported to be the main factor in increasing resistance against stress (Ako *et al*., 1994).

Results of this study indicate that suitable nutrition at the initial stages of *Oncorhynchus Mykiss* larvae is effective on growth, survival and resistance of this species. Since it provides necessary energy, larvae can pass better through external nutrition and as a result, production will increase. Therefore, to achieve higher production, nutrition optimization of Rainbow trout larvae especially by using HUFA in daily foods and transferring it to fish body through living food is advised to be used in Rainbow trout hatcheries.

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