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Study the Effect of *Artemia urmiana* Enriched with Selco Oil Produced in Iran on the Growth and Survival of Rainbow Trout Larva

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Abstract: Super Selco is an Artemia enrichment compound in aquaculture. Artemia has enjoyed great applications as a live food. However, it has low values of EPA and lack of DHA. Commercial emulsions such as selco have been made to enrich Artemia worldwide, but encountered to import limitations. In this study, enriched emulsion of Artemia was made with reverse engineering using waste materials and by products of aquatic animals harvest (Scomberidae, squids wastes and of olive oil extraction factories. Suspensions were tested and enriched Nauplii then were analyzed by G.C. The enrichment percentage of Nauplii in evidence and homemade samples were achieved as 27.47 and 22.14 %, respectively. Enriched live Artemia nauplii were biometrically measured containing 500 early feeding *Oncorhynchus mykiss* larvae which was consisted of 3 treatments and a control diet. The results revealed that treatments 1 and 2 showed a significant difference with treatment 3 and 4 on survival rate, growth coefficient and mortality rate Final results approved that there is the talent of selco enrichment oils production similar to foreign samples by interior potential.

Key words: Artemia Urmiana · GC · Rainbow Trout · Selco Oil

INTRODUCTION

Considering the fact that in most workshops there are more than 40% of mortality in the aqua larva stage [1], development and success in aqua husbandry industry depends on the success in larva breeding stage. Although artemia has been recognized in this industry as an exclusive living food, but it is poor in terms of level of essential fatty acids contents (EPA, DHA). Such non-saturated long chain fatty acids are quite essential for growth, survival, resisting against diseases, suitable pigmentation and removal of most of the disorders. Studies conducted by Turchini *et al.* [2], Hafezieh *et al.* [3] and Agh *et al.* [4] on metabolism of rainbow trout larva all approve this issue. Taking benefit from enriched artemia generates extraordinary results in larva in increasing growth, survival, reducing mortality, resistance against diseases and environmental stresses, Whereas artemia is a non selective filter feeder organism which merely eats a certain type of food in its environment which may be entered to the digestion system through the mouth in terms of size. This feature has caused that the enrichment methods are completed by using some various techniques namely the enrichment methods. The importance of using the enrichment compounds has caused that the global big study companies offer ready-to-use enriching emulsions all across the Globe; in which case INVE Company, of US-European nationality, may be mentioned which produces certain products under the trade names of Super Selco, A1Selco, DC DHA Selco and Easy Selco all over the worlds and trade the same with high prices (USD 100/ liter) [5]. On the other hand, these products are used extensively in the Iranian aqua farming industry. The current study was conducted with respect to the following objectives:

- Studying the potential of producing Artemia Selco enriching liquid similar to the imported one based on the Iranian facilities and capabilities.
- Studying the potential of using olive herbal oil production factories wastes as a source rich in non-saturated fatty acids to enrich the enriching emulsions,
- Production of this product results in generation of employment, social and economic development and optimized utilization of the wastes.

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MATERIALS AND METHODS

Artemia enriching oil was synthesized by taking benefit from the marine oils extracted from squid and olive herbal oil production factories wastes. In order to identify the relevant fatty acids, the Agiletn-6890 gas-based chromatograph machine, manufactured by Agilent Company was used. The machine data processing was conducted in Windows OS by using the relevant software.

The average diameter and distribution of sizes of oil phase particles were completed by a measurement instrument (Fritsch Analysette 22 GERMANY), which is calculated from the diffusion of laser. The prepared emulsion was transferred into zero degree centigrade fridge in capped glass jars covered with black covers to prevent any change in their combination structure.

Artemia urmiana cysts were obtained from Iran Artemia Research Center's Cyst Bank situated in Urmia (Iran). In order to remove their contamination, they were disinfected by using 200 ppm of sodium hypochlorite for 30 minutes in flowing state and in 1lt containers [6].

The required conditions for hatching the Artemia cysts were provided according to the method of Léger *et al*. [5]. In order to separate larva from cysts shells and other wastes, positive phototropism feature of artemia larva was used. After collection of the entire nauplii, averaging the total samples was made to calculate the number of nauplii per milliliter. In this study, the density of 200,000 instar I nauplius of artemia per liter of marine water was used. The level of needed water containing nauplii was calculated and the same were transferred into clean zouks containing fresh water with 35ppt salt with proper aeration for enriching purposes. Artemia enrichment was conducted based on Belgian enrichment techniques [7].

The used density is 0.4 g/lit of water containing 200000 nauplii in conic containers (capacity: 2.5 lit). The time of using the solution shall be 12 hours in fixed 25 degrees Celsius temperature with proper oxygenizing and 30 ppt salt and other conditions were the same. After preparation, the samples were injected into the chromatograph gas machine and the samples chromatogram was obtained.

The second stage of tests was conducted on 500 rainbow trout larva of recently-feeding nature, of which almost two-third absorbed through their yoke bag and were about to commence external feeding (avg. weight: 100 +/- 2 mtg, density: 25 pcs/lit (water cap.: 20 lit)) with respect to the following:

Treatment 1: Recently-feeding trout larva with enrichment free Artemia nauplius.

Treatment 2: Special traf for recently-feeding trout larva with Artemia nauplius enriched with an emulsion produced in Iran.

Incubation was performed under 12 degree centigrade temperature, 7 mg/lit oxygenizing and 7.8 pH. Calculation of the effective hatching percentage was found from $HE= N\times2000$ and $H\ \% = (N \times 100)\ (N + U + E)^{-1}$ formula, where:

N: avg. resulted nauplius.
U: avg. number of umbrella shaped state ones.
E: number of non-hatched cysts.

The collected nauplii were separated for a dose of 200000 pcs per liter of water, enriched or non- enriched and transferred into a fridge (4 degree centigrade temperature) for consumption in living conditions for a term of 2-day usage. The level of required food for each of the treatments was calculated with respect to Caldwell *et al*. [8] formula and was conducted 6 times per 24 hours. Based on which the level of food for each treatment was calculated based on 5% of its total biomass. Later on, per day 0.5 g was added to the total daily food. Therefore, 155ml of the one-liter volume of nauplii was collected daily and added in 56 stages round the clock at 07:00, 11:00, 15:00, 19:00 and 24:00, for a term of 10 days. In each time of feeding, the water flow was minimized for about half an hour so that the fish have the chance for being fed. Each morning the number of mortality of each pound was counted, recorded and the dead larva was slowly extracted from the trafe by siphon. Also the physicochemical factor of water was controlled on a daily basis and trough water exhaust filters and fences were cleaned on a daily basis. In order to learn about the larva performance, during the same period of husbandry, the fish feeding movements and behaviors such as method of swimming, taking pellet food and Artemia nauplii were observed and studied on a daily basis. Regarding larva biometry, by the end of the 10^{th} day and on a totally random basis the measures were taken and for larva biometry a digital scale with 0.01g accuracy and a millimeter-precise ruler were used [9].

RESULTS

The results of profile analysis and percentage of the composing fatty acids, enriching oil produced in Iran and non-enriched Artemia nauplii were obtained with respect to table 1.

Table 1: Results of Analysis of Profile and Percentage of Fatty Acids Treatments

Treatment/ % of type of fatty acid	Treatment 1	Treatment 2
SFA	17	21.47
MUFA	21.14	25.48
PUFA	14.45	37.47
EPA	0.57	7.65
DHA	00	17.51
ARA	13.08	0.78
LA	5.80	4.11
ALA	4.12	5.76

Table 2: Results of biometry studies

Test groups/ biometry indicators	Treatment 1	Treatment 2
Primary weight (g)	0.1± 0.002 a	0.1± 0.002 a
Wet weight (g)	0.55± 0.03 a	0.68±0.03 [b]
Total length (cm)	3.8±1.0 a	4.5±0.3 [b]
Special growth factor	3.158± a	4.5910± [b]
Food conversion factor	0.73 ±0.01 a	0.83±0.4 [b]
Fatness factor	5.5±0.3 a	6.1±0.2 [b]

Table 3: Results of survival percentage of fish larva under different feeding treatments in 10th day

Test groups/	Treatment 1	Treatment 2
Survival %	65± 5[a]	86 ±6[c]

Results of Workshop Tests: In the next stage the larva biometry study was conducted. In order to do so, 50 fish larva were subject to biometry on the first day of mixed feeding and their primary weight was obtained and then in the final stage (10th day) their biometry was completed, of which the results have been given in Table 2.

The results of survival percentage of fish larva under different feeding treatments on the 10th day of the final testing stage were obtained as table 3.

The results of survival rate by the end of test period (10th day) indicate that treatments 1 and 2 are of 65 and 86, respectively, which the highest survival during the growing term was related to treatment 2, while the lowest of the same was related to treatment 1 (65%). The difference of survival percentage between treatments 1 and 2 is meaningful ($p<0.05$) which indicates the effects of enriching emulsion.

DISCUSION AND CONCLUSION

Selco enriching oil is a non-homogenous emulsion of combining two liquids, in which one of the liquids is dispersed in the other in the form of drops. These systems have minimum sustainability, explained as per Stox Rule. For their sustainability, at least one emulsifier shall be available. Such emulsifier may be used as chemical and herbal type such as lycetine, glycerol, toin 80 and/or different types of other herbal gums applicable in food industries. Meanwhile, due to more proper consolidation and sustainability, 1~3% of different types of industrial and food emulsifiers were used as mixtures in this study.

The compounds of the emulsifiers used in production of artemia enriching solutions in aqua industry shall be as non-poisonous edible compounds and applicable in consumable food industries. Therefore, lycetine, glycerol, salep and toin 80 emulsifiers were used, which are totally along with Huck-Iriart *et al*. [10] studies in this regard.

In emulsion production, the same density factor up to 3%, environment pH, 50^C temperature and term of enriching have direct effects. Meanwhile, 1~3% compound density has been used in production of emulsion by using domestic facilities in Iran. The color of emulsion mixed with seawater is milky which indicates the oil particles diameters to be in micrometer level.

The olive oil, of which the production and extraction of the same dates back to 4000 BC and was used by the Hebrews for the first time, is now cultivated and processed in the northern area of Iran. Several factories extract olive oil industrially and/or traditionally. The wastes, which compose 6% of these factories productions, may be used as a proper source to be converted into higher-valued products. In this regard, these wastes may be used as an alternative for fish oil in aqua husbandry industry. Squids are belonged to cephalopods group, which compose more than 5% of the total fishing of southern shores of Iran as side products. Squids are used in some cases and they have high fat content, which is not really used. Around 13+/-3% of squid body is composed of fat, while 5.5 lit of premium quality was extracted from a 50 kg shipment of the same after being fished and transferred into Urmia Research Center. Squid may be used a proper and new source rich in omega 3 and omega 6; the non-saturated long chain fatty acids in production of enriching emulsions [2]. Due to movements, artemia stimulates larva in terms of feeding. Totally speaking, living food provides higher digestion and absorption capability in comparison to formulated ones, which may be explained with respect to the variability and level of enzymes existed in the same. The totally positive and meaningful effect of Artemia nauplii, whether enriched and non-enriched, with respect to the concentrated foods in high survival percentage indicates their importance in the relevant cost-effectiveness in the

aqua growing and breeding workshops and in aqua husbandry industry. This issue has also been reported by Rainuzzo *et al*.[11] whose study report on sea bass and sea bream fishes which confirms the results of the current study.

The results on survival rate at the end of the test period (10^{th} day) indicate that treatment 1 with 86% survival rate has the highest survival during the growing period. The final result of study shows that suspensions made in Iran may easily and successfully act as an alternative in enriching Artemia nauplii in aqua husbandry for the relevant imported commercial ones. Therefore, production of Selco enriching oil is possible in most of the countries having aqua husbandry based on their domestic capabilities and with similar quality as of the foreign ones and all the relevant field tests may be successful and the same may easily replace imported enriching oils.

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