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Production of Artemia enriching oil (Selco) by taking benefit from Iran's domestic capabilities

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Abstract
Artemia is a live food source which enjoys extensive applications in aquatic's breeding. However, it has little amount of Eicosapentaenoic acid and lacks Docosahexaenoic acid. Enriching artemia to improve its nutritious value is mandatory. Different commercial emulsions are prepared and offered all across the Globe, of which Selco produced by INVE-Aquaculture Company is the most famous one. This emlusion is highly used in Iranian aqua husbandry industry. Importation of this product into Iran faces certain problems and limitations. In this study, the said product was analyzed and made by using domestic capabilities of Iran by using reverse engineering of Selco imported to Iran. Shark liver oil and sunflower oil extraction factories as well as other compounds were used. These suspensions were tested with respect to the control sample. Artemia enriching works were made with emulsions as per the standard method. The results indicate that enrichment percentage in nauplii Artemia in the control and domestically produced samples have been 37 ± 3 and 25 ± 3. Then enriched Artemia nauplii were dropped on 500 recently-feeding larvae of trout fish on 4 treatments for growth factors biometry for 30 days in alive conditions and fed for 30 days. The results showed that treatments 1 and 2 had significant differences with treatments 3 and 4 in terms of survival percentage, growth factor, total length and level of disorders. However, no meaningful difference between treatments 3 and 4 was seen ($P \leq 0.05$). The final conclusion of authors indicates that production of enriching oils domestically by local capabilities is well possible with features similar to the foreign made ones and all the relevant field studies are successful which may replace foreign made enriching oils.

Keywords: Enriching oil, Selco, Artemia Urmiana, GC, Fatty acid profile

1. Introduction
Development and success in aquatic husbandry industry depends on successful nurturing in larvae stages of such aquatic, as in most of the sites there is a difference more than 40% in terms of larva mortality [1, 2]. In this industry, artemia is known as a unique living food source; however, it is poor in terms of essential fatty acids contents (DHA, EPA). These unsaturated long chain fatty acids are quite essential for growth, survival, resistance against diseases, suitable pigmentation and removal of most of the disorders. Studies conducted on sturgeon fish larva [3, 4] and on angel fish performance [5], on metabolism of trout fish larva [6], on Aras dam crayfish [7], and on trout fish [8] all confirm this issue. Using enriched artemia represents extraordinary results in increasing growth, survival, reducing mortality, resistance against diseases and environmental stresses among larva. As artemia is non-optional refining being which swallows any type of foodstuff in its living environment which may enter into its digestion system in terms of size, there are enrichment methods for the same. The importance of using enriched compounds has caused that big research companies all over the world offer ready-to-use enriching emulsions. In this regard INVE-Aquaculture Company with European-American nationality may be mentioned which produces certain products under the trade names of Super Selco, A1 Selco, DC DHA Selco, and Easy Selco all over the worlds and trade the same with high prices (USD 100/liter) [9]. Considering the above and that these products are highly used for Iranian aquatic husbandry industry, this study was conducted with respect to the following objectives:

1. May artemia enriching oil be produced by using local facilities and capabilities with similar features of the imported one?
2. Can shark liver oil is used as source rich in long chain fatty acids (EPA, DHA) to produce enriching emulsions?

3. Fungi Can the regional sunflower oil production factories wastes be used as a source full of unsaturated fatty acids to enrich the enriching emulsions?
4. Production of this product results employment promotion and economic-social development and optimized utilization of regional wastes and removing dependency on importation of this product and preventing funds exiting the country are from amongst the other objectives of this project.

2. Materials and Methods

Shark liver oil and sunflower herbal oil wastes were supplied from Chabahar and Khoy industrial oil processing factory. Vitamins compounds were purchased from veterinary pharmacy and foreign made enriching emulsion with INVE company brand for 2 liters was purchased from APT Co. All the needed chemicals including but not limited to chemicals solvents such as petroleum benzene, ethanol, emulsifiers of lecithin, glycerol, vitamins E, A, C and D and BHA, fibrous compounds, wheat fiber and Tween 80 were obtained. Other needed chemical solutions were provided from the laboratory facilities of Urmia University (materials dispose chemical laboratory), chemical and foodstuff disposing laboratory of natural resources studies center and livestock affairs of agriculture ministry of West Azerbaijan Province and state artemia research center were used.

2.1. Reverse engineering to determine Selco compound

Through conducting reserve engineering with standard method [10]. Selco compound structure was identified in analytical chemistry laboratory of Urmia University and the accuracy of the findings was compared with the results of other scholars, for which some analysis were performed.

The locally produced enriching oil was synthesized. Upon production, a magnetic blender was used and 50 degrees temperature is needed for distribution and homogeneity of two separate oil and water phases. In this project butyl hydroxyl anisole was used as antioxidant. In order to generate stability and lack of separation in synthesized emulsion several different hydro and oil soluble emulsifiers up to 3% were used (lecithin, glycerol, Tween, Tween 80) in combined manner.

2.2. Samples fatty acids analysis method and determination of their profile and percentages

In order to identify each and every fatty acids of the samples, gas based chromatographic machine model Agilent-6890 made by Agilent company was used. Processing the machine by using software in Windows environment was made.

2.3. Conducting samples field tests

2.3.1. Enriching works on artemia

The *Artemia urmiana* cysts were obtained disinfected from state artemia research center located in Urmia. In order to remove their pollution, they were disinfected, with 200 ppm sodium hypochlorite, for 3 minutes in floating state in 1lt con hatchery kit [11].

Hatching Artemia cysts: in order to do so, standard method was used [12]. In order to separate larva, cysts shell and other excessive materials, photophobic feature of artemia larva was used. After collecting the entire nauplius, totally the samples were averaged so that the number of naplius in a milliliter is calculated. In this study, 20000 densities of artemia nauplius instar 1 per liter of salty water were used. The needed water containing naplius is calculated and transferred into clean con hatchery kit; containing fresh water with 35 ppt saltiness with suitable aeration for enrichment [13].

Preparation of Artemia enrichment solutions and applying enrichment works: in order to enrich artemia based on Belgian enrichment techniques [14] as per the following three treatments was used:

Treatment 1: nauplius without treatment 2: enrichment emulsion: containing witness enrichment emulsion Treatment 3: nauplius containing locally produced enrichment emulsion: density used for each treatment 0.4 gr/lit of water containing 200000 nauplii in 2.5 lit conal shaped containers. The time used from the solution 12hr of fixed temperature of 25 degrees with suitable oxygenizing and saltiness of 30 gr /lit and other similar conditions were considered.

Conducting chemical analysis of enriched artemia to determine enrichment level with emulsions:

Artemia was taken from each treatments 1, 2 and 3 for 1 gr of enriched nauplii and washed distilled water and after dewatering the same was collected with spatula and transferred into micro-tubes and after insertion of specifications of each treatment on micro-tubes, in order to determine the profile and level of fatty acids, the same were transferred with ice mixture inside fiberglass ice container to Urmia-Jahad-Daneshgahi center.

2.3.2 Extraction fatty acids and determining their profile:

After preparation, samples were injected into chromatograph gas machine and the samples chromatogram was obtained by machine.

2.3.3 Second stage test from treatment field works:

Conducting the second stage was on 500 recently-fed fish larva in Ziveh cold water Trout Company located in 37th Km of Urmia, due to having sufficient facilities and conducting trout fish breeding works. In this stage, in breeding hall of this site was conducted of 5 rectangular tray with total capacity of each 40lit of water and each tray with 500 larva of fish which has absorbed almost 2/3 of its yoke and was about to start external feeding and with average weight of 100 ± 2mg, with density of 25 pieces per liter (capacity 2 liters) as per the following:

Treatment 1- recently fed larva with SFT concentrate food of Chineh co.

Treatment 2- recently fed larva with concentrate food enclosed with nauplius artemia without enriching

Treatment 3- special tray for fed larva with concentrate food enclosed with nauplii enriched with imported Selco oil

Treatment 4- special tray for fed larva with concentrate food enclosed with nauplii enriched from locally produced emulsion Temperature conditions; 12 centigrade degrees, oxygenizing (setting input water) equal to 7 mg/lit, 7.8 pH, for all the treatments were provided under similar conditions of site breeding salon.

Larva primary food is granular with 0.4 ~ 0.7 mm size with 48% protein, 12% raw fat, 13% ash, 2.5% fiber, 1.5% phosphorous and 11% moisture. In order to facilitate and decrease the error percentage, hatching operations of cysts, enriching nauplius and feeding larva in the same site was conducted for a month. Calculation the effective hatching

percentage is through relation HE= N × 2000 and H %= (N×100) $(N+U+E)^{-1}$.

N: average resulted nauplii

U: average number of umbrelliform state

E: number of non-hatched cysts of non-collected nauplii for 200000 pcs/lit of water, whether enriched and non- enriched as separated and for the fridge with 4 ºC temperature for consumption in alive-keeping manner used for 2 days. The needed daily food for each of the treatments was calculated and determined based on the following relation [15] and made for 6 times per 24 hours.

5% × average larva weight × number of larva of each treatment = daily food content in grams

Food content of this treatment was calculated based on 5% of total of its biomass weight. After each day, 0.5 gr was added to the total daily food. The used enriched nauplius content was calculated equal to 6% of nauplii dry weight and added to the total food content. Whereas each nauplius instar I of Urmia Lake artemia is equal to 3 to 4 micrograms of its dry weight, therefore each 250000 Artemia nauplius instar 1 of Urmia Lake has equal dry weight of a gram. Considering the moisture content in concentrate food used in the project, which is equal 10%, therefore, in each day equal to 2500 pcs of nauplii was added to food quota of treatments 2 to 4. Therefore, on each day 155 ml of nauplius per lit volume was collected and added within 10 days in 6 stages in hours of 7, 11, 15 and 24. In second 10 day period 200 ml and in 3rd one (from 20th to 30th day), 250 ml was given. In each time of feeding, almost half an hour the water flow was minimized to provide enough time for fish for feeding. Also water physiochemical factors were controlled on a daily basis and the trays water outlet filters and lace were cleaned. In order to learn about the treatments performance, each day during the period of husbandry, the fish feeding motion and behaviors such as method of swimming, grasping plated food and Artemia nauplius and the relevant disorders were observed and studied. In order to apply biometry the larva upon the end of the 30th day, it is acted on a totally random basis manner and for larva biometry a digital scale with 0.01 gr accuracy and a millimeter ruler was used.
Considering the values of total length and measured weights of fish, the growth indicators were calculated and assessed by taking benefit from the following relations:

$K\%=FBW/TL^3 \times 100$

100× (1n of primary weight – ln of final weight = (SGR) special growth rate percentage [3, 16].
Nutritious conversion factor = final weight – primary weight / consumed food.

TL: average final length in terms of cm per treatment
FBW: average final weight per treatment [17].

3. Results

Results of profile analysis and percentage of composing fatty acids, concentrate, Selco commercial enriching oil and emulsion locally produced and nauplii of non-enriched Artemia were obtained as table 2:

Table 1: Results of profile analysis and percentage of treatments fatty acids

Fatty Acid Index	Treatment 1	Treatment 2	Treatment 3	Treatment 4
C14:0	10.32	3.11	3.23	4.16
C14:1n5	0.18	1.05	0.08	1.05
C16:0	7.43	10.48	11.48	10.48
C16:1n7	10.53	6.54	5.53	7.54
C18:0	11.54	3.55	4.55	3.55
C18:1n9	11.86	13.54	16.86	12.54
C18:2n6cis	12.80	5.80	4.11	3.12
C18:3n3	1.37	3.37	0.87	2.35
C20:0	0.10	0	1.43	0
C18:3n6	1.87	0.87	4.87	1.75
C18:4n3	0.02	0.02	0.02	0.02
C22:0	0.24	0.24	0.24	0.24
C20:3n6	0.49	1.49	0.49	3.33
C20:3n3	0.02	1.02	1.21	5.54
C20:4n6	0	1.08	0.78	1.08
C20:5n3	0.54	0.54	0.65	0.57
C22:5n6	5.400	0	5.51	3.60
C22:5n3	0.87	0	2.28	5.80
C22:6n3	0.65	0	9.68	2.11
C24:0	0.54	0	0.54	0.45
SFA	30.03	1.39	21.47	18.83
MUFA	19.34	21.14	23.48	20.14
PUFA	19.58	14.45	37.47	25.30
EPA	0.54	0.57	7.65	3.58
DHA	1.95	0	17.51	11.08
ARA	0	1.08	0.78	1.08
LA	12.81	5.80	4.11	5.80
ARA	2.14	4.12	5.76	4.12

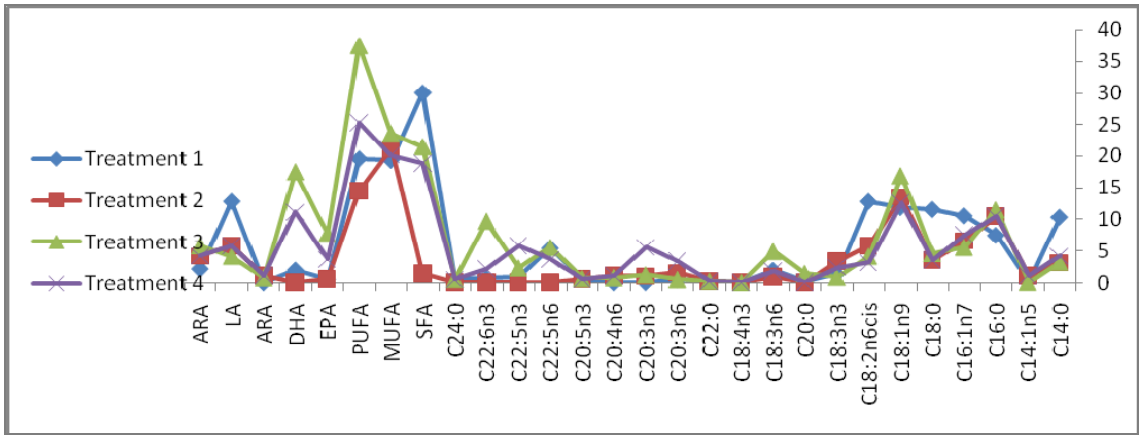

Chart 1: results of profile analysis and treatments fatty acids percentage

3.1. Results of site field test

In the next stage, larva biometry study was conducted. In order to do so, larva was subject to biometry on the first day starting mixed feeding and their primary weights were obtained which was conducted on 50 larva and then on the last stage their complete biometry was made, of which the results have been given in table 3:

Table 2: Results of examining rainbow trout fish larva biometry indicators

Biometric indictor test groups	Treatment one	Treatment two	Treatment three	Treatment four
Primary Weight (gr)	0.1±0.002 [a]	0.1±0.002 [a]	0.1±0.002 [a]	0.1±0.002 [a]
Wet Weight (gr)	0.55±0.03 [a]	0.58±0.03 [a]	0.68±0.03 [b]	0.63±0. 3 [b]
Total Length (cm)	3.8±0.1 [a]	4.1±0.2 [b]	4.5±0.3 [b]	4.3±0.2 [b]
Special Growth Ratio	3.15±8 [a]	4.63±8 [b]	4.59±10 [b]	4.32±3 [b]
Food Conversion Ratio	0.73±0.01 [a]	0.82±0.3 [b]	0.83±0.4 [b]	0.80±0.4 [c]
Fatness Factor	5.5±0.3 [a]	5.7±0.2 [b]	6.1±0.2 [b]	5.9±0.3 [b]

Results of fish larva survival percentage under different food treatments on 30th day of test period (final stage) were obtained as table 4.

Table 3: Results of fish larva survival percentage under different food treatments on 30th day

test groups	Treatment one	Treatment two	Treatment three	Treatment four
Survival rate %	a 65±2	b 78±4	c 85±3	c 80±3

Rain result upon the end of test period (30th day) indicated that treatments 1, 2, 3 and 4 were with 65, 78, 85, and 80 ratios, respectively, while the highest survival during husbandry period was related to treatment 3 and the lowest survival with 65% for treatment 1. Difference of survival percentage between treatment 1 and treatments 2, 3, and 4 was meaningful ($p \leq 0.05$). However, no meaningful difference from survival was seen between treatments 3, 4 which may express similar effects of locally produced enriching emulsions with the relevant foreign made commercial samples.

In this study, imbalanced swimming, skin wastes, lack of appetite, eye projection, wings wear considered as the general disorders and their number of the same were counted and analyzed. The disorders were controlled and studied during the test term and their numbers were recorded and analyzed, of which the results have been given in the following table:

Table 4: Disorders during test period

Test groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Observation of larvae underwith general disorders	33	27	21	19

General disorders (imbalanced swimming, skin wastes, lack of appetite, eye projection, wings wear) in testing groups indicate a meaningful difference among treatments, in a way that the highest and lowest of the same are in test groups 1 and 4 with 33 and 19 cases, respectively. Meanwhile, it shows a meaningful difference between treatments 1 and treatments 2, 3 and 4 while the same cannot be seen between treatments 2, 3 and 4.

4. Discussion

Selco enriching oil is a non-digestible double liquid and non-homogenous emulsion, in which one of the liquids is dispersed inside the other in the form of drops whose diameter is usually bigger 1 micron. Such systems with minimum sustainability shown with O/W relation explained as Stox Rule [18], for the sustainability of which at least one emulsifier shall exist. These emulsifiers may be used of chemical and herbal types such lecithin, glycerol. Tween 80 and various types of other herbal gums applied in food stuffs. Also in this study due to generation of stability and sustainability more suitable 1 to 3% of different types of industrial and food emulsifiers have been used in mixed manner. Emulsifiers compounds used in production of artemia enriching solutions in the aquatic farming industry shall be as non-poisonous and edible and usable types for foodstuffs. Therefore, lecithin, glycerol and Tween 80 emulsifiers were used which are fully in conformity to the studies of other researchers in this regard [18].

Upon synthesis of enriching solutions, whereas oil phase is dispersed in water phase, in order to achieve more proper result during synthesis and in thermal margin of 40 degrees and also by using a magnetic blender to establish a dense network through wander valance and hydrophobic forces of emulsifiers streams on the joint surface of water and oil. Temperature, percentage and types of emulsifiers directly affect the generation of dispersed network and its particles diameter and also in this project upon synthesis, heater temperature above 40 degrees and simultaneously a magnetic tank were used which are along with the reported study [19].

In production of emulsion, the relevant density factor up to 3%, ambient ph of 7.8, temperature of 50 degrees and enriching time directly affect while also in production of emulsion with domestic facilities, 1-3 compound density was used, and also the production of this study was synthesized based on ICES standard [20].

In southern waters of Iran there are more than 7 big families of sharks, of which thousands of tons are fished annually, 3% of their weights is shark liver. 35% of shark liver is rich in different types of unsaturated long chain fatty acids such as omega 3, omega 6 and even omega 9 [21], which is extracted traditionally. It has mainly non industrial consumptions may

be converted into more valuable products. In this study, from shark liver oil, total relevant saturated fatty acids equal to 24.5% of fatty acids forming thereof from total fatty acids of total single bank unsaturated fatty acids equal to 19.56 and total multi bank unsaturated fatty acids 34.23 and its Arachidonic acid is 3.45 and LA of 5.08% were achieved and used in formulation of enriching emulsion with local facilities with 30-40% [22].

Sunflower is a plant of starflower family, the seeds of which are used for oil extraction called as plant oil. This plant is highly cultivated in Iran and around 12% of the extracted oils are of sunflower. 7% of the relevant dried seeds weight is oil, cultivated in West Azerbaijan, Mazandaran, Guilan and other provinces [23].

Due to a variety of reason, sunflowers oil wastes is around 2 to 5% which may be a suitable source for formulation of products with higher added-value such as enriching oils used in aquatic industry.

Similar results have been reported from a domestic study [24] that surveys the sunflower oil value in fish nutritious health. Overseas have reported in improving level of HUFA, EPA and DHA comparatively with fish oil in enriching and replacing the same instead of fish oils in aquatic husbandry industry [25]. Comparison with the results of the report of the same study who have reported this oil in enriching *Artemia franciscana* and improving the term of growth and increasing the larva [25].

Studying the profile and structure and percentage of sunflower wastes oil fatty acids indicate that the relevant total fatty acids contents is equal to 7.14% of fatty acids composing from the total fatty acids the total single bank unsaturated fatty acids, multi bank, Arachidonic acid, LA and ALA were achieved as 56.20, 33.78, 1.21, 19.02 ad 5.85, respectively which may be used as one of the available sources from local facilities in formulation and production of enriching emulsion.

The reaction of receiving food in treatments 2, 3, 4 with respect to treatment 1 was more observed the same was not abundant and observable with respect to other among treatments 2, 3 and 4 and this point indicate the prey stimulation and importance in trout larva feeding behaviors. Report of previous authors also given a report on the role of living foods in qualitative improvement of nutrition in Sea bass and sea bream fishes larva [9, 26] and have totally similar results with those observed in this study, as Artemia nauplius stimulates larva nutrition due to motion [27].

Living foods enjoy higher digestion and absorption ability in comparison with the formulated ones, which may be explained as per the enzymes available in the same. This issue has been given in the study results 1987 on ocean fish larva. Higher growth factor in treatments 2, 3, and 4 with respect to treatment 1 explains this capability and proves the accuracy of study results of this project. Lack of solubility and toughness of excretions of larva fed by Artemia nauplius in treatments 2, 3 and 4 with respect to treatment 1 is significant and this advantage has a positive effect on incubators health and reducing bacterial load.

The quite positive and meaningful effect of Artemia nauplius, including enriched and non-enriched with concentrate in higher survival percentage indicate their importance as per their economic explicability in aquatic breeding and husbandry and in the relevant industry. This issue has been reported on Sea bream and Sea bass fishes [28] and confirms the results of this project.

Analysis of the results in the final stage (30th day) indicates that growth factors inter alia wet weight, total length, special growth factor, nutritious conversion factor and obesity factor in treatment 1 in comparison with treatments 2, 3 and 4 have meaningful difference ($p<0.05$) and this difference is more obvious in nutritious conversion factor. This result in totally similar to a study work of conducted on *Acipenser persicus* fish larva [29].

The results of level survival at the end of the test period (30th day) indicate that treatments 3 and 4 have highest survival during the husbandry term with 86.22 and 86.4 ratios, respectively. However, treatments 1, 2 had lowest survival for 65 and 82%. The difference between survival percentage between treatments 1 and 2 with treatments 3 and 4 is meaningful ($p\leq0.05$). However, no meaningful difference is seen from the survival percentage between treatments 3 and 4 which indicates similar effects of locally produced enriching emulsions with the relevant foreign made commercial samples. The level of survival of this study is along with a study work on trout larva [4]. Therefore, local suspensions in the same may also be completely with this study.

In this study, imbalanced swimming, skin wastes, lack of appetite, eye projection, wings wear considered as the general disorders and their number of the same were counted and analyzed. General disorders (imbalanced swimming, skin wastes, lack of appetite, eye projection, wings wear) in testing groups indicate a meaningful difference among treatments, in a way that the highest and lowest of the same are in test groups 1 and 4 with 33 and 19 cases, respectively. Meanwhile, it shows a meaningful difference between treatments 1 and treatments 2 and 4 while the same cannot be seen between treatments 3 and 4 and these are along with the results reported before [30].

5. Conclusion

The final result of this study indicate that locally produced suspensions may easily and successfully replace the relevant commercially imported samples in enriching Artemia nauplii for aquatic husbandry industry. Production of Selco enriching oil domestically with local capabilities with same features as of the relevant foreign samples is well possible and all the relevant field studies are successful which may easily replace the foreign made enriching oils.

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