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Application of Shark Liver Oil for *Artemia* Enrichment and its Comparison with Imported Selco Oil

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Abstract: In this study, enriched emulsion of Artemia was made with reverse engineering using home country potentials. The ingredients of imported Selco were analyzed in chemistry lab of Uremia University. Then different resources containing animal oils with highly unsaturated fatty acids, from waste materials and by products of south water resources aquatic animals harvest (liver oil of shark), vegetable oils provided from wastes of oil extraction factories and regional vegetable oils such as sunflower were studied. Fatty acid profiles of extracted oils from internal sources were analyzed by Gas Chromatography (GC). Provided emulsions were compared with foreign samples as control treatment. Then, the samples were analyzed by GC. The next step was to perform pilot project tests. In the pilot tests, first Artemia enrichment operation with enrichment emulsions was carried out by interior potential and foreign resources according to standard methods. All data were analyzed using SPSS software by one way ANOVA and Duncan test. The enriched nauplii were analyzed chemically by GC. The results showed that the absorption of emulsions in Artemia in interior and foreign samples were 37.47% and 25.30%, respectively. Then, enriched live Artemia nauplii immediately transferred to Ziveh cold water fish farm containing 500 newly feeding larvae of *Oncorhynchus mykiss* consisted of 3 treatments and a control larval treatment, in which some biologic performances like the mortality rate, growth, Feed conversion ratio and survival rate were determined. Abnormality in larvae, were studied. The results revealed that treatments 1, 2, had a significant difference with treatments 3, 4, on survival rate, growth coefficient, mortality rate and abnormality. The survival rate and growth coefficient and mortality rate and abnormality of treatment 3 had not any significant difference with control. Final results showed that there is the potential of Selco enrichment oils production similar to foreign samples by interior potential.

Key words: Selco · Enrichment Oil · *Artemia urmiana* · Gas Chromatography · Fatty Acid Profile

INTRODUCTION

Successful larviculture is a key factor for aquaculture investment and in most farms more than 40 % mortality in larval stages is recorded [1,2]. Artemia has known as a unique live food in this industry, but it is poor in essential fatty acids including EicosaPentaenoic Acid (EPA), Docosa Hexaenoic Acid (DHA). Highly Unsaturated Fatty Acids (HUFA) is essential for growth, survival, protection against diseases, enough pigmentation and elimination of abnormalities. This issue is confirmed by Turchini *et al*. [3], Hafezieh *et al*. [4] on sturgeon larvae, Manaffar [5] on Angel fish, Agh *et al.* [6] on *Oncorhynchus mykiss* larvae metabolism, Han *et al.* [7] on *Astacus leptodactylus* of Aras dam and Sarveh [8] on *Oncorhynchus mykiss*. The use of enriched Artemia results in a significant increase in growth, survival, mortality decrease, increase resistant against diseases and environmental stress in larvae. Artemia is a non-selective filter feeder that intake all around suitable sized food items. These enable us to enrich Artemia with different techniques. Nowadays large research institutes such as INVE-Aquaculture Company which provide, produce and sale enrichment emulsions including Selco, Super Selco, Selco A, DHA Selco and Easy Selco worldwide. Regarding that these products are used widely in Iran's aquaculture industry, the aim of this research was:

- If there is a possibility of production of Artemia enrichment solution from inland potential similar to imported sample.

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- If the shark liver oil can be used as a rich source of HUFA (EPA, DHA) to produce enrichment emulsions.
- Increased employment and social economic improvement and beneficial use of waste products, independence of importing these products.

MATERIALS AND METHODS

Shark Liver Oil Was Provided from Chahbahar: Vitamins and enrichment emulsion were provided from INVE Company. Chemical materials including petroleum benzene, Ethanol, Lecithin emulsifier, Glycerol, Vitamins A, C, D3 and E and BHA, Fiber, wheat fiber and Tween 20 provided from Laboratories of Urmia University, Iranian Artemia Research Center and Ministry of Jahad-e-Keshavarzi.

Reverse Engineering for Determining Selco Composition: The imported Selco composition was decomposed by Reverse engineering and re-synthesized. To determine fatty acid profile GC apparatus Agilent-6890 was used. All data were analyzed using windows software.

Artemia Enrichment: *Artemia urmiana* cysts were provided from Iranian Artemia Research Center and disinfected by 200 ppm sodium hypochlorite for 30 min. Artemia cysts were hatched according to. Positive photo taxis were used to separate larvae from cyst shells and waste materials. 200000 Instar I Artemia nauplii per 1 liter of salt water with salinity of 35 ppt and aeration were provided according to Bengetson *et al.* [2]. Artemia enrichment was performed based on Belgian technique [9] and three treatments were as follow:

Nauplii without enrichment emulsion, nauplii with enrichment emulsion as control and nauplii with inland enrichment emulsion. The concentration of emulsion was 0.4 g per liter containing 200000 naupli for 12 hours. Constant temperature (25°C), salinity (30 g/l) and well aeration were provided. 1 g enriched nauplii were obtained from each treatments and washed with distilled water and transferred to micro tubes and were sent to laboratory of Jahad-e-daneshgahi Urmia wrapped in the fiberglass ice pack to determine fatty acid profile. Prepared samples were injected to GC apparatus model Agilent-6890 and samples chromatograms were obtained. 500 newly fed larvae of *Oncorhynchus mykiss* were provided from Ghezel-Mahi-Sardabi-Ziveh. Five cubic trays with capacity of 40 l each were used to rear 500 larvae that newly absorbed their yolk sacs with mean weight of 100±2 mg at a density of 25 larvae per liter (20 l capacity) as follows:

Treatment 1: Larvae that newly absorbed their yolk sacs with concentrate SFT00 Chineh Company.
Treatment 2: Larvae that newly absorbed their yolk sacs with concentrate together with Artemia nauplii.
Treatment 3: Larvae that newly absorbed their yolk sacs with concentrate and enriched naupill with imported Selco oil.
Treatment 4: Larvae that newly absorbed their yolk sacs with concentrate and enriched nauplii with inland emulsion (Table 2).

Other parameters such as temperature 12°C, dissolved oxygen 7 mg/l, pH 7.8 were the same in all treatments. The starter diet of larvae were granulated with 0.4-0.7mm in size and proximate composition of protein 48 %, crud fat 12 %, ash 13%, fiber 2.5 %, phosphorus 1.5 % and humidity 11%. Cyst hatching and nauplii enrichment and larvae feeding were carried out at the same farm for one month. Collected nauplii were maintained at 4°C up to 2 days. Daily diet for each treatment was calculated according to Narciso [10]. Larvae feeding were done six times per day.

The diet was set up as 5% of total biomass weight. 0.5 g was added daily to diet. Enriched nauplii were added as 6% of dry weight to consume diet. Dry weight of each instar I nauplius of Artemia from Urmia Lake was 3-4 microgram. According to 10% humidity of concentrate meal, 25000 nauplii were added to treatments 2 to 4 daily. 155ml of 1 l nauplii was collected and added at 7, 11, 15, 19 and 24 during 10 days. In second and third ten days 200 ml and 250 ml were added, respectively. In each feeding time water movement was reached to minimum that fish had the opportunity for feeding. Mortality rate of each tray were recorded and dead fish were removed daily. Feeding behaviors such as swimming mode, diet intake and abnormalities were assessed. At the end of the period (day 30^{th}) biometry of all larvae were done using 0.1 g scale and ruler. Growth indices were calculated according to Sistani [11] and Towfighian *et al*. [12] as follows:

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

$100 \times SGR = (\text{final weight} - \text{initial weight})$

RESULTS

The results of fatty acid profiles of treatments were shown in Table 1.

Table 1: The results of fatty acid profiles of treatments

Fatty acid	Treatment 1	Treatment 2	Treatment 3	Treatment4
C14:0	10.32	3.11	3.23	4.16
C14:1n5	0.18	1.05	0.08	1.05
C16:0	7.43	1.48	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	58	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	0.872	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	54	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	29.58	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
ALA	2.14	4.12	5.76	4.72

Table 2: The results of biometrical characteristics of examined *Oncorhynchus mykiss*

Examined Groups Biometrical Indices	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Initial weight (g)	a 0.0020±0.1	a 0.002±0.1	a 0.002± 0.1	a 0.002± 0.1
Wet weight (g)	a 0.03±0.5	a 0.03 ± 0.58	b 0.03 ± 0.68	b 0.3 ± 0.63
Total length (cm)	a 0.1± 3.8	b 0.2 ± 4.1	b 0.3± 4.5	b 0.2± 4.3
Specific Growth Rate	a 8± 3.15	b 8± 4.63	b 10± 4.59	b 3± 4.32
Feed conversion Rate	a 0.01± 0.73	b 0.3 ± 0.82	b 0.4± 0.83	c 0.4± 0.8
Fattening coefficient	a 0.3± 5.5	----------	----------	b 0.3± 5.9

Table 3: Survival rate of larvae treatments at the end of period (day 30th)

Examined Groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Survival Rate %	c 1± 65	a 3± 82	b 4± 86	b 2± 85

Table 4: Abnormalities during experiment period

Tested Groups	1	2	3	4
Number of abnormalities	33	27	21	19

The results of biometrical characteristics of tested *Onchorhynchus mykiss* were shown in Table 2.

Also, the results of survival rate of larvae treatments at the end of period (day30th) were shown in Table 3.

The results of survival rate at the end of experiment (day 30) for treatment 1 to 4 were 65, 82, 86 and 85%, respectively. The greatest and lowest survival were related to treatment 3 (86± 4) and treatment 1 (65± 1), respectively. The difference in survival rate between treatment 1 and other treatments was significant (P=0.05). Also, no significant difference was observed between treatments 3 and 4 that can be due to the same effects of inland and imported enrichment emulsions. Abnormal swimming, skin lesions, anorexia, exophthalmia, fin erosion considered as general abnormalities. The results of abnormalities during experiment period were summarized in Table 4.

General abnormalities showed significant differences among treatments as the greatest and lowest of them were observed in test group 1 and 4 with 33 and 19 cases, respectively. Also, treatment 1 had a significant difference with other groups, but groups 2, 3 and4 had not significant difference.

DISCUSION

Selco enrichment oil is a non-homogenous emulsion of two separate liquid that in which one liquid is dispersed in another one as tears more than 1 μ liter. These systems have the least stability and at least one emulsifier is needed to stabilize them. These emulsifiers can be chemical and herbal such as lecithin, glycerol, Tween 80 and other herbal gum applied in food industry. In this study 1-3 % of food and industrial emulsifiers were used due to suitable stability. Emulsifiers were used in production of Artemia enrichment solutions in aquaculture should be non-toxic and edible and can be used in food industry. Therefore, lecithin, glycerol and Tween 80 were used according to Khodaparast [13]. At synthesis period, 40°C and magnetic reservoir was used that was similar to method used by Huck-Iriart *et al.* [14]. To produce inland emulsion, the maximum 0f 1-3 % concentration and ambient pH 7.8, temperature 50°C were used with regard to ICES standard [15]. There are more than 7 families of sharks that thousands ton of them were harvested yearly. Shark liver is equal to 3 % of its body weight and 35 % of shark liver weight is rich in HUFA omega 3 and omega 6 even omega 9 that extracted traditionally [16-17]. Usually it is used as non-industrial purposes and it can be transformed to higher value products. In this study on shark liver oil, total saturated fatty acids consisted of 24.5 %, single band unsaturated fatty acids equals to 19.56%, total unsaturated fatty acids with multiple band 34.24%, Arachidonic acid 3.45%, LA 4.46% and ALA 5.08 %, respectively. The rates of food intake were higher in treatments 2, 3 and 4 compared to treatment 1. These criteria had not significant difference in treatments 2, 3 and 4that revealed prey movement and its importance in feeding behavior of *Oncorhynchus mykiss* larvae [3] and Dhert *et al.* [18] showed the role of live food in improving feeding quality in Sea bass and Sea bream larvae that is similar to our findings because Artemia nauplii can cause feeding stimulation due to its movement.

Live food is more ingestible and digestibility compared to formulated food that can be explained by their enzymes as reported by Leger *et al.* [19] on ocean fish larvae. Higher growth coefficient in treatments 2, 3 and 4 compared to treatment 1 can explain this ability and confirm our results. Insolubility and hardness of excrements of larvae feeding Artemia nauplii on treatments 2, 3 and4 compared to treatment 1 have a positive influence on incubators hygiene and decrease in bacterial load. Positive significant influence of Artemia nauplii compared to concentrate on higher survival rate revealed their importance in economic aquaculture that confirmed by Rainuzzo *et al.* [20] on Sea bream and Sea bass. The results showed that growth indices including wet weight, total length, specific growth rate, food conversion coefficient,fating coefficient in treatment 1 has a significant difference with other treatments at the end of experiment (day 30th) (P<0.05). That is prominent in food conversion ratio. This is similar to finding of Agh *et al*. [6] on *Acipencer persicus.* The results at the end of experiment (day 30th) revealed that treatments 3 and 4 with 86.22 and 86.4 had the most survival rate and treatments 1 and 2 with 65 and 82 % had the least survival rate, respectively. Survival rate showed a significant difference between treatments 1, 2 and 3, 4 (p = 0.05). But there was not any significant difference between treatments 3 and 4 on survival rate that can be due to similarity between inland and foreign commercial enrichment emulsions. Obtained survival rate was similar to the results of Hafezieh *et al.* [4] on *Acipenser percicus*. Abnormal swimming, skin lesions, anorexia, exophthalmia, fin rot considered as general abnormalities that showed a significant difference among treatments as the most of them were in treatment 1 (33 samples) and the least were

in treatment 4 (19 samples). Also, treatment 1 showed a significant difference with treatments 2, 4 but there was no difference between treatments 3 and 4 that was similar to results of Watanabe *et al*. [21]. The results revealed that inland made suspensions can be used successfully and easily to enrich Artemia nauplii in aquaculture instead of foreign commercial ones. Production of Selco enrichment oil with inland potential can be achievable and all field tests were successful and can be replaced with foreign enrichment oils.

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