Effect of different levels of n-3 HUFA on larvae culture performances of Beluga *(Huso huso)* fish

Dadgar Sh.*

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Abstract

The effects of n-3 highly unsaturated fatty acids (n-3 HUFA) were studied on Beluga (*Huso huso)* fish larvae by feeding fish larvae with live food enriched with 4 different oils (ICES 30/4, tuna eye oil, flaxseed oil and cottonseed oil) containing different levels of n-3 HUFA including 27.19, 20.33, 12.71 and 0.39 mg g^{-1} dry weight of oil, respectively. Fish larvae weighing 30.00±2.00 mg were randomly divided into 12 groups of 150 fish each and triplicates fed the 4 experimental diets for 3 weeks. There were no differences in survival rates, but significant differences were found in growth rates and fatty acid composition of the fish larvae after 30 days. Fish fed low levels of dietary n-3 HUFA (0.39 mg g^{-1} DW) showed poor n-3 HUFA contents but these fatty acids improved with the elevation of the n-3 HUFA levels in the diet up to 20 mg g^{-1} DW. Fish fed the flaxseed oil showed the highest level of crude lipid 15.00±3.01). Results showed that tuna eye oil and ICES30/4-enriched live food (4.83 ±1.29 and 4.61 ±0.99 mg g^{-1} DW, respectively) showed the highest n-3 HUFA content for sturgeon fish larvae.

Keywords: *Huso huso*, n-3 HUFA, Growth and survival rate, Fats and fatty compounds, Tuna eye oil and enriched live food.

Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran

* Corresponding author's Email: shdadgar@ifro.ir

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Introduction

Lipids play an important role in fish nutrition for the provision of body energy and essential fatty acids (EFA) (Sargent *et al*., 1989). The n-3 highly unsaturated fatty acids (n-3 HUFA) in marine organisms contain primarily eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). These fatty acids are required to ensure membrane permeability and plasticity, enzyme activation, prostaglandin production and other functions (Sargent *et al*., 1989).

Yone and Fujii (1975) reported that supplementation of a corn oil diet with linolenic acid (18:3n-3), an EFA for freshwater fish, did not improve the growth of red sea bream juveniles and that only n-3 HUFA were effective as EFA for the fatty acid metabolism of this species. Many species of marine finfish are now widely believed to have an essential requirement for n-3 HUFA (Owen *et al*., 1972; Cowey *et al*., 1976; Kanazawa, 1985; Hafezieh *et al*., 2010). Marine fish juveniles (Fujii *et al*., 1976; Takeuchi *et al*., 1990) and larvae (Gatesoupe *et al*., 1977; Watanabe *et al*., 1989; Izquierdo *et al*., 1990; Hafezieh *et al*., 2010) have all shown optimal growth when fed dietary levels of n-3 HUFA ranging from 0.5 to 2.0%. This does not mean that marine fish are necessarily incapable of converting 18:3n-3, via the desaturation/ elongation pathways, to higher-molecular-weight n-3 fatty acids (n-3 HUFA). The conversion has been demonstrated for red sea bream (Yone and Fujii, 1975) and Atlantic salmon (Hardy *et al*., 1987). However, in species that it occurs, the conversion may proceed at a rate that is insufficient to prevent EFA deficiencies.

Previous studies in turbot (Linares and Henderson, 1991) and sturgeon fishes (Mourente and Tocher, 1993) have indicated HUFA must be present in the diet. It is also known that EFA requirements differ not only between species but also between growth stages (Gatesoupe and Le Milinaire, 1985). The essential and minimum dietary levels of eicosapentaenoic and docosahexaenoic acids were investigated in larvae and fingerlings of different species (Koven *et al*., 1989, 1990, 1992; Kalogeropoulos *et al*., 1992; Mourente and Tocher, 1993; Rodriguez *et al*., 1993, 1994; Hafezieh *et al*., 2010). However, the requirements for n-3 HUFA of larvae, juvenile or adult *H. huso* fish are still unknown.

The aim of the present study was to investigate the effects of HUFA oil-enriched live food on growth, survival rates and body composition of larvae of *H. huso* which is the most important group of Iranian sturgeon fishes for aquaculture.

Material and methods

Preparation of sturgeon fish larvae

The "settled stage" *H. huso* larvae from Salamati sturgeon propagation center were moved to a private fish farm in 20

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L plastic bags containing 1/3 water and 2/3 oxygen. After adaptation (21 minute per°C), they were transferred to two 2000 L tanks with suitable water flow for 5 days.

Upon the completion of yolk sac absorption, the larvae were transferred to 12, 40L experimental tanks (50x40x30 cm^3). Each tank was stocked with 150 larvae. All tanks were washed and disinfected with 10gL^{-1} sodium hypochlorite or sun dried, and filled with water (30 liter). The water was prepared by adding aged, de-chlorinated tap water.

Water quality monitoring

Water quality parameters including temperature, dissolved oxygen, pH, ammonia nitrogen and salinity were monitored 2 to 3 times weekly using a maximum- minimum thermometer, YSI oxygen meter model 57, Schott Gerate pH meter model CG 837 all with ±0.1 degree accuracy, Lamotte Low Range Ammonia Kit and a hand-held Atago refractometer model 8808, respectively. Water flow was 0.8 l/min with aeration (DO, during the culture period ranged from 7.1 to 7.8 mg/L, while the water temperature ranged from 20.4 to 20.7°C).

Feeding

For adaptation and monotony in feeding, all larvae were fed with unenriched *Artemia urmiana* nauplii during the first three days. From day four, they were randomly assigned to 15 treatment triplicates. The control group was fed with unenriched *Artemia urmiana* nauplii and the others were fed enriched *Artemia* with the experimental oils the fatty acid compositions of which are shown in Table 1. Feeding was carried out at a rate of 6 times a day for the first five days of the trial. From day 6 to the end of the period, the fish were fed 5 times/day (feeding rate was based on biometry of 5 sub samples of larvae) (Watanabe, 1993).

Larval performance

Larval performances were monitored for survival rate, specific growth rate (SGR), feed conversion ratio (FCR), and condition factor (CF). Survival percentage was calculated by counting the dead larvae during the experiments. Lengths were measured with rulers, weight with a digital scale with 0.01 g accuracy and SGR, FCR and CF were also calculated by the standard formulae (Rasowo, 1995).

Table 1: Fatty acid compositions of the live food enriched with different oils.

	ICES 30/4	Tuna eye oil	Flaxseed oil	Cottonseed oil
DHA/EPA	3.32±0.42	0.67±0.02	0.36±0.02	0.00±0.00
Σ Saturated	30.08±2.32	25.00±2.02	26.00±2.12	43.02±2.50
Σ Monoens	19.77±2.60	33.03±3.25	38.98±3.12	18.23±2.42
Σ n-3 HUFA	27.19±2.02	20.33±2.40	12.71±2.32	0.39±0.02

The averages of fatty acid contents were measured in mg g^{-1} dry weight.

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Proximate composition

Samples for biochemical analyses were washed with minimum volume of distilled water and after removal of the excess water, they were transferred into eppendorf tubes and frozen at -20°C for 24 h period followed by -80°C until further preparative work. Samples were then freeze-dried for 48 h, ground with a glass homogenizer, and kept at -80°C (Rasowo, 1995).

Extraction of fish larval lipid and analysis of fatty acids

Enriched and unenriched sturgeon fish larvae were oven dried at 60˚C for 24 h, ground, and double extracted using diethyl ether. The ether was let to evaporate and the remaining lipid residues were collected. The lipid residue was converted to fatty acid methyl ester (FAME) derivatives by adding N-Heptanes (1 mL) and 2N NaOH (0.05 mL). It was shaken continually for a period of 15 min (Harel *et al*., 2000).

The fatty acid analysis was carried out with GC under the following running conditions: injector temperature 200˚C; nitrogen carrier gas flow rate 1 mL min-1; injection volume l-1; splitting ratio 1:25; CBP20 Shimpak column; column temperature increased from 100 to 180˚C with a period of 30 min by linear gradient. After 40 min, 19 peaks were detected. These peaks were compared with related FAMEs standard and identified. All assays were done in triplicates.

Results

Water quality

No significant differences (p>0.05) were observed for all water parameters, WT, pH, DO, and Nitrate measured during the experiment.

Growth and survival results

The effects of dietary lipid on mean wet weight, feed efficiency and survival percentages are summarized in Table 2.

Fatty acid composition

Fatty acid composition of fish in the four treatment focusing on Arachidonic acid (ARA) C20:5n3 (EPA), C22:6n3 (DHA), saturated, monoens, Σ omega 3 and DHA/EPA and ω- 3/ ω- 6 ratios are presented in Table 3.

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Table 2: Results of four weeks feeding trial.

	Wet weight (mg) (WW)
Initial wet weight	30±2.00
After five days adaptation	46.8±3.03

Final 30 days

	ICES30/4	Flaxseed oil	Tuna eye oil	Cottonseed oil
WW (mg)	303.55±42.41[b]	324.71±37.82[c]	315.91±23.63[c]	294.34(75.62[a]
Survival(%)	90.17±3.08[a]	89.53±2.85[a]	89.21±3.12 [a]	88.98±2.32 [a]
FCR	1.20±0.15[a]	1.21±0.12[a]	1.23±0.10[a]	1.24±0.09[a]
CF	0.44±0.03[a]	0.43±0.02[a]	0.44±0.02[a]	0.44±0.03[a]
SGR	12.67±0.70[a]	12.84±0.70[a]	12.69±0.49[a]	12.70±0.47[a]

Numbers in rows having different letters indicate that treatments were significantly different at $p<0.05$. "Mean ± SD, n =30".

Table 3: Crude lipids and fatty acids composition of different larval treatments

	Initial	ICES30/4	flaxseed Oil	tuna eye Oil	Cottonseed oil
Lipid % DW	**10.12±1.06[d]**	**12.86±2.02[c]**	**15.00±3.00[a]**	**14.14±1.10[a]**	**13.00±1.00[b]**
Fatty acids mg g^{-1} DW					
C20:4n6 (ARA)	1.48±0.07[a]	0.89±0.12[b]	0.89±0.22[b]	1.13±0.24[a]	0.76±0.24[b]
C20:5n3 (EPA)	0.90±0.13[d]	2.90±0.36[a]	2.22±0.32[b]	2.35±0.34[b]	1.91±0.27[c]
C22:6n3 (DHA)	0.30±0.18[c]	2.47±0.34[a]	1.58±0.27[b]	2.30±0.23[a]	1.15±0.19[b]
DHA/EPA	0.30±0.42[c]	0.86±0.27[a]	0.69±0.24[b]	0.82±0.09[a]	0.65±0.19[b]
Σ Saturated	27.49±1.14[b]	27.80±1.82[b]	28.91±2.59[a]	29.22±3.32[a]	26.40±3.23[c]
Σ Monoens	32.30±1.04[a]	32.61±4.39[a]	29.05±3.31[b]	31.17±2.32[a]	27.23±2.66[c]
Σ n-3 HUFA	1.27±0.30[c]	4.61±0.99[a]	3.64±0.73[b]	4.83±1.29[a]	3.01±0.87[b]
ω- 3/ ω- 6	0.85±0.30[c]	5.25±0.43[a]	4.30±0.33[b]	4.35±0.19[b]	4.09±0.22[b]

Values are the averages of three replications ±SD
Numbers in rows having different letters indicate that treatments were significantly different at $p<0.05$.

Discussion

The effect of dietary fatty acids on *H. huso* larvae growth became noticeable after the 4th week of feeding ($p<0.05$). However, no marked differences in feed efficiency and survival were observed among the groups during the feeding trial ($p>0.05$). These results are in agreement with Dong-Fang *et al.* (2003) who reported the same results in *Acipenser transmontanus* larvae. The best wet weight was obtained in fish receiving tuna eye oil-enriched live food containing levels of 20.33 mg g^{-1} DW n-3 HUFA (324.71±37.82). However, no significant differences were observed among fish larvae fed tuna eye and flaxseed oils–enriched diet. Cottonseed oil-enriched live food produced the minimum levels of n-3 HUFA (294.34±75.62) in sturgeon fish larvae.

To our knowledge, there is very little information on fatty acids composition of sturgeon fish larvae. In the present study the largest increase in lipid content was observed in fish fed flaxseed oil (Table 3) which had the

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mediate percentage of total lipids. This finding is similar to that obtained for 1g gilthead sea bream fingerlings (Kalogeropoulos *et al*., 1992).

The fatty acid composition of lipid in fish is assumed to be markedly influenced by the diet. This may, however, apply mostly to natural and commercial diets high in total lipid which inhibit *de nouo* synthesis of fatty acids (Sargent *et al*., 1989). Fish fed low-lipid diets may modify the digested fatty acids extensively. Feeding diets containing a low n-3 HUFA level resulted in a high level of 18: In-9 and a low content of n-3 HUFA.

The percentage of 18: ln-9 acid was effectively reduced by supplementation with n-3 HUFA, while 20:5n-3 and 22:6n-3 were increased (Table 3). Our data in general, are in agreement with those reported for rainbow trout (Takeuchi and Watanabe, 1976,1977a), red seabream (Takeuchi *et al*., 1992), *Oncorhynchus mason* (Thongrod *et al*., 1990), *Pagrus major* (Takeuchi *et al*., 1990) and *Acipenser persicus* larvae (Hafezieh *et al*., 2010).

Fatty acid profiles from marine finfish, exhibited an increase in 18: ln-9, which in general, is one of the characteristics of EFA deficiency (Watanabe, 1982). The amount of 18: ln-9 in sturgeon fish larvae decreased from 19.17 to 13.86 mg g^{-1} DW by feeding the EFA-deficient diet (cottonseed oil) for 4 weeks and the concentration of EPA and DHA increased from 0.90±0.13 to 1.91±0.27 and from 0.30±0.18 to 1.15±0.19 mg g^{-1} DW, respectively (Table 3). Feeding diets ICES30/4, flaxseed oil and tuna eye oil resulted in an increase in HUFA content in sturgeon fish larvae and a reduction in 18: ln-9 levels to values similar to those of the initial sample. Consequently, the ratio of 18: ln-9 to n-3 HUFA which is indicative of the nutritive condition (Fujii *et al*., 1976) decreased (Table 3).

In conclusion, the present study suggests that the optimum n-3 HUFA requirement for Sturgeon fish larvae is about 20.10 mg g^{-1} diets on a dry weight basis.

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