Effect of dietary fish oil on growth responses of severum *(Heros severus)*

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Abstract. The purpose of this study was to present a suitable diet for the severum (*Heros severus*) ornamental fish, with an emphasis on normal lipid level and use of fish oil (FO) while incorporating growth indices of this fish in an aquarium environment. To achieve this objective, 420 fish with average weight of 0.5±0.02 g and average length of 2.2±0.25 cm were randomly released into 21 aquariums. In this study, fish were divided into 7 test groups including 6 treatment groups receiving diets containing 3% (T1), 5% (T2), 8% (T3), 10% (T4), 12% (T5), 14% (T6) fish oil and a control group (CG) (receiving oil-free diet), each with 3 replications, and were observed and assessed for an 82-day test period. Biometric assessment of all fish in treatment groups and control group was performed once every 15 days. At the end of test period, these biometric data were used to calculate the growth indices and feed indices in order to assess the growth performance. Statistical analysis was performed by calculating the average value of each parameter in Excel software and then importing them into SPSS software (v.20). That software was then used to search for significant differences between the results of different test groups. In this study, weight gain significantly increased with up to 10% increase in lipid level, so that the highest weight gain, specific growth rate, length gain, and daily weight gain were observed in treatment group T4, where all these parameters were significantly higher than other test groups (P <0.05). The survival rate was over 90% in all test groups and showed no significant difference (P> 0.05). Results of this study which was focused on the growth and development of ornamental fish *H. severus* fed with different levels of fish oil showed that the optimal level of this type of oil in their diet is 10%.
Key Words: ornamental fish, fish nutrition, growth parameters, fish oil, lipid.

Introduction. Keeping ornamental fish is a very popular and appealing hobby, but diet of this type of pets is one of the most neglected subjects of research (Earle 1995). Ornamental fish industry is well established in Asia (Shim 1988) and generates stable profit for countries engaged in this business (Parameshwaran et al 2002). In the Far East, keeping ornamental fish is an ancient practice and dates back to one thousand years ago, but since the 17th century this hobby has also become increasingly popular in Western world. There are about 4,000-5,000 species of ornamental fish, but only fewer than 100 species can be considered as common house pets (Pannevis 1993). Fish feed plays an important role in maintaining the health of ornamental fish as well as their natural behavior, color, appearance, and also their growth, development and reproduction (Erdogan et al 2012). But current data about these species is not enough to provide a properly formulated diet (Boonyaratpalin & Lovell 1977). Fish need of essential fatty acids (EFA) is different between different species depending on the temperature at which they live and also their natural dietary habits (Cowey & Sargent 1979). Fish oil and fish meal are two important biological sources and diet ingredients needed by carnivorous fish species (Carson 1962). The proper use of fish oil in aquaculture is the key to solve the future problems and ensuring the sustainability of this industry. Fish oil, extracted from the oceans, provides the main source of long-chain polyunsaturated fatty acids (Turchini et al 2009) but it have a high cost and high risk of oxidation means that it should be supplied and used in limited quantities (Dosanjh et al 1988). Traditionally, aquaculture diets have mainly relied on the use of fish oil as a lipid source to provide energy (Tacon & Metian 2008). Grisdale-Helland et al (2002) have studied the growth factors of Atlantic salmon *(Salmo salar)* at different temperatures. In their study, they found that fish fed with fish oil had a considerably increased weight gain compared with other test groups (fed with a diet of soybean oil and a mixture of fish oil and soybean). In a study conducted by Kalogeropoulos et al (1992) focused on the effects of diets containing different levels of cod liver oil and soybean oil (SO) on growth performance and body composition of Gilthead bream *(Sparus aurata)*, authors found that diet containing 12% fish oil (cod liver), puts the weight gain of this group of fishes in second place compared with other treatment groups (fed with a mixture of two oils with different percentages). In a study conducted by Twibell et al (2012) focused on assessing the growth response and fatty acid composition of juvenile Coho salmon *(Oncorhynchus kisutch)* fed with diet containing fish oil and fish meal, authors reported that the weight gain and feed efficiency for fish fed with diet containing 11 and 12 percent fish oil were considerably higher than those in other test groups (fed with a diet containing vegetable oil). In a study conducted by Nielsen et al (2005) on the effect of fish oil on growth and fatty acid composition of rainbow trout *(Oncorhynchus mykiss)* they found that specific growth rate (SGR) in fish fed with a diet containing the maximum amount of fish oil (23%) and a mixture of fish oil and vegetable oil (11.1% rapeseed oil + 11.9% fish oil) was significantly higher and fish fed with a diet containing 11.9% fish oil was next in the ranking.

The purpose of this study is to present a proper diet for *H. severus*, with an emphasis on normal lipid level and with the use of fish oil (FO) while incorporating growth parameters of this fish in the aquarium environment.

Material and Method

Preparation of test diets with different lipid levels. In this study, 6 isonitrogenous experimental diets was formulated with fish oil levels of 3, 5, 8, 10, 12, 14 percent and a control group (CG) (fish oil-free diet) were prepared. Diets were prepared by ingredients listed in Table 1, based on a formula generated by Win Feed 2.8 software.

Table 1

Ingredients and proximate composition of experimental diets (%)

Ingredients	*Experimental diets (%)*					
	T1 (3)	*T2 (5)*	*T3 (8)*	*T4 (10)*	*T5 (12)*	*T6 (14)*
Fish meal	18.9	19	19.3	19.4	18.3	16.8
Wheat meal	14.3	13.5	12.2	11.4	7.5	2.7
Wheat gluten	20.2	20.5	20.9	21.2	21.2	21.2
Soybean meal	17.5	17.1	16.5	16.1	19	22.8
Oil	1.5	3.6	6.7	8.8	11.1	13.4
Vitamin premix	2	2	2	2	2	2
Mineral premix	1.5	1.5	1.5	1.5	1.5	1.5
Additives	10.5	10.5	10.5	10.5	10.5	10.5
Dicalcium phosphate	13.6	12.3	10.4	9.2	9	9.1
	Proximate analyses (%, DM)					
Dry matter	91.85	92.12	92.61	92.85	93.44	93.76
Crude protein	43.42	43.48	44.32	44.57	45.52	46
Crude fat	12.91	13.82	14.74	15.10	16.23	17
Ash	11.95	12.52	12.59	12.70	13.21	14.10

Additives: Astaxanthin 3%, antioxidants 0.1%, binder 3%, moderate inhibitor 0.4%, D L methionine 1%, lysine 1%, garlic powder 2%; DM - dry matter.

First, ingredients were ground by an electric mill and then were sieved to avoid any effect of ingredients particle size on consistency, formation and digestion of diet. Ingredients required for each diet were then weighed, and prepared according to the formula. Water was added and mixed with each group of ingredients to form a nonstick paste. Once paste was formed, the amount of required oil, determined by formula, was weighed and added to the paste. Resulting paste got passed through a meat grinder with a pore plate with 2 mm pores (in diameter) and resulting paste strings were placed in aluminum trays. After that, trays were placed in the oven at 65°C for 8 hours and then were dried under the hood in sterile conditions. These strings were cut to the size suitable for fish's mouth by a cutter, were put in special plastic packs and were kept in a refrigerator at 4°C.

Test design and supply of fish. In this study, 420 juvenile *H. severus* (all from the same parent) with a mean weight of 0.5±0.02 g, and an average length of 2.2±0.25 cm were tested. 21 aquariums with dimensions of 50 x 40 x 30 cm and capacity of 60 L were prepared and an air stone was used in each aquarium for aeration. Fish were divided into 7 groups including 6 treatment groups with diets containing 3% (T1), 5% (T2), 8% (T3), 10% (T4), 12% (T5), and 14% (T6) fish oil, each with 3 replications and one control group (oil-free diet) also with 3 repetitions, and then evaluations were made over an 82-day period. Fish were randomly released into the aquariums (20 in each aquarium) and treatments and group numbers were assigned to aquariums. To ensure proper adaptation, fish were fed with a basic diet and were kept in their new aquariums for 10 days. 50% of the water was changed once every four days and filters were also used to maintain and protect water quality throughout the test period. Standard water temperature was adjusted and regulated by automatic heaters installed in each aquarium. All aquariums were aerated by a central air pump. Throughout the test period, the amount of oxygen dissolved in the water was measured by specialized test kit every day and water pH was also measured by a pH meter once every two days. Fish were fed 3 times a day (at morning, noon and sunset) by a daily diet equal to 4% of body weight based on biomass obtained in each biometric assessment and with respect to fish feeding behavior. Once adaptation period was finished, the main stage of test started and continued for 82 days; in this period, biometric assessments were conducted once every 15 days. Weights of fish were measured by a scale with a precision of 0.01 grams and sizes of fish were measured by a precision ruler. Feedings were stopped for 12 hours before and after biometric assessment to reduce the fish stress in the course of procedure.

Calculation of growth indices, feed indices and survival rate. Growth indices including length gain, survival rate, specific growth rate, weight gain, and condition factor and also feed indices including feed conversion ratio and voluntary feed intake were measured to evaluate the growth performance with respect to measured biometric data.

Condition factor: CF = [Final weight (g) / fork length 3 (cm)] × 100
Feed conversion ratio: FCR = Feed intake (g) / weight gain (g)
Weight gain: WG (g) = [(Final weight - initial weight) / initial weight] ×100
Specific growth rate (%): SGRW= [(final body weight - initial body weight) / days of experiment] ×100
Specific growth rate (%): SGRL= [(final body length - initial body length) / days of experiment] ×100
Daily growth rate: DGR (g) = [(Final body weight - initial body weight) / initial body weight] × days
Length gain: LG (mm) = [(Final length – initial length) / initial length] ×100
Survival rate (%): SR = (Final fish number/Initial fish number) × 100
Voluntary feed intake: (VFI) = Dry feed intake / [(initial + final fish biomass)/2]

Carcass analysis. Once test period was finished, 5 specimens were randomly selected from each treatment group fed with diet containing 3% (T1), 5% (T2), 10% (T4), 12% (T5), and 14% (T6) fish oil and also from control group (CG) (with oil-free diet) and were transferred to the laboratory. The total carcass protein content was measured by Kjeldahl method, and crude fat content was measured by soxhlet method with accordance to

AOAC (1990) instructions. Moisture was also measured by method described by AOAC (1990) (Table 2).

Table 2

Proximate composition (%) of whole body of *Heros severus* fed diets containing fish oil

Parameters	*Experimental diets (%)*					
	T1 (3%)	T2 (5%)	T4 (10%)	T5 (12%)	T6 (14%)	CG
Crude protein	67.3±0.36	65.3±0.41	62±0.5	63.5±0.37	64.2±0.46	65.1±0.38
Crude lipid	10.1±0.19	15.7±0.38	23.1±0.33	22.4±0.33	19.95±0.4	8.9±0.2
Moisture	24.5±0.38	27.7±0.35	28.8±0.27	27.1±0.16	27.5±0.08	25.1±0.17
Ash	6.9±0.07	7.1±0.13	7±0.17	7.5±0.14	7.6±0.16	8.1±0.11

Statistical analysis. The mean values related to different groups of data were calculated in Excel and then were imported into SPSS software (v. 20) to be searched for the presence of significant differences between results related to different treatments. Kolmogorov-Smirnov test showed the normality of data distribution, and then presence or absence of significant difference between treatments was evaluated by One-Way ANOVA method. Once significant differences were observed, Post hoc LSD in the ($P<0.05$) level was used to assess significant differences between groups.

Results. Water temperature in all aquariums was 28±1°C; water had an almost neutral pH in the range between 6.8 and 7.8; dissolved oxygen was fluctuating in the ranges between 6.8-8.3 ppm in all aquariums. Calculations showed that nitrite content and total hardness were 0.02 mg/L and 215 mg^{-1} respectively. Growth indices, feed conversion ratio and survival rate of *H. severus* fed with diet containing different levels of fish oil are shown in Table 3. At the end of test period, weight gain (WG), which is a function of the final weight, was significantly different ($P<0.05$) in different test groups. The highest weight gain was observed in the fish fed with the diet containing 10% fish oil (T4). At the end of test period, this group (T4) also had the highest specific weight growth rate (SGRW) which was significantly higher ($P<0.05$) than other treatment groups. The lowest weight gain and lowest specific weight growth rate were observed in the group fed with the diet containing 14% fish oil (T6) (Figure 1). Trend of growth in *H. severus* specimens obtained from 5 biometric assessments over 82 days of test period with respect to different levels of fish oil in their diet is presented in Figure 2. The daily growth rate (DGR) was also calculated for different test groups which showed that the highest value for this parameter was observed in the group fed with the diet containing 10% fish oil (T4) which had a significant difference ($p<0.05$) with other test groups; the lowest value for this parameter was observed in the group fed with the diet containing 14% fish oil (T6). Lowest values of feed conversion ratio (FCR) was observed in the test groups fed the diet containing 5% (T2) and 10% (T4) fish oil (difference between FCR values of these groups was negligible). The highest feed conversion ratio was observed in the test group fed the diet containing 12% (T5) fish oil. At the end of test period, there was no significant difference in survival rate of test groups ($P>0.05$) and this value was over 90% in all groups and there was no sign of disease in any of the specimens. Statistical analysis and data presented on Table 3 also showed that at the end of test period, specific length growth rate (SGRL) in the test group fed with the diet containing 10% (T4) fish oil was significantly higher ($P<0.05$) than other groups. Lowest values of specific length growth rate were observed in the test groups fed with the diet containing 14% (T6) fish oil. Condition factor (CF) was also calculated for different test groups and it was found that highest value for this parameter was observed in the group fed with the diet containing 14% fish oil (T6) which was significantly different ($p<0.05$) from other groups.

Table 3

Growth parameters of *Heros severus* fed the experimental diets containing fish oil for 82 days

Growth parameter	*The fish oil content of experimental diets*					
	3%	*5%*	*8%*	*10%*	*12%*	*14%*
FCR (%)	1.61±0.08b	1.39±0.03c	1.46±0.1c	1.41±0.11c	1.84±0.15a	1.8±0.1a
SGRW (%)	17.68±1.77c	24.76±2.02b	22.56±2.29b	28.7±4.07a	13.62±1.61d	12.92±2.07d
SGRL (%)	58.7±13.65c	62.11±6.25b	60.57±7.72b	65.04±18.23a	54.02±7.99d	48.86±14.67d
WG (g)	14.5±1.45c	20.3±1.66b	18.5±1.87b	23.53±1.79a	11.16±1.32d	10.6±1.7d
LG (mm)	48.13±11.2c	50.93±5.13b	49.66±6.33b	53.33±14.94a	44.3±6.55d	40.06±12.03d
SR (%)	93.33±5.43a	100±0.00a	100±0.00a	100±0.00a	93.33±5.43a	100±0.00a
CF (%)	13.26±2.73d	15.3±1b	14.99±0.92c	15.38±1b	12.84±1.27e	16.25±0.68a
VFI	66.22±3.65c	57.33±1.6d	59.72±4.18d	57.9±4.76d	75.51±6.64a	74.1±4.49b
DGR (g)	69.74±1.14c	72.91±0.64b	72.07±0.89b	73.88±0.92a	66.73±1.35d	65.84±1.94d

* Different superscript in each row indicate significant differences among treatments (P<0.05).

The highest amount of voluntary feed intake (VFI) was observed in the test groups fed with the diets containing 12% and 14% fish oil respectively (difference between VFI values of these groups was very small), and lowest value for this parameter was observed in the groups fed with the diets containing 12% and 14% fish oil (with very small difference). Test group fed with the diet containing 10 percent fish oil had the highest growth rates which was significantly different ($p<0.05$) from other groups, and the groups fed with the diet containing 14% and 12% fish oil showed the lowest growth rate (Table 3).

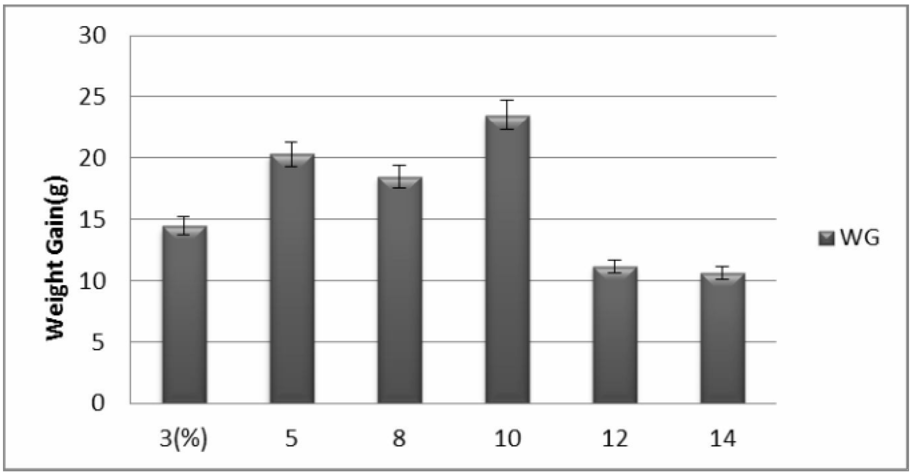

Figure 1. Weight gain (Mean±SD) of *Heros severus* fed with formulated diets containing fish oil during the 82 days.

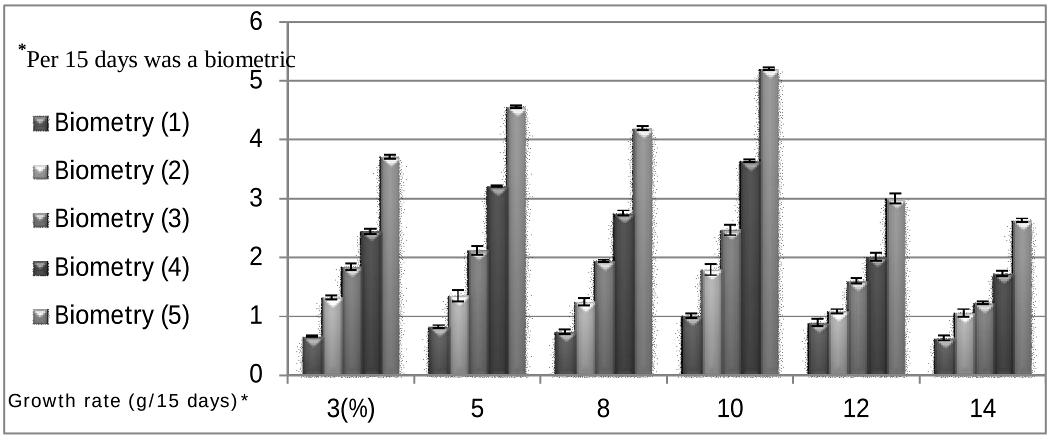

Figure 2. Trend of growth (Mean±SD) of *Heros severus* fed with formulated diets containing fish oil during the 5 biometrics.

Discussion. According to results, growth rate of *H. severus* juveniles fed with diets containing 10% fish oil was a higher than that of other test groups which were fed with other levels of fish oil (Figure 1). The use of various oils in the fish diet is as effective as other energy sources on their growth, survival rate and body composition (Sener & Yildiz 2003; Almaida-Pagan et al 2007). This is the first report on the impact of different dietary lipid levels (emphasized on fish oil) on growth parameters, feed efficiency and body composition of *H. severus*. The results obtained by this study show that fish fed with the diet containing 10% fish oil (T4) had a higher weight gain and also a higher specific growth rate compared with other test groups, which indicate that substances in this diet more successfully provided the nutritional needs for faster growth of the subjects. This means that the increase in oil and energy provided by diet fed to group (T4) led to lower share of proteins and higher share of fat in their tissue, and in other words it led to a growth caused by increased fat tissue. Nielsen et al (2005) reported that difference between FOmax fish and the fish fed the other diets (rapeseed oil and mixture of fish and rapeseed oil) was expected due to the difference in fatty acid content of the diets. The lowest SGR was observed in fish fed with the diet containing 14% and 12% oil; this indicate the lack of necessary fat required for growth and also an imbalance between protein and fat content in these diets. Dietary lipid and protein levels are the most important factors in the P:E ratio of various types of brown-marbled grouper *(Epinephelus fuscoguttatus)* (Shapawi et al 2014). Similarly, in a study conducted by Al-Souti et al (2012) on red hybrid tilapia *(Oreochromis sp.),* lowest SGR level was obtained for 12% oil content. In the present study, the highest value obtained for SGR was observed in test group fed the diet containing 10% oil (T4). SGR decreases with the increase in length, so low values of specific growth rate (SGR) observed in some studies can be explained by the growth and development of the fish (Sunde et al 1998). In a study by Jiang et al (2013) on growth parameters of darkbarbel catfish *(Pelteobagrus vachelli)* fed with diets containing fish oil and soybean oil, they found that fish fed with diets containing 8% fish oil did not show any significant difference in specific growth rate (SGR) compared with other groups tested over an 80-day growth period. Peng et al (2008) reported the weight gain (WG) of juvenile black seabream fed with diets containing 9% fish oil as 3.07 grams which is almost similar to results obtained in this study. Özşahinoğlu et al (2013) assessed the growth parameters of European sea bass *(Dicentrarchus labrax)* fed with diets of fish oil, and obtained the best results for growth and lowest change in the nutrients composition in the body and muscles. In this study, the best growth and FCR for *H. severus* was obtained by using diets containing 5% and 10% fish oil. The reason behind the better growth in these treatment groups can possibly be that using these diets with these levels of fish oil led to optimum consumption of nutrient substances by fish. In the present study, the highest feed conversion ratio (FCR) was observed in the group fed with the diet containing 12% fish oil which is similar to results obtained in a study conducted by Nielsen et al (2005) on growth parameters of rainbow trout *(Oncorhynchus mykiss)* where they reported the highest value of FCR in test groups fed with diets containing 11.9% fish oil. In this study, the weight gain significantly increased along with up to 10% increase in lipid level. In a study by Subhadra et al (2006), authors suggested that changes in fish growth can be a result of a combination of diet and feeding period. In this study, a weight loss was observed with the increase in oil level from 10% to 14% while growth factors showed no significant difference, so we can say that using these amounts of fish oil in the *H. severus* diet is not desirable and leads to additional costs and meager and insignificant increase in growth performance. In a study by Regost et al (2003) authors reported a significant difference in final weight and growth rate of Turbot *(Scophthalmus maximus)* fed with diets containing 9% fish oil. They found that treatment groups fed with diets containing fish oil showed a greater impact on growth compared with other oils. In this study, the highest length gain (LG) values was observed in the groups fed with diets containing 10% and 5% oil, (difference between LG values of these groups was very small). In a research by Hardy et al (1987) on growth parameters of Atlantic salmon *(Salmo salar)* they found that fish fed with diet containing 9.6% menhaden oil had the highest length gain among all test groups, this difference however was not statistically significant ($P>0.05$). In this study, condition factor (CF) in fish fed with the diet containing 14% oil (T6) was highest among all test groups, which is possibly because of lower involvement of protein in *H. severus* body structure and higher involvement of fat in the body tissue, which led to hampered longitudinal growth with the increase in body weight (because of decreased impact of protein on bone growth) (Mooraki & Moieni 2013). Study conducted by Bell et al (2010) also reported the highest CF in fish fed with highest level of fish oil, which is similar to results obtained in this study. Meanwhile, lowest condition factor (CF) was observed in test groups fed with the diets containing 12% (T5) and 3% (T1) oil. In this study, the maximum voluntary feed intake (VFI) was observed in fish fed diets containing 12% and 14% oil which also had the lowest growth rate. In a study by Peng et al (2014) on the growth parameters of the *S. maximus* juvenile fed with diets containing different rates of fish oil and soybean oil, authors found that fish fed with diets containing the highest levels of fish oil had the highest feed intake (FI) among all test groups. Previous studies have shown that fish are able to regulate their feed intake according to diet quality with a self-feeding device when the duration of feed availability is sufficiently long. On this basis, diets with poor acceptability are often rejected and caught again several times before being abandoned, and this feeding behavior is responsible for increased feed waste (Medale et al 1998). No sign of any disease was observed throughout the experiment; the lowest survival rate however was observed in group fed with the diet containing 12% and 3% fish oil; but given the lack of significance in the difference between mortality rates, it seems to be independent from the dietary lipid level. The use of lipid sources such as fish oil in the diet of coho salmon *(Oncorhynchus kisutch)* also has not caused any significant difference in the survival rate of this fish (Twibell et al 2012).

Conclusions. Results of this study which was focused on the growth and development of *H. severus* fed with different levels of fish oil showed that the optimal level of this type of oil in fish diet is 10%. Additional research must include the physiological assessment of the above observation and laboratory analysis of changes in fatty acids in the liver and muscles which both seem necessary to obtain the exact optimal level of oil in the diet of this specie.

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