# Evaluating the effect of dietary soybean oil on growth performances of juvenile severum (*Heros severus*)

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**Abstract**

Evaluating the effect of dietary soybean oil (S.O) on growth performances of juvenile ornamental fish Severum *(Heros severus)* was the main objective of this study. To achieve this objective, 462 fish were considered which were divided into 7 test groups including 6 groups of fishes (with an initial average weight of 0.6 ± 0.05 g and length of 2.3±0.35 cm) fed diets containing 6 levels of S.O ($D_1$(3), $D_2$(5), $D_3$(8), $D_4$(10), $D_5$(12), $D_6$(14)%) and the control group (CG) (receiving an oil-free diet) for 12 weeks with biometry intervals of 15 days. At the end of the test period, growth performance and nutritional parameters were measured. The study showed that the maximum weight was in fish fed diet containing 8% of S.O and also the maximum specific growth rate (SGR) ($D_3$, $p<0.05$) was obtained for this group. The highest rate of daily weight gain (DWG) was obtained for $D_3$ which was significantly ($p<0.05$) much more than that for $D_4$, $D_6$ and $D_5$ (the lowest increase). The highest length gain (LG) was observed in $D_3$ which was significantly more ($p<0.05$) in comparison with the other treatments tested. The survival rate was over 80% in all test groups and showed no significant difference ($p>0.05$). At the end of the period, fish were evaluated for estimated analysis of the tissue. The results of this study which was focused on the growth and development of ornamental fish *H. severus* fed with different levels of soybean oil suggest that the optimal level of this type of oil in their diet is 8% based on the growth parameters.

**Keyword:** Soybean oil, Lipid, Growth performances, Diet formulation, Severum (*Heros Severus*)

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## Introduction

Nowadays, in addition to edible fish culture, breeding of different types of ornamental fish is taken into consideration and research projects are conducted enthusiastically in this field. The economic importance of ornamental fish is not lower than that of edible fish. In this regard, an important aspect of aquaculture is nutrition that fish farmers should pay special attention to, since a large part of the fish production costs is allocated to this part (Firouzbakhsh *et al.*, 2011). However, little information is available on the nutritional requirements of ornamental fish (Kruger *et al.*, 2001; Miller and Mitcholl., 2008; Güroy *et al.*, 2012).

The reproduction and breeding industry of ornamental fish is developing in line with the growth of aquaculture industry in the world. More than 1000 types of freshwater fish, which consist of 100 families, are commercial ornamental fish (FAO, 2003). In this regard, this field of fisheries science has become a large industry and a profitable business. On the other hand, health and nutrition of ornamental fish are two decisive factors in the ornamental fish industry (Ghosh *et al.*, 2008). Thus, food has a key role in health, appropriate natural behavior, improving the phenotype, the color of ornamental fish and also their growth, development and reproduction (Erdogan *et al.*, 2012). Also, the apparent growth is affected by the amount of food intake and food absorption into the body (Buurm and Diana, 1994).

In this regard, formulated commercial diets for salmon often consist of 40-50% crude protein and 12-17% crude lipid that are used in ornamental fish farms regardless of nutritional behavior and ecology of the natural nutrition of this fish. As a result, it leads to decreasing growth performance, and economically, it is not affordable (Royes *et al.*, 2006).

Trading volume index of ornamental fish is about one billion dollars in the world (Erdogan *et al.*, 2012). The global supply of vegetable oils clearly represents the largest source of lipid for animal diets (Soyatech, 2009). Soybean oil (S.O), as a source of fat in the diet, is used in published studies on fish such as sturgeon (*Acipenser schrencki*) (Xiao and Cheng, 2001), common carp (*Cyprinus carpio*) (Fontagne *et al.*, 2000), Japanese flounders (Lee *et al.*, 2000) (*Paralichthys olivaceus*). The use of S.O in the above studies cannot indicate practical and reliable application of this fat source, and S.O should be evaluated based upon target species (Turchini *et al.*, 2011).

S.O has about 60 g of EFA /100 g of fatty acids and EFA in S.O is dominated by 18:2n-6 group (Tacon, 1990). S.O has been considered among available vegetable oils because of its availability, low cost and high amounts of essential fatty acids (Bell *et al.*, 2001; Caballero *et al.*, 2003). Grisdale-Helland *et al.* (2001) examined the soybean oil diet on growth performance and feed in Atlantic trout (*Salmo salar*). They found that fish fed the diet containing 27.8% of S.O is in the second rank of growth among the diets tested, and they did not show any significant differences in different test groups (containing 27.8% fish oil and in a 1:1 ratio for fish oil and soybean oil each one 13.9%). Ng *et al*. (2012) did not observe any significant differences in their study on growth parameters in red hybrid tilapia fish (*Oreochromis* sp.) fed diets containing vegetable oils (soybean, palm and flax) and fish oil. Jiang et al. (2012) examined the effects of diets containing soybean oil and fish oil on the growth of catfish (*Pelteobagrus vachelli*). In their experiment, they found that diets containing 8% S.O did not show any significant differences in weight gain (WG) during the 80 days of feeding. Lochman and Brown (1997) examined the average weight gain in goldfish (*Carassius auratus*) fed with a diet containing 4% fish oil (cod liver oil) and S.O (4%) for a period of 42 days. They found that there was no significant difference on weight gain in gold fish fed with a diet containing 4% S.O throughout this period. Regost *et al*. (2003) studied the growth performance of Turbot (*Psetta maxima*) fed with diets containing 9% S.O and 9% fish oil, and observed a significant difference in final weight and growth rate of the fish. Deng *et al.* (2013) examined the growth factors of Pacific threadfin (*Polydactylus sexfilis*) fed a diet containing fish oil, S.O and in a 1:1 ratio for fish oil and S.O.

Their results suggested that diets containing 8% S.O should provide adequate levels of essential fatty acids to support normal growth of juvenile Pacific threadfin under the current feeding conditions.

The purpose of this research is to produce proper diets with emphasis on optimum levels of fat using S.O for ornamental species ofseverum (*Heros severus*) according to the growth parameters in the aquarium environment.

## Materials and methods

### *Experimental diets*

In this study, 6 experimental diets of formulated isonitrogenous with different levels of S.O (3, 5, 8, 10, 12, 14%) and a control group (experimental diet without oil) were prepared. Diets with raw material (Table 1) were prepared in accordance with the formula written by software 2.8 Win Feed (Cambridge, UK). The raw materials were ground (diameter 1 mm) using an electric mill. According to the provided formula the level of each one was calculated and weighed. Then, these materials were mixed to form a paste by adding water. The amount of oil needed was weighed according to the formula and then added to the paste mixture.

**Table 1: Ingredient and proximate composition of experimental diets.**

| Ingredient(g/10g) | Experimental diets | | | | | | |
|---|---|---|---|---|---|---|---|
| | $D_1$ (3) | $D_2$ (5) | $D_3$ (8) | $D_4$ (10) | $D_5$ (12) | $D_6$ (14) | CG |
| Fish meal | 1.89 | 1.9 | 1.93 | 1.94 | 1.82 | 1.68 | 1.91 |
| Wheat meal | 1.43 | 1.35 | 1.22 | 1.14 | 0.75 | 0.27 | 1.52 |
| Wheat gluten | 2.02 | 2.05 | 2.09 | 2.12 | 2.12 | 2.12 | 1.99 |
| Soybean meal | 1.75 | 1.71 | 1.65 | 1.61 | 1.9 | 2.28 | 1.75 |
| Oil | 0.15 | 0.36 | 0.67 | 0.88 | 1.11 | 1.34 | - |
| Vitamin premix | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Mineral premix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| *Additives | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 |
| Dicalcium phosphate | 1.36 | 1.23 | 1.04 | 0.91 | 0.9 | 0.91 | 1.42 |
| **Proximate analyses (%, DM*)** | | | | | | | |
| Dry matter | 90.88 | 91.11 | 91.51 | 91.85 | 92.04 | 92.38 | 89.62 |
| Crude protein | 44.42 | 44.80 | 45.30 | 45.80 | 46.20 | 46.83 | 43 |
| Crude fat | 6.31 | 7.70 | 10.22 | 12.80 | 15.01 | 16.55 | 4.59 |
| Ash | 14.70 | 14.85 | 13.62 | 12.50 | 12.75 | 13.10 | 17 |

*Additives : Astaxanthin 3%, antioxidants 0.1%, binder3%, moderate inhibitor 0.4%, D L methionine 1%, lysine 1%, Garlic Powder 2%; *DM, dry matter.

The paste was passed through a meat grinder with a pore plate with 2 mm pores (in diameter) and the resulting paste strings were placed in aluminum trays. After that, trays were kept in an oven at a temperature of 65°C for 8 hours, and then dried under the hood in sterile conditions. Finally, these threads were cut by the cutter according to the size of the fish's mouth, sealed in plastic bags and kept at 4°C until usage.

*Design of Experiment*

In this study, 462 numbers of Severum with an average weight of 0.6±0.05 g and an average length of 2.3±0.35 cm were obtained (all from the same parent). After preparation of 21 aquaria with dimensions of 30×50×70 cm and volume of 100 liters, the aquaria were equipped with an aeration system and heater to maintain the water temperature. Fish were put into 7 experimental groups; 6 groups with diets containing S.O at levels of 3 ($D_1$), 5 ($D_2$), 8 ($D_3$), 10 ($D_4$), 12 ($D_5$), 14 ($D_6$) and 1 control group (CG) (oil-free diet) for 12 weeks; each one was replicated 3 times. Fish were stocked randomly inside the aquaria (22 per aquarium) and treatments and group numbers were assigned to aquaria according to the type of food. For adaptation, fish were fed a basic diet and were kept in the conditions of new aquaria for 8 days. To protect water quality during the period, bio-filters were used and 50% of water was replaced every three days. The dissolved oxygen and pH of the water were measured every two days using specialized test kits via the colorimetric method. Fish were fed 3 times a day (08:00, 11:00 and 16:00 hours) on the basis of biomass obtained in each biometric assessment, and the amount of food was calculated according to appetite of fish and based upon 4% of the fish weight. The main stage of the test started and continued for 84 days after completion of the adaptation period, and biometric assessments were conducted once every 15 days. To measure weight and length, a scale with a precision of 0.01 g and a precision ruler were used, respectively. Feedings were stopped for 15 hours before and after biometric assessment to reduce fish stress in the course of the procedure.

*Calculation of growth indices, feed indices and Survival rate*

At this stage, according to data obtained from biometric assessments, growth parameters which include specific growth rate, the percentage of body weight gain, the percentage of body length gain, survival rate of average daily gain, condition factors and nutritional indices including optional food intake and feed conversion ratio were measured as follows (De Silva and Anderson, 1995; Austreng, 1978; Yanbo and Zirong, 2006; Gümüş, 2011).

Specific growth rate (%); $SGR_W$=[ln final body weight _ ln initial body weight / days of experiment] ×100.

Feed conversion ratio; FCR=Feed intake (g) / weight gain (g).

Weight gain; WG (g)=(Final weight - initial weight) / (initial weight) ×100.

Length gain; LG (mm) = (Final length− initial length) / (initial length) ×100.

Condition factor; CF=(Final weight (g) / (fork length (cm)) $^3$ × 100.

Daily growth rate; DGR (g) = ((Final body weight) - (initial body weight) / initial body weight × days.

Voluntary feed intake; (VFI) = Dry feed intake / [(initial + final fish biomass)/2]

Specific growth rate (%); $SGR_L$= [ln final body length - ln initial body length / days of experiment] ×100

Survival rate (%); SR = (Final fish number/Initial fish number) × 100.

*Carcass analysis*

At the end of the feeding trial, 8 fish were randomly selected from treatments fed with diets consisting the levels of 3($D_1$), 5($D_2$), 8($D_3$), 10($D_4$), 12($D_5$), 14($D_6$) oil content and the control group (without oil) (CG), and transferred to the laboratory. The total carcass protein content of body and crude oil were measured using the Kjeldahl method and Soxhlet method according to the instructions of AOAC (1990), respectively. Moisture was also measured by the method described in AOAC (1990) (Table 3).

*Statistical analysis*

The mean values related to different groups of data were calculated in Excel and then imported into SPSS version 20 to be checked for the significant differences between results related to different treatments. Normal scattering of data was determined using the Kolmogorov-Smirnov test, and then the significant differences between treatments were investigated using One-Way ANOVA. After observation of significant difference, post hoc LSD in the ($p<0.05$) level was used for investigation of significant differences between the groups.

## Results

The parameters of water quality were maintained as follows: water temperature=28.2°C, pH = 6.8 to 7.8, dissolved oxygen: 6.8 to 8.3 ppm, nitrite = 0.02 $mgL^{-1}$, and total hardness: 215 $mgL^{-1}$. Growth indices, feed conversion ratio and the percentage of survival in severum (*H. severus*) fed with diets containing different levels of S.O are shown in Table 2.

The percentage of body weight gain (WG), which is a function of final weight, was significantly different ($p<0.05$) among the studied groups at the end of the period. In fact, the highest weight gain was recorded in fish fed diets containing 8% oil ($D_3$) in that the specific growth rate of weight ($SGR_W$) of this group was considerably higher than the studied treatments at the end of the period ($p<0.05$ ). However, the lowest weight gain was in fish fed diets containing 12% oil ($D_5$). The daily weight gain rate (DGR) was calculated in fish of different treatments and found that the maximum value was in fish fed a diet containing 8% oil, which showed significant differences ($p<0.05$) with treatments $D_4$, $D_5$ and $D_6$, and the lowest value was in fish fed a diet containing 12% oil ($D_5$).

Feed conversion ratio (FCR) of fish fed diets containing 8 and 10% oil was the lowest at the end of the period. Also, highest FCR was related to fish fed the diet containing 12% oil ($D_5$). At the end of the experiment, survival rate (SR) in all treatments had no significant differences, and was more than 80%. Also, no sign of disease was observed in all fed fish ($p>0.05$). With statistical analysis and study of Table 2 in terms of specific growth rate of length ($SGR_L$), fish fed a diet containing 8% oil ($D_3$) had higher $SGR_L$ than the rest of treatments at the end of the period ($p<0.05$). The lowest $SGR_L$ was in fish fed a diet containing 12% oil ($D_5$). Condition factor (CF) of fish of different treatments was calculated and it was found that the maximum amount was for fish fed a diet containing 10% oil ($D_4$), which was significantly different ($p<0.05$)with that in other treatments and the lowest was for fish fed the diet of 12% oil ($D_5$). The highest and lowest amounts of voluntary food intake (VFI) were in fish fed diets of 12% oil ($D_5$) and 8% oil (D3), respectively. Fish fed diets of 8% oil ($D_3$) showed the highest growth that had significant differences with that in the other treatments ($p<0.05$), while fish fed a diet containing 12% oil showed the lowest growth rates (Table 2).

**Table 2: Growth parameters of *Heros severus* fed the experimental diets containing soybean oil for 84 days.**

| Growth parameters | 3 (%) | 5(%) | 8(%) | 10(%) | 12(%) | 14(%) | CG (%) |
|---|---|---|---|---|---|---|---|
| FCR (%) | 1.69±0.1[b] | 1.55±0.05[d] | 1.53±0.11[c] | 1.53±0.11[c] | 2.5±0.41[a] | 1.69±0.1[b] | 1.64±0.06[e] |
| $SGR_W$ (%) | 17.48±1.97[b] | 18.7±0.64[a] | 19.39±2.42[a] | 16.5±2.18[b] | 9.51±3.17[d] | 14.59±0.96[c] | 15.53±1.73[c] |
| $SGR_L$ (%) | 54.88±12.62[b] | 56.26±4.68[a] | 58.54±21.38[c] | 53.41±12.27[c] | 49.62±29.4[e] | 54.65±13.19[d] | 42.19±24.02[f] |
| WG (g) | 14.33±1.61[b] | 15.3±0.52[a] | 15.9±1.98[a] | 13.53±1.79[c] | 7.80±2.6[e] | 11.96±0.38[d] | 13.18±0.84[c] |
| LG (mm) | 45±10.34[a] | 46.13±3.84[a] | 48±17.53[b] | 43.8±10.06[b] | 40.68±24.11[d] | 44.81±10.81[c] | 34.64±11.54[f] |
| SR (%) | 100±0.00[a] | 100±0.00[a] | 93.33±5.43[a] | 100±0.00[a] | 80±9.42[b] | 93.33±5.43[a] | 100±0.00[a] |
| CF (%) | 15.61±0.45[b] | 15.62±0.88[b] | 14.49±3.62[c] | 15.94±0.71[a] | 11.22±3.55[e] | 13.47±2.18[d] | 13.21±3.24[d] |
| VFI | 69.25±4.31[b] | 63.59±2.09[c] | 62.88±4.9[c] | 63.12±4.84[c] | 102.55±16.98[a] | 69.33±4.11[b] | 62.62±2.81[c] |
| DGR (gr) | 69.56±1.32[a] | 70.48±0.33[a] | 70.75±1.31[a] | 68.9±1.37[b] | 59.83±4.48[c] | 67.8±0.38[b] | 68.27±0.81[b] |

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

**Table 3: Proximate composition (%) of whole body of *Heros severus* fed diets containing soybean oil.**

| Diet | Experimental diets (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | D1(3) | D2 (5) | D3 (8) | D4 (10) | D5 (12) | D6 (14) | CG |
| Crude protein | 68.1±0.18 | 67.30±0.17 | 65.52±0.32 | 62.70±0.34 | 62.80±0.33 | 63.48±0.27 | 65.1±0.38 |
| Crude lipid | 9.7±0.31 | 13.95±0.4 | 20.50±0.36 | 34.69±0.29 | 17.50±0.31 | 23.70±0.43 | 8.9±0.25 |
| Moisture | 24.3±014 | 25.60±0.33 | 25.50±0.36 | 25.70±0.32 | 25.50±0.32 | 27.65±0.8 | 25.1±0.52 |
| Ash | 6.45±0.15 | 6.70±0.09 | 6.90±0.14 | 7.20±0.13 | 7.40±0.34 | 7.50±0.35 | 7.70±0.33 |

## Discussion

This research was conducted to determine the optimum level of lipid using S.O for growth and maintenance of ornamental severum (*H. severus*). The results show that infants of severum fed the 8% oil diet had higher growth rates than the other levels. The use of oil in the diet of fish such as energy sources in diets of fish had effects on growth, survival and body composition (Sener and Yildiz, 2003; Almaida-Pagan *et al*., 2007).

This is the first study on the impact of diets containing different lipid levels based upon S.O on growth and food productivity and body composition of *H. severus* ornamental species. The results of this study suggest that fish fed diets containing 8% oil ($D_3$) with a higher weight gain showed better specific growth rates than other groups. It indicates that nutrients available in the diet $D_3$ provide the nutritional requirements needed for faster growth of severum, compared to other groups. It means that increase in the amount of oil and energy in the diet of treatment ($D_3$) decreases protein of tissue and raises fat. In other words, it increases the fat of tissue. Piedecausa *et al.* (2007) reported that the highest level of fatty acids, 18: 2n-6 in the S.O can increase fat storage in the fish body, and also Ng *et al*. (2003) claimed that a high content of fatty acids in the diet leads to increasing the storage of these acids in muscle tissue. Hence, we can say that there is a positive association between dietary fat and fat content of total body for many fish species (Chou and Shiau, 1996; Du *et al*., 2005; El-Marakby, 2006; Martins *et al*., 2007).

Fish fed with diets of 12% ($D_5$) showed the lowest SGR which indicates that the diet did not have the adequate fat for growth and also there is an imbalance between fat and protein content. In the present study, the maximum value of CGR was in fish fed diets of 8% oil ($D_3$) that is similar to the study which was carried out by Deng *et al*. (2013) on Pacific threadfin (*P. sexfilis*). The highest SGR was obtained in S.O 8%. Since SGR decreases with increasing fish size, lower values observed for specific growth rate (SGR) in some studies can be explained by the evolution of fish (Sunde *et al*., 1998).

Peng *et al*. (2008) reported the amount of weight gain for young fish of black marine wire (*Acanthopagrus schlegeli*) fed diets of 9% S.O was 2.20 g. In this study, the best FCR for Severum was achieved using diets of 8 and 10% oil. The potential reason which can be considered for better growth of $D_3$ and $D_4$ is that in these are the best diet sources of nutrients to be consumed by fish. In this work, the highest FCR was for fish fed with the diet of 12% S.O which was similar to the results obtained in the investigation of Ricardo *et al.* (2001) on the performance and composition of fatty acids of fish Surubim (*Pseudoplatystoma coruscans*) fed with vegetable oils and animal.

The highest FCR was observed in treatments fed a diet containing 12% S.O and other vegetable oils tested (Corn oil and Linseed oil). In this research, the percentage of weight gain rises along with the increase of fat to the level of 8%. Subhadra *et al*. (2006) suggested that changes in fish growth can be the result of a combination of diets and the length of feeding period. In this study, by increasing oil level from 8 to 14%, weight loss was observed and growth factors had no significant differences. So, we can say that the use of this amount of S.O in the diet of *H. severus* is not desirable. Lachman and Brown (1997) in their study found that the average weight gain in goldfish (*Carasius auratus L.*) fed a diet containing 4% S.O for a period of six weeks cannot show a significant difference. In this study, the largest percentage of length gain (LG) was in fish fed diets of 8% oil. By contrast, Jiang *et al.* (2012) found that catfish (*P. vachelli*) fed diets containing different levels of S.O did not show any significant differences in growth parameters (weight and height) after 80 days of test period. In this work, the condition factor was higher in fish fed diets of 10% oil ($D_4$) in comparison with the other treatments. The cause of high CF in this treatment could be because of the lower amount of protein and higher amount of fat in the body of Severum fish which was involved. As a result, by increasing body weight, because of the lower effect of protein on bone growth, it did not have good growth (Vesal *et al.*, 2016). In this experiment, CF in fish fed the diet of 12% S.O ($D_5$) was lower compared to other treatments.Deng *et al.* (2013) found that the CF was the lowest for pacific threadfin fish (*P. sexfilis*) fed diets containing 8% S.O which showed significant differences that were not in accordance with the findings of this research. Regost *et al.* (2003) in their study observed a significant difference between the final weight and growth rate of Turbot fish (*P. maxima*) fed diets of S.O 9%. They found that the treatments fed diets containing vegetable oils cannot only have a significant impact on the growth of the fish. In this study, the maximum amount of voluntary food intake (VFI) was in fish fed the diet containing 12% oil ($D_5$) which had the lowest growth rate and the highest FCR.

Peng *et al*. (2014) investigated the growth parameters in the Turbot fish (*Scophthalmus maximus L.*) by replacing the different levels of fish oil with S.O, and found that the use of 7.5% S.O in the diet had an intermediate effect on food efficiency and food consumption of this fish, compared to other diets tested (mixed 1:3 fish oil+2:3 S.O and vice versa). Also, fish fed the lowest level of S.O had the highest food intake (FI) in comparison with the other groups ($p$<0.05).

Previous studies have shown that fish are able to adjust their food consumption based upon diet quality using a self-feeding device when the time of food availability is not long enough. Accordingly, feeding with a diet, which is faced with a weak adoption, has often been rejected and we can say that this nutritional behavior of fish is responsible for the amount of uneaten food (Vesal *et al*., 2016).

There were no signs of disease among fish. However, the lowest survival rate was obtained in the diet containing 12% oil ($D_5$). But, due to very little differences, it seems that the mortality rate is independent of the fat level in the diet. It has been reported that the use of S.O in the diet of *A. schlegeli* cannot have any effect on the survival of this species (Peng *et al*., 2008).

In this study, the efficiency growth of *H. severus* fed different levels of S.O in the diet showed that the optimal level of fat for this species is using 8% of this oil. The present investigation offers a preliminary approximation of the lipid requirements of *H. severus* regarding the use of S.O. Nonetheless, according to the current results, the quantitative necessities of essential fatty acids have not been evaluated and require further investigations.

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