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Growth parameters in juvenile gold severum (*Heros severus*) fed diets containing fish oil and soybean oil

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Abstract: The aim of the present study is evaluating the effect of different dietary lipid sources (in a 1:1 ratio for fish and soybean oil) in diet of gold Severum *(Heros severus)* juvenile, an ornamental fish, based on growth performance and feeding efficiency. In this study, fish were divided to 7 experimental groups including 6 Groups fed with diets containing (T_1 (3), T_2 (5), T_3 (8), T_4 (10), T_5 (12), T_6 (14) %) of oils blend and a control group (CG) (receiving oil-free diet). 441 fish with initial average weight and length of 0.7 ± 0.12 g and 2.4 ± 0.14 cm, respectively, were divided into 21 aquariums randomly and fed with experimental diets for 85 days. Biometry was done once every 15 days. The results showed that T_3 (8% oils blend), had the highest WG, LG, SGR and DGR, significantly different with the other treatments ($p<0.05$). The survival rate in all treatments was over 85% in all test groups and showed no significant difference ($P>0.05$).The results of this study which were focused on the growth and development of ornamental fish *H. severus* fed with different levels of this type of mixed oils propose that the optimal level in their diet is 8%.

Keywords: Gold Severum (*Heros severus*), Fish oil, Soybean oil, Diet formulation

Introduction

The industry of ornamental fish is rearing parallel to aquaculture activities around the world seem to grow ever increasing and be pleased by many societies as an amusing occupation. More than 1000 species of freshwater specimens belong to 100 families are among the vast list of commercial ornamental fish (FAO, 2003). In this regard, nutrition and preparing feed are two important aspects of the whole procedure of having a healthy, colorful residents in aquarium, also with normal behavior (Erdogan *et al*., 2012); Although, compare to the commercial fish industry, little is known about the nutritional needs of ornamental fish (Kruger *et al*., 2001; Miller and Mitchell, 2008; Güroy *et al*., 2012). By the way, the optimal feed formulation which fulfilling all the nutritional needs of the fish should be modified in a way to have the potential of being to manufactured (Halver and Hardy, 2002). Fish meal and fish oil are two important ingredients which used to supply the essential nutritional needs including EAAs and EFAS; but regard to the statistics, the extraction and production volume of these, reduced significantly during the past decades and the aquaculture industry could not count on these two sources as a reliable rise; the researchers not only warn about the decreasing of fish resources as an important ingredient for animal feeding industry, but also seeking for substitutions among a variety of ingredients (Tocher, 2015).

In this circumstance, ongoing researches are concerning on the possibility and feasibility of replacing fish meal and fish oil with other herbal substitutions are followed up (Turchini *et al*., 2011). Parameshwaran *et al*., (2002) evaluating the effect of replacing cod liver oil with soybean oil and coconut oil on *Carassius auratus L*. larvae; which determined that the replacement does not affect the growth performance through the low levels of PUFA needed by specimens, which these levels could be also provided by synthesizing LC-PUFAS from C18-PUFA.

Lochman and Brown (1997) investigated the effect of feeding *Carassius auratus* with a diet containing four percent cod liver oil and four percent soybean oil for a period of six weeks; it is revealed that the mean weight gain does not show a significant difference among the treated groups and the control group which fed by a diet containing only fish oil. In 1997, the effect of applying canola oil in the diet of *C. aratus* was also observed and specified that applying canola oil as a complete substitution for fish oil does

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not suppress the growth rate of larvae (Pozemick and Wiegand., 1997). The obtained results were similar to the results presented by Parmaeshwaran *et al*. (2002) who worked on the replacement of fish oil by vegetable oils in the diet of gold fish larvae. The efficacy of using soybean oil and the mixture of soybean and fish oil were being to test on Tread fin fish *(Polydacty lussexfilis)* and the outcomes revealed that feeding *Polydacty lussexfilis* through a diet formulated by using 8% soybean oil could provide all the nutritional needs of fish (Deng *et al*.,2013). The applied diet contains 0.48% EPA, 0.44 % DHA and 1% n-3LC-PUFA.

Turbot (*Psetta maxima*) was also fed with a diet contain equal share of (9%) soybean oil and fish oil and the results show that there is a significant difference between the final weight and growth rate of treated fish and the control group which fed the fish oil diet (Regost *et al*., 2003); although this reduction in growth performance was relatively low. The diet mixture of fish oil and soybean oil was also studied in diet prepared for Seabream juveniles who indicated; that a proportion of 50 percent replacement of fish oil by soybean oil in diet of treated sea breams over a period of 92 days did not affect the growth trend (Peng *et al*., 2008).

The aim of the present study, with respect to the high popularity of *Heros severus* (gold Severum) as a freshwater member of many aquariums, was to formulated a practical diet regards to determine the optimum level of lipid by mixing soybean oil and fish oil, considering the growth performance and feeding efficiency of fish during the trial.

Materials and methods

Fish

In the present trial 441 specimens of *Heros severus* were used, with initial average weight and length of 0.7 ± 0.12 g and 2.4 ± 0.14 cm, respectively. The trial conducted based on introducing 7 experimental groups including 6 groups were fed with diets containing (T_1(3), T_2(5), T_3(8), T_4(10), T_5(12), T_6(14)%) of oils blend (in a 1:1 ratio of fish and soy oil) and a control Group (CG) which were fed with diet contain no oil (Tab.1). In all groups, each treatment (different percent of oil) considered as triplicates. Specimens were randomly divided in 21 aquarium with 50 liter volume (50×40×30 cm), and after ten days of acclimatization, the fish were fed with prepared diets for a period of 85 days.

Feed preparation

The groups of diet with equal ratio of protein (g) / Energy (kcal) and different levels of a mixture of equal share of the two oils (fish and soybean oil) were formulated by win feed 2.8 software (Cambridge, UK) and prepared by using of ingredients listed in table 1 as follow:

The dried, grinded- sieved (1mm in size) ingredients based on the formula were mixed and the oils blend and water were added to form dough. The prepared dough was pelleted through a 1mm die of a meat grinder and ultimately the pellets oven dried for 8 hours at 60°C, cooled in strilled condition, bagged and stored in refrigerator until usage. Approximate analyses were conducted to ensure that the prepared diets having the expected quality (Tab.1).

Tab. 1: Composition of experimental diets containing different levels of mixture of fish oil and soybean oil (1:1 ratio) used for feeding *Heros Severus* for 85 days.

	Experimental diets						
Ingredient (%)	**T_1 (3%)**	**T_2 (5%)**	**T_3 (8%)**	**T_4 (10%)**	**T_5 (12%)**	**T_6 (14%)**	**CG**
Fish meal	18.9	19	19.3	19.4	18.3	16.8	19.1
Wheat meal	14.3	13.5	12.2	11.4	7.5	2.7	15.2
Wheat gluten	20.2	20.5	20.9	21.2	21.2	21.2	19.9
Soybean meal	17.5	17.1	16.5	16.1	19	22.8	17.5
Oils blend	1.5	3.6	6.7	8.8	11.1	13.4	-
Vitamin premix	2	2	2	2	2	2	2
Mineral premix	1.5	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	10.5
Dicalcium phosphate	13.6	12.3	10.4	9.2	9	9.1	14.2
	Proximate analyses (%, DM)						
Dry matter	90.19	90.51	91.25	91.55	92.43	92.86	90.56
Crude protein	43	44.65	45	45.75	46.45	46.65	43.20
Crude fat	5.99	8.54	11.31	12.65	14.50	15.43	4.44
Ash	16.45	15.65	13.95	13.45	13.05	12.90	17.20

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

To maintain the water quality, bio filters and a central oxygen pump and heaters were used in aquarium, 50 percent of water in each aquarium was replaced every three days at the beginning and every five days at the end of the trial. Temperature, dissolved oxygen, pH, ammonia were measured daily (Tab. 2).

Tab. 2: Daily average of physicochemical parameters of water during the trial.

Parameter	Extent of variation
pH	6.6 – 7.6
Temperature	29±1°c
Dissolved Oxygen	6.6 – 8.1 ppm
Nitrite	0.01mg/l
Total Hardness	210mg/l

During the rearing period all the groups were fed three times a day (6 a.m., 12 a.m., and 6p.m.) at the base of 4 percent of the biomass and also with respect to their eagerness and appetite. For evaluating the growth and feeding performance, biometry was done once every 15 days. The weight was measured by using of digital balance (bearing 0.01g) and the length were obtained by using of caliper; 12hours before conducting biometry feeding of fish were cut to reduce and avoid stress and oxygen depletion.

Growth and feeding performance regard to the obtained data were measured by calculating the equations as bellow:

- Feed conversion ratio; FCR = Feed intake (g)/weight gain (g).
- Specific growth rate (%); SGR_W= (ln final body weight_ ln initial body weight/days of experiment) ×100.
- Specific growth rate (%); SGR_L= [(ln final body length- ln initial body length)/days of experiment] ×100.
- Specific growth rate (%); SGR_W = [(ln final body weight - ln initial body weight) / days of experiment] ×100
- Weight gain (g); WG= [(Final weight-initial weight) / initial weight] ×100.
- Survival rate (%); SR = (Final fish number/Initial fish number) × 100.
- Condition factor; CF= [Final weight (g) / fork length (cm)]3 × 100.
- Daily growth rate (g); DWR = [(Final body weight - initial body weight) / initial body weight] × days.
- Voluntary feed intake; VFI = [Dry feed intake / (initial + final fish biomass)] / 2

Tissue analysis

At the end of the trial with respect to the best and worst growth performance, FCR, CF and VFI, five specimens selected randomly from T_1, T_2, T_3, T_4, T_5, T_6 and CG respectively and transferred to the laboratory for carcass analysis. The content of crude protein and crude lipid were determined through Kjeldahl and Soxhlet procedure (AOAC, 1990), respectively. The moisture content was also measured regards to AOAC (1990) (Tab. 3).

Tab. 3: Proximate composition (%) of whole body of *Heros severus* fed diets containing different levels of mixture of fish oil and soy oil (1:1 ratio).

	Experimental diets (%)						
Parameters	T_1 (3%)	T_2 (5%)	T_3 (8%)	T_4 (10%)	T_5 (12%)	T_6 (14%)	CG
Crude protein	67.17 (±0.26)	66.20 (±0.21)	64.05 (±0.38)	64.95 (±0.15)	65.30 (±0.23)	65.88 (±0.15)	65.1 (±0.38)
Crude lipid	16.20 (±0.42)	18.82 (±0.17)	19.50 (±0.23)	24.56 (±0.33)	25.50 (±0.11)	26.85 (±0.23)	8.9 (±0.25)
Moisture	26.40 (±0.18)	26.50 (±0.31)	26.70 (±0.46)	25.60 (±0.27)	25.70 (±0.12)	26.35 (±0.19)	25.1 (±0.52)
Ash	5.95 (±0.43)	6.30 (±0.13)	6.80 (±0.28)	6.95 (±0.33)	7.20 (±0.21)	7.35 (±0.18)	7.70 (±0.33)

Statistical analysis

The obtained data with normal distribution, pretested through Kolmogorov –Smirnov, were analyzed by One-way ANOVA path and probable differences between treatments being compared by post-hoc LSD at $p<0.05$ confidence level. The content of crude protein, crude lipid and moisture were compared through one_sample t-test.

Results

Growth, feeding performance and Survival rate of *Heros severus* fed with diet containing different levels of oils blend (in 1:1 ratio of fish and soy oil) are presented in Table 4. The percent of weight gain

which affected by final weight at the end of the trial as the initial weight of specimens did not have a significant difference at the beginning of the trial which was different between the treatments at the 95 percent confidence level. As it is shown in table 4 the maximum weight gain was observed in fish fed with diet contained 8 percent mixture oils(T_3), the maximum specific growth rate (SGR_w) was also belonged to T_3 ($p<0.05$). While the lowest SGR_w was calculated for the fish fed with diet contain 12 percent oil (T_5). The highest amount of daily growth rate (DGR) belonged to T_3 which was significantly higher, compared to T_1, T_2 and T_4 and the lowest increment was observed in T_5 ($p<0.05$). The best feed conversion ratio was calculated for fish fed with diet contain 8 percent oil (T_3) at the end of the trial; and the worst FCR belonged to T_5 (diet contain 12 percent oil). At the end of trial the survival rate of all treatment were calculated up to 85 percent with no significant difference ($p>0.05$) between the groups, and also no sign of disease were reported.

Tab. 4: Growth parameters of *Heros severus* fed the experimental diets containing different levels of mixture of fish oil and soybean oil (1:1 ratio) for 85 days.

Growth parameters	3%	5%	8%	10%	12%	14%	CG
FCR (%)	1.52 (±0.05^d)	1.78 ±0.23^c)	1.32 (±0.01^d)	1.91 (±0.06^b)	2.58 (±0.3^a)	1.91 (±0.02^b)	1.64 (±0.06^e)
SGR_W (%)	22.39 (±1.44^b)	15 (±2.75^c)	25.57 (±0.56^a)	12.52 (±1.09^c)	7.56 (±1.4^e)	10.85 (±0.3^d)	15.53 (±1.73^c)
SGR_L (%)	61.38 (±7.52^b)	55.75 (±24.1^c)	63.66 (±2.53^a)	50.24 (±9.68^d)	46.34 (±18.5^f)	49.39 (±15.52^e)	42.19 (±24.02^f)
In. W. (g)	0.7 (±0.12^a)	0.7 (±0.12^a)	0.7 (±0.12^a)	0.7 (±0.12^a)	0.7 (±0.12^a)	0.7 (±0.12^a)	0.7 (±0.12^a)
Final W. (g)	20.86 (±1.54^b)	14.8 (±1.89^c)	23.46 (±2.33^a)	12.76 (±2.12^d)	8.7 (±1.5^f)	11.4 (±2.33^e)	16.33 (±1.75^d)
In. L. (mm)	24 (±0.14^a)	24 (±0.14^a)	24 (±0.14^a)	24 (±0.14^a)	24 (±0.14^a)	24 (±0.14^a)	24 (±0.14^a)
Final L. (mm)	36.01 (±1.36^a)	34.98 (±2.02^b)	36.54 (±2.85^a)	33.9 (±1.78^c)	33.13 (±2.38^c)	33.73 (±2.83^c)	32.32 (±1.8^d)
SR (%)	100 (±0.00^a)	93.33 (±5.43^a)	100 (±0.00^a)	100 (±0.00^a)	86.66 (±8.42^b)	93.33 (±5.43^a)	100 (±0.00^a)
CF (%)	14.41 (±1.29^b)	12.72 (±2.73^d)	14.74 (±0.11^a)	14.75 (±2.23^a)	11.73 (±3.29^e)	13.53 (±2.06^c)	13.21 (±3.24^d)
VFI	62.50 (±2.33^d)	73.31 (±9.42^c)	54.03 (±0.47^e)	78.33 (±2.57^b)	105.94 (±12.60^a)	78.53 (±0.9^b)	62.62 (±2.81^c)
DGR (g)	72.10 (±0.59^b)	67.53 (±2^c)	73.25 (±0.17^a)	65.78 (±1.12^d)	57.68 (±2. 84^e)	64 (±0.38^d)	68.27 (±0.81^b)

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

Regard to the results of statistical analysis (Table 4), fish belongs to T_3 had also higher length specific growth rate (SGR_L) compare to other treatments ($P<0.05$); the lowest SGR_L obtained for fish fed 12 percent oil (T_5). Condition Factor index was also revealed that the ratio of weight / length in fish fed with 10(T_4) and 8(T_3) percent oils blend had the maximum amount, respectively and the lowest value belongs to treatment fed with 12 Percent oil (T_5) ($P<0.05$). With respect to the results, it is obvious that applying 8 percent oils blend (soy and fish oil (1:1)) in formulating diet for *Heros severus* would be the best condition for rearing fish in aquarium and have an ideal growth pattern and also having the lowest FCR (1.32±0.01) while the fish try to consume less feed (VFI= 54.03±0.47) compared to other treatments in the groups and ultimately it has the best ratio of Weight / Length. The results show that consuming 8 percent mixture oils (soy and fish oil) has the best effect on *Heros severus* growth pattern but condition factor does not support the trend and it was observed that the specimens gained more weight compare to length increment; but the evaluated FCR had the best condition between the other treatments (1.32 ± 0.01). As a conclusion the best growth performance was observed for T_3 (consuming 8 % of oils blend). Moreover, the best condition factor was evaluated for treatment T_3 which had significantly different with other treatment ($p<0.05$).

Discussion

The present study was conducted to determine the

optimum level of lipid using fish oil and soybean oil (oils blend 1:1 ratio) in diet of gold Severum *(Heros severus)* juvenile, an ornamental fish, based on growth performance, survival rate and feeding efficiency. The results indicated that fish fed diet containing 8 percent oils blend had the best growth performance compared to other treatments. The obtained results through literature revealed that in the case of consuming diet with P:E (Lipid) ratio of 0.9 (Akiyama *et al*., 1997; Wilson, 2002) the source of oil could affect the growth performance significantly which can be due to bioavailability of essential fatty acids especially n-3 long chain fatty acids, but this concept should be considered more as the results of carcass analysis showed that the share of crude lipid content in tissue muscle increase at the maximum level of 19.50 percent and the crude protein adversely decreased to 64.05 percent in T_3 compare to $T_1$and T_2. In other words, increasing in weight gain could be explained through increment of fat content of the tissues. Piedecausa *et al*., (2007) reported that 18: 2 n-6 content of soybean oil could elevated the fat proportion of fish tissue. The increment of fat composition of tissue because of consuming diets with high proportion of fatty acid was also confirmed by Ng *et al*., (2003). The effect of consuming different sources of oil on carcass composition of fish was also reported by Sener and Yildiz (2003) and Almaida-Pagan (2007).

The fish fed with diet contained 12 percent lipid (consist of equal share of fish oil and soy oil) had the lowest SGR, this could be a caused of an imbalance in the content of n-3 and n-6 essential fatty acids. This condition improved as the lipid content of the diet decreasing to 8 percent of the oils blend. It shows that the optimum level of lipid could be altered with respect not only to the ratio of protein: Energy (lipid) (Shapawi *et al*., 2014), but also to the fatty acid composition. The maximum value of SGR was calculated for thread fin fish fed with diet contain 8 percent soybean oil (Deng *et al*., 2013). Peng *et al*., (2008) reported that the weight gain of Black sea bream fed with diet containing mixture of 3.6 percent fish and 5.4 percent soybean oil was equal to 3.13 gr; which is concord with the present results, as the possibility of replacing soybean oil with fish oil verified at the level of 8 percent mixture of 50% of the two oils. This verification was also approved regard to the FCR as the best ratio obtained for the aforementioned Treatment (T_3).The explanation of obtaining a good growth performance and FCR for T_3 in the present study, could be the provision of proper balance of n-3 / n-6 F.A. which absorb by fish tissue. Furthermore, Peng *et al*., (2008) observed that there was no different between the amounts of weight gain of black sea breams' fed on diet contain 100 percent fish oil and those fed on diet contain 40 and 60 percent fish oil and vegetable oil, respectively. In this regard, feeding darkbarble with a diet contain a mixture of fish oil and soybean oil had a positive effect on growth performance of the specimens compared to those which fed on diets contain fish oil or soybean oil, separately (Jiang *et al*., 2013). But it should be considered that not only the composition of diet (Subhadra *et al*., 2006) but also the species will affect the results of oil substitution as Chou and Shiau (1996) represented a positive effect of feeding tilapia hybrids (*Oreochromis niloticus x Oreochromis aureus*) with diet contain a blend of fish oil and vegetable oil, but this result did not achieve for Nile tilapia (Trushenski *et al*., 2009; Szabó *et al*., 2011).

The condition factor of fish fed with 8 percent mixture oils (T_3) also approved the results of carcass analysis, as the condition factor of this treatment was significantly higher than the other treatments, which means the lower amount of protein and higher amount of fat in the body of *Heros severus* which was involved (Vesal and Vosooghi., 2016). The measured condition factor for thread fin tail fed by diets contain an equal proportion (4 percent) of Pollack oil and soybean oil and also for treatment fed by diet contain 8 percent soybean oil indicate that condition factor has the lowest value compared to the other treatment (Deng *et al*., 2013), which is not concordance with the present study. The results indicated that increasing the content of oil in diet from 8 % to 14 % oil did not affect the growth performance of fish. The same result obtains for Black sea bream (possibility of replacing 50% of fish oil with vegetable oil) (Peng *et al*.,2008).The replacement of fish oil, more than 60 percent, by vegetable oils in the diet of European bass was also successfully reported by Montero *et al*.(2005). Moreover this replacement in the diet of gold fish larvae could be done completely by using of soybean or coconut oil (Parameshwaran *et al*., 2002). The results of the present study are in concordance with results acquired after replacement of fish oil with soybean oil in the diet of darkbarble *(P. Vachelli)*; this diet did not have an adverse effect on growth performance of this member of cichilidae family after 80 days of rearing.

The diet containing mixture of fish oil and soy oil

(1:1 ratio) in Turbot diet was also caused an increment in final weight and growth performance of fish; moreover survival rate did not have a significant difference among the treatments. However, the maximum loss was observed in the group fed with 12 percent soy oil, it revealed that oil replacement does not have any effect on survival rate of fish (Regost *et al*., 2003); the results in the present study also show 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% oils blend; but given the lack of significance in the difference between mortality rates, it seems to be independent from the dietary lipid level. The alteration of oil source did not also affect the survival rate of Thread fin fish (Peng *et al*., 2008).

Conclusion

As a conclusion the best growth performance and feeding indices were observed for the treatment used diet containing 8 percent oil; so 8 percent mixture of fish oil and soybean oil (1:1 ratio) could be reported as the optimum level of oil need to be supply for preparing diet for *Heros severus*. The present research provides a preliminary estimation of lipid requirement of *Heros severus* regards to use of mixture of fish oil and soybean oil (1:1 ratio); however quantitative requirements of essential fatty_ acids cannot be determined based on the current results; and this will need further investigation.

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