

## The comparison of growth, survival rate and behavior of Oscar fish (*Astronauts ocellatus*) fries fed by *Artemia urmiana*, earthworm and beef heart supplemented diets

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### Abstract

This study was conducted to evaluate the effect of different fresh feed supplemented to commercial diet on growth and survival of Oscar fish (*Astronauts ocellatus*) (initial weight: 1.58-1.70 g) during 60-day period. The husbandry environment consisted of glass aquariums appropriate to growth with a photoperiod cycle of 12 L: 12 D. The water temperature was kept at  $28 \pm 1^\circ\text{C}$ . 360 fries of Oscar (initial weight: 1.58-1.70g) were randomly distributed in four treatments with three replicate aquaria per treatment fed by different diets including: 1: commercial concentrate (crud protein: 46-54%, crud fat: 11–15 %, maximum moisture: 11 %), 2: commercial concentrate feed + chopped earthworm, 3: commercial concentrate feed + adult *Artemia urmiana* and 4: concentrate feed + chopped beef heart. Daily feeding was performed at a rate of 3 % body weight. The results showed that *Artemia*, earthworm and beef heart used as additive feed to commercial concentrate diet significantly increased growth indices compared to the concentrate feed (control diet) ( $p < 0.05$ ). The lowest survival rate was related to the treatment fed by earth worm ( $70.53 \pm 2.67$  %) ( $p < 0.05$ ), however, no significant difference was observed between treatments fed by *Artemia*, earthworm and commercial diet ( $p > 0.05$ ). The number of total bacteria, lactic acid bacteria, mesophyll bacteria and enterobacteriaceae were higher in fish fed with earthworm compared to the other groups. Aggressive behavior (take out the eyes of other fish, tearing and biting the caudal fins of smaller fish) was observed in the group fed with earthworm. In conclusion, feeding with *A. urmiana* and beef heart additives is recommended for Oscar fish due to their highest survival rates and growth indices.

**Keywords:** *Artemia urmiana*, *Astronauts ocellatus*, Feeding, Fresh feed

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## Introduction

Ornamental fish farming is one of the highest value added fisheries activities worldwide (Ahmadifard *et al.*, 2016) which can lead to economic growth in developing countries (Olivotto *et al.*, 2006). In Iran, the development of aquaculture industry and the growing public interest in ornamental fish have led to the production, trade and export of ornamental fish in a thriving market. The amount of ornamental fish production in Iran was about 31 million fish in 2005, which in 2017 increased to more than 244 million fish (Ghorbanzadeh and Nazari, 2013; Ghorbanzadeh and Nazari, 2017). Oscar (*Astronauts ocellatus*, Agassiz, 1831), belongs to the cichlid family, is native to the Amazon River basin including Brazil, Peru, Colombia and Guinea (Tavares-Dias and Nevis, 2017). This fish is very popular and of great economic interest among aquarium fish lovers and sport fisheries in different countries of the world due to its attractive color and natural reproduction in captivity. Oscar is an omnivorous species, although its diet consists mostly of fish, crustaceans, gastropods and aquatic insects larvae (Froese and Pauly, 2017; Tavares-Dias and Nevis, 2017). In addition to environmental and managerial factors, adequate feed supply is an essential factor and its optimization can have a significant impact on fish increasing growth, survival and reducing production costs (Raseduzzaman *et al.*, 2014). Numerous researchers have investigated the effects of different diets on growth, survival and reproduction of

different fish species (Keramat Amir Kolaei and Ebrahimi, 2008; Kasiri *et al.*, 2012; Raseduzzaman *et al.*, 2014; Seidgar *et al.*, 2015). However, little information is available on nutritional demands of breeding and early stages of ornamental fish.

*Artemia* is used as live food with appropriate size (depending on its life cycle phase), easy for consumption and transportation in the aquarium trade and proper for breeding marine and freshwater fish and crustacean larvae (Lim *et al.*, 2001). While the adult form of *Artemia* is primarily used as a frozen or dry frozen food in the aquarium business, the nauplii is used exclusively in breeding farms (Tamaru *et al.*, 2001). To minimize the risk of pathogens, most ornamental fish breeders in Singapore use healthier *Artemia* nauplii instead of moina to feed their fish (Lim *et al.*, 2003; Koru and Turkmen, 2014). Earthworm has also become popular as a source of protein, essential amino acids especially lysine or a tool for organic waste management (Kasiri *et al.*, 2012). Earthworm biomass (*Eisenia foetida*) with high crud protein (52-70% dry weight), lysine and methionine essential amino acids, simple and low cost production, different sizes in different periods of life, is a suitable food for aquatic animal's nutrition (Fadaee, 2012; Ahmadifard *et al.*, 2016). Earthworm has been used at low levels in *Macrobrachium rosenbergi* culture to accelerate spawning (Correia *et al.*, 2002), white leg shrimp *Litopenaeus vannamei* post larvae to increase growth (Apolinario *et al.*, 1998), *Oncorhynchus*

*mykiss* (Velasquez *et al.*, 1991; Oscar-Pereira and Gomes, 1995), *Cyprinus carpio* (Nandeeshia *et al.*, 1988) and African catfish (*Clarias gariepinus*) (Dedeke *et al.*, 2013) to improve growth performance. Minced beef heart is also used as a main food in carnivorous aquarium fish. However, compared to other foods, it results in a rapid decline in water quality (Heidary and Akbary, 2013). Mohammad Nejad Shamoshaki *et al.* (2011) revealed that fish fed with beef heart had higher growth and survival rate compared to those fed with bloodworms, compact worms, *Artemia* and *Gammarus*. The aim of this study was to compare the effects of *Artemia urmiana*, earthworm, beef heart and a commercial feed on the growth, survival rate and behavior of Oscar (*Astronauts ocellatus*) fries.

## Materials and methods

### *Growth and survival characteristics*

The experiments were performed in accordance with national and research guidelines for keeping and studying animals. Tiger Oscar fish (N=360, initial weight: 1.58-1.70 g) were reared in Azarmahi ornamental fish farm (Gowgan, East Azarbaijan Province, Iran) for 60 days in identical and controlled conditions in four feed treatments, each treatment in triplicate (12 aquaria with dimensions of 120×60×45 cm watered with 40 cm depth). The study treatments were as follows: group 1 (commercial feed), group 2 (67% commercial feed and 33% earthworm), group 3 (67% commercial feed and 33% *A. urmiana*) and group 4

(67% commercial feed and 33% beef heart). The commercial food belonged to 21 Beyza Company. Frozen earthworm was purchased from Nademi Vermicomposting Company and stored at -20°C. *Artemia* biomass was obtained from National Artemia Research Center (Urmia, Iran) and stored at -20°C. Beef heart was bought from the market and after separating its fats; it was cut into pieces and kept in -20°C until experiment. In all treatments temperature, pH (5.5-7), dissolved oxygen (7–8 mg/L), aeration and other factors were provided under optimal conditions. In order to prevent the accumulation of ammonia and other toxic compounds, one third of the water in the aquariums was exchanged every 4 days and in all treatments the uneaten feed was siphoned daily before feeding. During the rearing period, water temperature was kept in 28±1°C, and the light regime was adjusted to 12 L: 12 D. Daily feeding was performed in three meals at 3% body weight rate, so that they consumed food in five minutes. Growth indices were recorded every two weeks. Daily mortality was recorded to determine the survival rate. The following formulas were used in the calculations (Mehrad and Sudagar, 2010; Ahmadifard *et al.*, 2016)

Weight gain (g) = final weight - initial weight

Increase in length (mm) = final length - initial length

Percentage of growth rate = (final weight - initial weight) ÷ initial weight × 100

$\frac{\ln W_2 - \ln W_1}{t_2 - t_1} \times 100 = \text{SGR}(\%)$

Where, W=weight in g,  $t_2$ = end time (day),  $t_1$ = initial time(day)

$$K = (W / L^3) \times 100$$

Where, K=condition factor, W= weight in g, L= total length(cm)

Survival rate%= (number of fish at the end of the experiment ÷ number of fish at the beginning of the experiment) ×100

### *Gut bacterial flora*

To determine the changes in microbial population in the gut, three fish were randomly selected from each aquarium at the end of the study. In order to prevent stress, clove powder (150 ppm) was used for anaesthesia. Immediately after death of the fish, intestines of fish were separated and 1 ml of their contents was removed using a sampler and transferred to a tube containing phosphate buffer and mixed well. Plate count agar was used to determine the total number of gut bacteria (incubation for 48 hours at 37°C), mesophilic bacteria (incubation for 48 hours at 37°C) and psychrotrophic bacteria (incubation for 10 days at 7°C). Violet Red Bile Lactose Agar was used for measuring the number of enterobacteriaceae (incubation for 24-48 hours at 37°C), and MRS Agar was used to determine the number of acid lactic bacteria (incubation for 24-48 hours at 37°C). Colony counting method was used for enumeration of these species. Prototypes of -1 to -7 dilutions were prepared. Phosphate buffer solution was used as the diluent. In this way, 7 test tubes with 9 ml buffer were prepared. 1 ml of the prototype containing 1 g of sample was soaked in 10 ml buffer using

a pipette, transferred to tube no.1 and thoroughly mixed. This was repeated until dilution 7 (Miller and Wolin, 1974).

### *Statistical analysis*

The data were obtained from each treatment in triplicate and the results were presented as mean ± SE. Data were analyzed using SPSS 22 software. The normal distribution of data was checked using Kolmogorov-Smirnov test and the homogeneity of variances was checked using Levene test. Analysis of Variance (ANOVA) followed by Duncan's test was used to identify statistical differences among different treatments. The correlation between length and weight of fish in different treatments was carried out using Pearson correlation test. P value less than 0.05 was considered statistically significant.

### **Results**

The results of Oscar fish biometry during the study period are shown in Table 1. At the beginning of the study, total length and weight of fish did not vary significantly ( $p > 0.05$ ). Subsequently, feeding the fish with earthworm, *Artemia* and beef heart additives significantly increased the length and weight of the fish compared to commercial concentrate diet ( $p < 0.05$ ). Also, during the first 30 days of the study, earthworm treatment showed better performance compared to *Artemia* and beef heart treatments ( $p < 0.05$ ), however, there were no significant difference between the

*Artemia* and beef heart treatments ( $p>0.05$ ). At the end of the study (Day 60), maximum length and weight were observed for beef heart treatment ( $p<0.05$ ).

**Table 1: Comparison of total length and weight (Mean  $\pm$  SE) of Oscar fish in different treatments during the study period.**

| Variable    | Treatment                               | Day                           |                               |                               |                               |                               |
|-------------|---|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|             |   | 1*                            | 15                            | 30                            | 45                            | 60**                          |
| Length (mm) | commercial concentrate feed             | 28.52 $\pm$ 1.23 <sup>a</sup> | 35.00 $\pm$ 0.64 <sup>a</sup> | 43.54 $\pm$ 0.64 <sup>a</sup> | 51.57 $\pm$ 1.03 <sup>a</sup> | 64.53 $\pm$ 1.49 <sup>a</sup> |
|             | commercial concentrate + <i>Artemia</i> | 28.97 $\pm$ 1.28 <sup>a</sup> | 41.13 $\pm$ 1.41 <sup>b</sup> | 51.16 $\pm$ 1.07 <sup>b</sup> | 58.93 $\pm$ 1.32 <sup>b</sup> | 73.54 $\pm$ 1.39 <sup>b</sup> |
|             | commercial concentrate + earth worm     | 28.94 $\pm$ 1.28 <sup>a</sup> | 45.56 $\pm$ 1.06 <sup>c</sup> | 56.96 $\pm$ 1.13 <sup>c</sup> | 66.00 $\pm$ 1.41 <sup>c</sup> | 73.18 $\pm$ 1.64 <sup>b</sup> |
|             | commercial concentrate + beef heart     | 28.80 $\pm$ 1.25 <sup>a</sup> | 38.83 $\pm$ 1.40 <sup>b</sup> | 52.22 $\pm$ 1.61 <sup>b</sup> | 63.98 $\pm$ 0.77 <sup>c</sup> | 78.77 $\pm$ 1.40 <sup>c</sup> |
|             | <i>P</i> value                          | 0.99                          | 0.00                          | 0.00                          | 0.00                          | 0.00                          |
| Weight (g)  | commercial concentrate feed             | 1.58 $\pm$ 0.10 <sup>a</sup>  | 1.91 $\pm$ 0.03 <sup>a</sup>  | 2.35 $\pm$ 0.05 <sup>a</sup>  | 2.84 $\pm$ 0.08 <sup>a</sup>  | 4.60 $\pm$ 0.28 <sup>a</sup>  |
|             | commercial concentrate + <i>Artemia</i> | 1.65 $\pm$ 0.10 <sup>a</sup>  | 2.14 $\pm$ 0.09 <sup>a</sup>  | 2.61 $\pm$ 0.12 <sup>ab</sup> | 3.92 $\pm$ 0.21 <sup>b</sup>  | 7.55 $\pm$ 0.22 <sup>b</sup>  |
|             | commercial concentrate + earth worm     | 1.68 $\pm$ 0.11 <sup>a</sup>  | 2.48 $\pm$ 0.12 <sup>b</sup>  | 3.45 $\pm$ 0.17 <sup>c</sup>  | 5.24 $\pm$ 0.24 <sup>d</sup>  | 7.74 $\pm$ 0.46 <sup>b</sup>  |
|             | commercial concentrate + beef heart     | 1.70 $\pm$ 0.10 <sup>a</sup>  | 2.17 $\pm$ 0.11 <sup>a</sup>  | 2.85 $\pm$ 0.20 <sup>b</sup>  | 4.70 $\pm$ 0.15 <sup>c</sup>  | 9.13 $\pm$ 0.47 <sup>c</sup>  |
|             | <i>P</i> value                          | 0.88                          | 0.00                          | 0.00                          | 0.00                          | 0.00                          |

The letters a, b, c...in each column indicate a significant difference among different treatments ( $p<0.05$ ). \*initial, \*\*final

The result of correlation between length (mm) and weight (g) of Oscar fish in different treatments during the study period is shown in Table 2. Generally,

the Pearson correlation test showed positive correlation between length and weight of Oscar fish in different treatments during the study period.

**Table 2: The correlation between length (mm) and weight (g) of Oscar fish in different treatments during the study period**

| Treatment                                | Day                    |                          |                          |                        |                        |
|--|------------------------|--------------------------|--------------------------|------------------------|------------------------|
|  | 1                      | 15                       | 30                       | 45                     | 60                     |
| Commercial concentrated feed             | r = 0.523<br>p = 0.149 | r = 0.209<br>p = 0.589   | r = - 0.049<br>p = 0.900 | r = 0.732<br>p = 0.025 | r = 0.808<br>p = 0.008 |
| Commercial concentrated + <i>Artemia</i> | r = 0.309<br>p = 0.418 | r = 0.589<br>p = 0.003   | r = 0.788<br>p = 0.012   | r = 0.922<br>p = 0.000 | r = 0.925<br>p = 0.000 |
| Commercial concentrated + earth worm     | r = 0.274<br>p = 0.475 | r = - 0.539<br>p = 0.134 | r = 0.800<br>p = 0.010   | r = 0.908<br>p = 0.001 | r = 0.928<br>p = 0.000 |
| Commercial concentrated + beef heart     | r = 0.415<br>p = 0.267 | r = 0.024<br>p = 0.951   | r = 0.852<br>p = 0.004   | r = 0.832<br>p = 0.005 | r = 0.916<br>p = 0.001 |

A comparison of the mean indices of GR, SGR and CF of fish fed with different diets is shown in Table 3. At the end of study period, treatments fed by earthworm, *Artemia* and beef heart had significantly higher weight gain compared to commercial concentrate diet ( $p < 0.05$ ). Feeding with beef heart supplemented diet resulted in the highest weight gain ( $p < 0.05$ ), but there was no significant difference between *Artemia* and earthworm treatments ( $p > 0.05$ ). Feeding with earthworm, *Artemia* and beef heart significantly increased the fish length compared to concentrate diet ( $p < 0.05$ ), but no significant difference was observed among these three treatments ( $p > 0.05$ ). Also, feeding with earthworm, *Artemia* and beef heart supplements showed significantly higher growth rate and specific growth rate compared to concentrate diet ( $p < 0.05$ ), but there was no significant difference among treatments containing these supplements in terms of GR and SGR ( $p > 0.05$ ). K value was significantly higher in treatments fed with *Artemia* and earthworm compared to concentrate diet ( $p < 0.05$ ), however, beef heart did

not show a significant difference with concentrate or *Artemia* and earthworm ( $p > 0.05$ ).

The results of the survival rate of Oscar fish fed with different diets during the study period (60 days) are given in Table 4. The highest survival rate (%) was observed in commercial concentrate feed ( $94.47 \pm 1.65$ ), *Artemia* ( $93.43 \pm 1.65$ ) and beef heart ( $90.43 \pm 1.33$ ) treatments, while the lowest survival rate ( $70.53 \pm 2.67$ ) was related to earthworm treatment and this difference was significant compared to other groups ( $p < 0.05$ ). Based on the results of survival rate and growth parameters, it can be concluded that for early stages of Oscar fish, a commercial diet containing *Artemia* is recommended and with increasing growth, changing to commercial concentrate feed with beef heart additive induced the highest growth.

The results of different bacteria counting in the intestine of fish fed with different diets are shown in Table 5. The number of total bacteria, lactic acid producing bacteria, mesophilic bacteria, psychrotrophic bacteria and

entrobacteriaceae in the intestine of fish were higher in treatment fed with earthworm compared to other treatments ( $p<0.05$ ).

**Table 3: Comparison of Oscar growth indices in different feeding treatments during the study period (Mean  $\pm$  SE, n=9).**

| Treatment                               | Weight increase (g)          | Length increase (mm)          | Growth rate (%)                 | Specific growth rate (SGR)   | Condition factor (K)          |
|---|------------------------------|-------------------------------|---------------------------------|------------------------------|-------------------------------|
| commercial concentrate feed             | 3.01 $\pm$ 0.33 <sup>a</sup> | 36.01 $\pm$ 1.95 <sup>a</sup> | 206.33 $\pm$ 39.30 <sup>a</sup> | 1.77 $\pm$ 0.18 <sup>a</sup> | 1.70 $\pm$ 0.07 <sup>a</sup>  |
| Commercial concentrate + <i>Artemia</i> | 5.90 $\pm$ 0.15 <sup>b</sup> | 44.56 $\pm$ 1.80 <sup>b</sup> | 370.60 $\pm$ 28.62 <sup>b</sup> | 2.55 $\pm$ 0.09 <sup>b</sup> | 1.90 $\pm$ 0.05 <sup>b</sup>  |
| Commercial concentrate + earth worm     | 6.05 $\pm$ 0.45 <sup>b</sup> | 44.24 $\pm$ 2.44 <sup>b</sup> | 379.86 $\pm$ 51.71 <sup>b</sup> | 2.55 $\pm$ 0.15 <sup>b</sup> | 1.96 $\pm$ 0.04 <sup>b</sup>  |
| Commercial concentrate + beef heart     | 7.43 $\pm$ 0.42 <sup>c</sup> | 49.97 $\pm$ 1.80 <sup>b</sup> | 448.87 $\pm$ 31.14 <sup>b</sup> | 2.81 $\pm$ 0.09 <sup>b</sup> | 1.85 $\pm$ 0.04 <sup>ab</sup> |
| <i>P</i> value                          | 0.00                         | 0.00                          | 0.001                           | 0.00                         | 0.02                          |

The letters a, b, c...in each column indicate a significant difference among different treatments ( $p<0.05$ ).

**Table 4: Comparison of Oscar fish survival rate (Mean  $\pm$  SE) fed with different treatments.**

| Variable indice   | Commercial concentrated feed  | Commercial concentrated + <i>Artemia</i> | Commercial concentrate + earth worm | Commercial concentrate + beef heart |
|-------------------|-------------------------------|--|-------------------------------------|-------------------------------------|
| Survival rate (%) | 94.47 $\pm$ 1.65 <sup>a</sup> | 93.43 $\pm$ 1.65 <sup>a</sup>            | 70.53 $\pm$ 2.67 <sup>b</sup>       | 90.43 $\pm$ 1.33 <sup>a</sup>       |

The letters a, b, c... indicate a significant difference among different treatments ( $p<0.05$ ).

**Table 5: Mean number of intestinal bacteria (Mean $\pm$ SE) of fish fed with different treatments (log CFU/g).**

| Treatment                               | Total bacteria               | Acid lactic bacteria          | Mesophilic bacteria          | Psychrotrophic bacteria      | Enterobacteriaceae           |
|---|------------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|
| commercial concentrate feed             | 6.26 $\pm$ 0.09 <sup>a</sup> | 1.25 $\pm$ 0.04 <sup>ab</sup> | 3.34 $\pm$ 0.01 <sup>a</sup> | 2.25 $\pm$ 0.01 <sup>b</sup> | 0.00 $\pm$ 0.00 <sup>a</sup> |
| commercial concentrate + <i>Artemia</i> | 6.72 $\pm$ 0.02 <sup>c</sup> | 1.22 $\pm$ 0.04 <sup>a</sup>  | 3.47 $\pm$ 0.02 <sup>b</sup> | 2.27 $\pm$ 0.01 <sup>b</sup> | 0.00 $\pm$ 0.00 <sup>a</sup> |
| commercial concentrate + earth worm     | 6.81 $\pm$ 0.02 <sup>c</sup> | 1.44 $\pm$ 0.06 <sup>c</sup>  | 3.53 $\pm$ 0.01 <sup>c</sup> | 2.04 $\pm$ 0.02 <sup>a</sup> | 1.16 $\pm$ 0.04 <sup>b</sup> |
| commercial concentrate + beef heart     | 6.56 $\pm$ 0.01 <sup>b</sup> | 1.17 $\pm$ 0.08 <sup>a</sup>  | 3.37 $\pm$ 0.01 <sup>a</sup> | 2.47 $\pm$ 0.05 <sup>c</sup> | 0.00 $\pm$ 0.00 <sup>a</sup> |
| <i>P</i> value                          | 0.000                        | 0.053                         | 0.000                        | 0.000                        | 0.000                        |

The letters a, b, c...in each column indicate a significant difference among different treatments ( $p<0.05$ ).

The results of monitoring the behavior of fish fed with different diets are shown in Table 6 and Figure 1. At the beginning

of the breeding period, fish fed a diet containing earthworm showed aggressive behavior as up to a weight of

3 g, they take out the eyes of other teammate fish and from the weight of 3 to 6 g the tails of smaller fish were bitten and torn. The rupture of the finfish fed

earthworm was 17%, while such behavior was not observed in other groups.

**Table 6: Aggressive behavior and biting of fish fed with different treatments.**

| Treatment                | Aggressive behavior and biting |
|--------------------------|--------------------------------|
| Concentrate feed         | -                              |
| Concentrate + Artemia    | -                              |
| Concentrate + earth worm | 17 %                           |
| Concentrate + beef heart | -                              |



**Figure 1: Aggressive behavior and biting of caudal fins (arrows) in earth worm supplemented fed treatment**

### Discussion

The aim of this study was to investigate the effect of *A. urmiana*, earthworm and beef heart supplements on growth and survival of *A. ocellatus* fries. The highest survival rate was observed for fish fed with commercial concentrate feed, *Artemia* and beef heart supplemented diets, respectively and the lowest was related to earthworm supplemented feed. Feeding with *Artemia*, earthworm and beef heart supplements resulted in

higher length, weight and SGR yields compared to commercial concentrate diet that is in consistent with the carnivorous feeding regime of Oscar fish, which feeds on aquatic insects and worms as natural feed (Yilmaz and Arslan, 2013). In line with our results, Sontakke *et al.* (2019) observed that live foods including tubifex, *Artemia*, enriched *Artemia*, *moina* and enriched *moina* performed better on growth *N. chitala* fries compared to commercial



food (Sontakke *et al.*, 2019). Seidgar *et al.* (2015) investigated the effects of the diets supplemented with *A. urmiana*, fairy shrimp (*P. spinosa*) and commercial dry concentrate on growth and survival of *Carassius auratus* fries and stated that the most weight and length gain were observed in treatment containing fairy shrimp and *Artemia* supplements (Seidgar *et al.*, 2015). Also, Kamrunnahar *et al.* (2019) concluded that manufactured and commercial feeds are not good alternatives for live foods (Kamrunnahar *et al.*, 2019). Keramat Amir Kolaei and Ebrahimi (2008) investigated the effects of replacing live feed with dry feed on the growth and survival of *Betta splendens* larvae and concluded that feeding with live or dead *Artemia* resulted in 10 fold higher growths and a higher survival rate compared to dry feed.

Better performance of earth worm compared to concentrate diet may be related to its higher protein content especially Methionine and Lysine amino acids, Arachidonic acid and essential fatty acids (Ako *et al.*, 1999). Chakrabarty *et al.* (2011) used vermiwash and commercial feed to study the growth and survival of *Poecilia sphenops* and concluded that vermiwash significantly increased growth rate of juvenile fish compared to live plankton and commercial diet. The highest survival (98%) was belonged to vermiwash, while survival rate in fish fed by live plankton and commercial diet was 80 and 75%, respectively (Chakrabarty *et al.*, 2011). Ahmadifard *et al.* (2016) revealed that growth and

survival indices of *Xiphophorus helleri* larvae fed with commercial diets and replacing it with 25, 50 and 75% earth worm were not affected by diet type but were significantly affected by sex and they recommended to use low levels of 25% earth worm powder instead of commercial diet. They suggested that earth worms compared to commercial food could not increase the growth and survival rate of sword tail (Ahmadifard *et al.*, 2016) that is agreed with negative effect of earth worm on the survival rate of Oscar obtained in our study. This could be due to the poor minerals and amino acids content, presence of anti-nutritional factors; reduced food consumption and low protein digestibility especially in higher levels of earth worm in the diet (Storebakken *et al.*, 2000; Ahmadifard *et al.*, 2016).

*Artemia* as a live feed has had a positive effect on the higher growth and survival of carnivorous fish such as Oscar (Sontakke *et al.*, 2019). The positive effect of *Artemia* on growth and survival of *N. chitala* fry (Sontakke *et al.*, 2019), *Carassius auratus* fry (Seidgar *et al.*, 2015), *Betta splendens* (Keramat Amir Kolaei and Ebrahimi, 2008) have been reported. Growing *Artemia* biomass is rich in protein (67 % dry weight), and low in row fat (4 % dry weight). It is highly digestible and has a thin exoskeleton rich in essential amino acids (Leger *et al.*, 1989). Watanabe (1987) concluded that freshwater fish species essentially require linoleic acid or linolenic acid or both. Although growing *Artemia* has deficiency in terms of  $\alpha$ -LA, enriched *Artemia* has high

LNA Essential fatty acids such as DHA and EPA, which are essential for marine organisms (Dhont and Lavens, 1996). Heidary and Akbary (2013) examined the effect of *Artemia* nauplii on spawning, fecundity, fertility and growth of Angel fish (*Pterophyllum scalar*) and suggested that comparing with diets including cheneh (Lufak) dry feed, *Artemia* nauplii and minced cow heart, maximum number of eggs, hatching percentage and the highest weight and length of larvae were obtained from spawners fed by *Artemia* nauplii. They mentioned that adding live feed as a supplement will make fish healthier. Adult *Artemia* has been found to have more essential amino acids and protein than *Artemia* nauplii (Claus *et al.*, 1979).

Minced beef heart is also used as a main food item in carnivorous aquarium fish including *A. acillatus*, but it imposed a rapid decline in water quality due to contamination (Heidary and Akbary, 2013). Mohammad Nejad Shamoshaki *et al.*, (2011) observed that *Heros severus* fed with cow heart had higher growth and survival compared to blood worms, compact worms, *Artemia* and Gamarus that is in accordance with our study on better performance of beef heart compared to other diets.

The results of this study showed that despite the highest growth performance during the first 45 days, earth worm presented the lowest survival rate, therefore, it is not recommended for early stages of feeding. In the early stages, a diet containing *Artemia* or beef heart is recommended and especially

with increasing weight, changing the diet containing beef heart brings more growth. Segner *et al.* (1993) noted that the use of dry diet alone is not recommended in the early stages of predator fish due to their incomplete digestive system. Therefore, it is better to provide a combination of dry and live food to larvae to increase digestion and absorption efficiency, increase food palatability and achieves a better performance (Akbary *et al.*, 2008; Heidary and Akbary, 2013). Nandeesh *et al.* (1994) reported that combined diets were preferable to diets with only one protein source.

The amount of whole gut bacteria, lactic acid producing bacteria, mesophilic bacteria and entrobacteriaceae were higher in fish fed by earthworm complement ( $p < 0.05$ ). At the beginning of the rearing period, fish fed diets containing earth worm supplements showed aggressive behavior, so that up to the weight of 3 g, took out the eyes of other fish and in weights of 3-6 g, caudal fins of smaller fish were bitten and torn. Fin rupture in fish fed with earth worm supplement was 17%, while such behavior was not observed in other treatments. Such behavior can be attributed to size variation of fish in this treatment and nature of this feed item. Also, it has been reported that the use of handmade food in *B. splendens* larvae in the short term leads to reduced growth and abnormal behavior in the fish and it was attributed to the rejection of handmade feed in the early stages of this fish (Keramat Amir Kolaei and Ebrahimi, 2008).

## Conclusions

In conclusion, feeding with commercial diets together with *Artemia*, earthworm and beef heart supplements had positive results on growth parameters of Oscar fish. The use of earth worm supplement reduced the survival rate of Oscar. Therefore, feeding with diets containing *A. urmiana* and beef heart supplements is recommended for Oscar fish due to their highest survival rates and growth indices. Moreover, it seems that for early stages feeding, a diet containing *Artemia* is recommended and with increasing the weight, the diet containing beef heart causes more fish growth. Further research is needed to optimize an effective diet with a lower cost for this species.

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## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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