Poeciliid Research

The effect of using earthworm meal (*Eisenia foetida*) as protein supplement for the growth of *Xiphophorus hellerii* juveniles

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Abstract. Many studies have highlighted the possibility of using an alternative source to substitute fish meal, as the protein obtained from earthworm meal. Its special attributes have been demonstrated by chemical analyzes, animal experiments and digestibility test. The aim of this work was the establishment of the effect of supplementation in different proportions the granulated feed of *Xiphophorus hellerii* juveniles with meal obtained from earthworm biomass (*Eisenia foetida*). For this purpose the fry from a *X. hellerii* female were distributed in three aquariums and were fed differently. The fish from the control group were fed with commercial granulated feed, while for the experimental groups, two mixtures of granulated feed which was supplemented with 10% and 20% earthworm meal were used. To evaluate the impact of the earthworm meal supplementation, the fish were weight after 120 days. Analyze of the obtained results was made by comparison of mean body weight and the biggest values were registered at the group fed with 10% earthworm meal.
Key Words: swordtail, protein supplement, earthworms, supplementing meal.

Introduction. The insurance of optimal protein level in fish feed is achieved by using protein rich raw materials, and fish meal is considered the main supplier. Earthworms represent a good protein source (Hilton 1983) and an alternative regarding the replacement of fish meal in feeds used in aquaculture (Beg et al 2016; Ibanez et al 1993; Prayogi 2011; Pucher et al 2014; Reinecke et al 1991; Sogbesan et al 2008).

Protein from earthworm meal can be produced at a low cost due to the fact that the worm feed on the organic residues, grow and multiply rapidly (Dynes 2003), they can also be grown on a small scale in boxes made from different materials (Kostecka & Paczka 2006). The obtained earthworm biomass can be used in raw state or it can be dehydrated, as earthworm meal. The usage as a meal has the advantage that it can be stored optimally and qualitatively for longer periods.

The special qualities of earthworm meal (*Eisenia foetida*) have been demonstrated by chemical analyses, which reveals a high crude protein content (60-70%) and essential amino acids (Reinecke et al 1991; Sigh et al 1978; Sakthika et al 2014) comparable to fishmeal. The advantages of earthworm meal usage have been demonstrated by studies and researches aiming both ornamental fish and species for human consumption.

Regarding the usage of earthworm meal in different proportions in fish feed, it has been demonstrated a positive influence on growth rate and on protein and energy efficient usage (Pucher et al 2014). Protein from earthworm meal influences reproduction performances and stimulates fry appetite (Nguyen et al 2010) also observing a reduction of lipid content of the carcasses (Pereira & Gomes 1995).

Usage of *E. foetida* in ornamental aquaculture also represents an interesting theme, and the more and more intensive character and the magnitude of which it extends demand the finding of new protein sources with high biological value but also with reduced costs (Mandal et al 2010; Rezvani et al 2011). Thus it has been found the positive effect on gonad development and reproduction performances at angle fish

Astronotus ocellatus (Rezvani et al 2011) and the research of Kostecka & Paczka (2006) show that feding guppy fish *(Poecilia reticulata*) with earthworm biomass influences both growing and body development and number of the obtained products.

Starting from these aspects and regarding the importance of diversifying diets used in aquarium fish feeding, the research objective consisted in establishing the effect of supplementing swordtail (*X. hellerii*) feed with meal obtained from earthworm (*E. foetida*) biomass.

Material and Method. The experiment was conducted between February-May 2016 in the laboratory of Ecology and Environmental Protection of the Animal Sciences and Biotechnologies Faculty, UASVM Cluj-Napoca. Three groups were formed: control group (M) and the two experimental groups (E_1 and E_2), each with 10 fry of *X. hellerii* (red variety) with the age of 7 days from the same female on her second gestation. The fish were assigned randomly in three glass aquariums, each with a usable volume of 18 L of water. The aquariums have been equipped with individual oxygen water filtering system, thermostat (24°C, pH 7.5) and common source of artificial light (6 hours/day).

To feed the fish, commercial granulated feed was used (TROCO PRIM) in proportion of 100% for the control group M, and in the case of experimental groups, this feed was supplemented with *E. foetida* meal. To obtain the earthworm meal we proceeded in the following way: earthworms were harvested (Figure 1) from semi-fermented cattle manure raised in the Teaching Resort of UASVM Cluj-Napoca. The obtained biomass has been manually washed with water (Figure 2). In the followings, the earthworm biomass has been dried and dehydrated in the oven (Figure 3) at the temperature of 42°C for 48 hours. The dry biomass has been milled resulting earthworm meal (Figure 4) which has been incorporated in the granulated feed and two mixtures were formed: commercial feed plus 10% percent earthworm meal for experimental group E_1 and commercial feed plus 20% earthworm meal for experimental group E_2.

Figure 1. Harvested earthworms.

Figure 2. Washing of worms.

Figure 3. Dehidration on oven.

Figure 4. Earthworm meal.

The fish have been fed with these diets three times a day, for a period of 120 days, and at the end of this period, they were weighed with the electronic analytic balance (Explorer Pro, capacity 110 g, precision 0.1 mg).

Raw data has been statistically processed (GraphPad Prism 7 program) and the differences test was used between two means (test t, $p < 0.05$).

Results and Disscusion. The obtained individual values and means for each group (Table 1) have been superior in the case of fish fed with the earthworm supplemented feed by 10%. Percentage increase (210%) compared to the control group shows a very good evolution of fish, which practically have doubled their bodyweight. Mean bodyweight of group E_1 was of 0.356±0.017 g, recording a difference of 0.187 g compared to the control group. If we refer to the group E_2, the fish have achieved a mean bodyweight of 0.195 g±0.019 and the difference from the control group was of only 0.026 g. Between the two experimental groups E_1 and E_2 there's a difference of 0.161 g in the favor of group E_1 which benefited of 10% protein supplementation.

The calculated variation coefficients express variability: in normal limits (14.04%) of variation at group E_1, mean (17.74%) at control group M and high (30.70%) for fish in group E_2. Therefore the variability analysis indicates the uniformity of fish which were fed with feed mixture of 10% earthworm meal. This can suggest that the fish had an increased appetite for this type of feed. On the other hand, the big variability of fish from group E_2 indicates the fact that supplementation of feed with 20% earthworm meal doesn't influence growth and development performances.

Table 1

Individual values, mean and dispersion indices, the significance of bodyweight differences at *Xiphophorus hellerii* juveniles depending on treatment

BWM g)	*BWE_1 (g)*	*BWE_2 (g)*
0.2057	0.3023 ♂	0.0919
0.1927	0.3355	0.1119
0.1501	0.3810	0.1578
0.1644	0.4140	0.1640
0.2293	0.3051 ♂	0.2300
0.1588	0.3688	0.2333
0.1501	0.3504 ♂	0.2272
0.1620	0.3338 ♂	0.2269
0.1420	0.4690 ♂	0.2326
0.1362	0.3001	0.2792
Mean 0.1691	0.3560	0.1955
Increase 100%	210%	116%

Treatment	*Mean±SEM*	*Limits*	*V%*	*t*	*d*	*p*
M	0.169±0.009	0.136-0.229	17.74	2.091	0.187	< 0.05
E_1	0.356±0.017	0.300-0.469	14.04	0.277	0.026	< 0.05
E_2	0.195±0.019	0.092-0.279	30.70	1.532	0.161	< 0.05

BWM = individual bodyweight of fish from control group M; BWE_1 = bodyweight of fish from group E_1; BWE_2 = bodyweight of fish from group E_2; SEM = standard error; V = variation coefficients; t = test; d = differences between mean.

An important observation is the fact that in group E_1 a number of 5 individuals appeared (marked ♂ in Table 1) which developed the tail in form of a sword (Figure 5), characteristic for males of *X. hellerii*. It is known that sexual dimorphism at this species is manifested by the extension of the tail fin, hence the name swordtail. After 3-4 months from birth they achieve sexual maturity (Tamaru et al 2001), but on the other hand at this age the sex is not stable and can be affected by internal and external factors (Ortega-Salas et al 2013). The fact that the exteriorization of sexual dimorphism doesn't appear in the case of the other two groups shows that in case of group E_1 the fish have achieved sexual maturity. The observation can be sustained based on the results of other authors, whom highlight the influence of earthworm meal both on growth and body development as on reproduction performances at aquarium fish (Kostecka & Paczka 2006; Rezvani et al 2011).

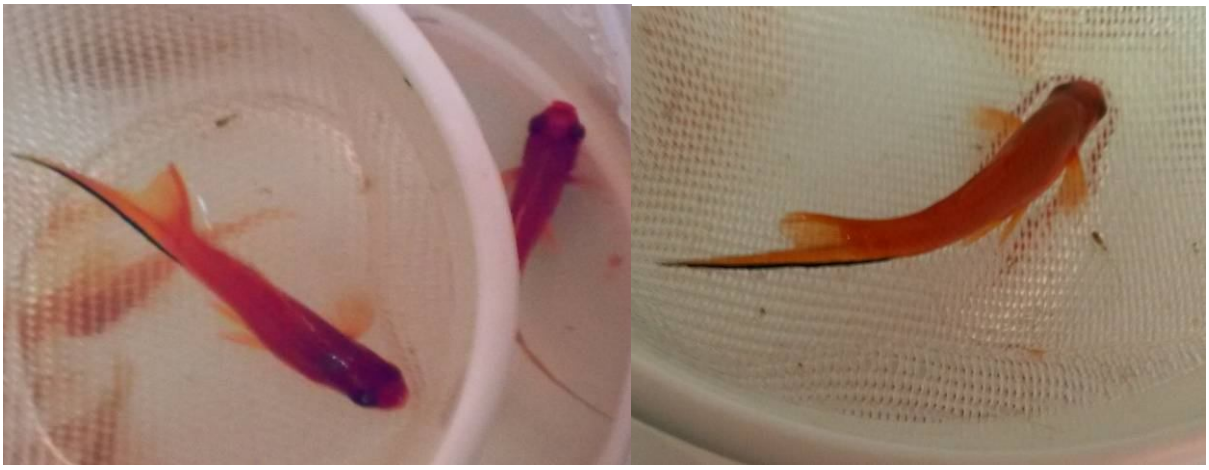

Figure 5. Individuals from group E_1 which present the extension of tail fin.

Conclusions. In the case of *X. hellerii* juveniles, the best results have been obtained at group E_1 which has been fed with granulated feed supplemented with 10% earthworm meal. Percentage increase (210%) compared to the control group shows a very good

evolution of fish, which practically have doubled their bodyweight. The supplementation of granulated feed with 20% earthworm meal didn't have the same effect.

The much higher uniformity level of fish from group E_1 which also had the best growth, suggests the preference and increased appetite for the administrated feed.

On the basis of the obtained results, in which the best growth performances of *X. hellerii* fry was achieved by supplementing the feed with 10% earthworm meal, we recomand this ratio to be the most optimal and efficient

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