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Original Article

Effects of Live Food Feeding on Growth Performance and Some Histological Parameters of *Herotilapia multispinosa* and *Amatitlania nigrofasciata*

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ABSTRACT

Aquarium fish feeding is an expensive hobby. Live foods such as *Artemia* are highly expensive and widely used. Therefore, aquarists look for alternative live food sources to feed their fish. Nematodes and annelids are good sources of protein and are easy to produce. In this study, we evaluated the effects of nematode and annelid feeding on the on growth performance and some histological parameters of *Herotilapia multispinosa* and *Amatitlania nigrofasciata* compared with commercial feeds. *A. nigrofasciata* fish groups fed with live and commercial food showed the highest survival rates of 91% and 100%, respectively. However, *H. multispinosa* fish groups fed with live food showed inhibited growth. Histologic examination of the liver and intestines revealed negative effects on these aquarium fish. Thus, right feeding regime practices should be established for using nematodes and annelids as fish food sources.

Keywords: Annelids, nematodes, aquarium fish, *Herotilapia multispinosa*, *Amatitlania nigrofasciata*

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INTRODUCTION

Aquariums are generally accepted as a hobby all around the world. Yet, the aquarium fish industry is a very big and important industry in terms of fish production. The aquarium fish trade costs about 659 million US dollars in the world. Supporting industries like fish feeds, filters, water chemicals and other equipment totals up to a 30 billion US dollars industry. Over 4000 freshwater and 1400 marine species of fish are in circulation for aquarium trade and an estimated 1 billion fish is sold every year (Whittington and Chong 2007, Tolon and Emiroğlu, 2014). It is evident that, aquarium fish are very cost effective compared to other fisheries products (Galib and Mohsin 2010, Saxena 2003). Growing aquarium industry increased the demand for ornamental fish which increased the need for aquarium fish culture establishments. Presently, freshwater species used in aquariums are 90% cultured and 10% wild captured whereas marine species are only 5% cultured. Yet marine fish are much more profitable and sought for by the hobbyists (Hekimoğlu 2005; Gümüş et al. 2014; Türkmen and Aktuğ, 2011).

Cichlid species are the most common species used for aquariums as well as the most cultured aquarium species. Most of them are preferred by the middle class and low income consumers for their affordable prices. One of the most important problems for these consumers is the cost of feeding. Quality feeds are important for both fish producers and home consumers for fish health, breeding and growth performance and visual quality (Naylor et al., 2009). High quality feeds are available as commercial products with high prices. Especially, larval feeding products like artemia and granulated feeds are very high priced feeds which are commonly used. The most used live food for larval feeding is Artemia sp. Which is collected from the salt lakes around the world like Utah, Great salt Lake, Iran, Urmia Lake, İzmir, Çamaltı Saltworks. These sources are limited and dependent on natural stocks and growing need for artemia by aquaculture hatcheries increases the prices every year (Korkut et al., 2003; Tosun et al., 2015). This high prices results in the need for alternative live foods with low production costs.

Figure 1. Nematod culture media and climbing nematodes on the walls

Nematodes and Annelids are important alternatives for fish feeding with their low production costs and nutritional qualities. *Panagrellus redivivus* is a free living non-parasitic nematode which can be suitable for larval feeding. *P. redivivus* is the preferred nematode species for most of the aquarists with their ease of production and nutritional composition (Bruggemann, 2012). *Enchytraeus sp.* are annelid species used in aquarium fish feeding. Easy and economic growth of these live foods gets the attention of both fish producers and scientists for further research and production techniques (Tosun et al., 2015; Şahin et al., 2017). Starter culture costs for these live feeds are low and they are easily produced with low cost raw materials like water and oatmeal. One of the important aspect of producing annelids and nematodes is the ease of nutritional composition enhancement. Inclusion of fish meal and oil results in culture mediums results in high quality nutritional quality both for nematodes and annelids (Delbare and Dhert, 1996; Ricci, 2001; Santiago et al., 2003; Reyes et al., 2011; Schlechtriem et al., 2004; Kumlu et al., 1998; Santiago et al., 2004; Ercan et al., 2018).

In this study, we evaluated the effects of annelid and nematode feeding on *Amatitlania nigrofasciata* and *Herotilapia multispinosa* growth performances and some histologic parameters in aquariums.

MATERIAL AND METHOD

Nematodes

Panagrellus redivivus cultures used in this study were obtained from the Live Feed Laboratory of Aquaculture Department, Faculty of Aquatic Sciences, İstanbul University. *P. redivivus* cultures were inoculated in 1l plastic cases (Figure 1). The culture medium consisted of oat meal, water and yeast (baker's yeast) 200 L mixture (Tosun et al., 2015). Culture media was kept at 24°C. 10 plastic cases were used for nematode production and all received 2 ml starter cultures. Plastic cases were kept in Live Feed Culture Laboratory, Faculty of Aquatic Sciences. Microworms climbing the walls were harvested with a spatula to feed fish (Figure 1).

Annelids

Enchytraeus albidus was obtained from the Live Feed Laboratory of Aquaculture Department, Faculty of Aquatic Sciences, İstanbul University. *E. albidus* starter culture was inoculated in two 6.8 l (33 cm x 23 cm x 9 cm) plastic cases. Coconut turf was used as growth media with weekly additions of baby formula, milk, yoghurt and kefir (Memiş et al., 2004). Climbing worms were easily harvested and used as food (Figure 2).

Control Feed

Commercial aquarium flake feeds (AHM – Tropical mix flake) were used for control groups. Proximate composition of the feed is given in Table 1.

Fish

Herotilapia multispinosa (rainbow cichlid) *and Amatitlania nigrofasciata* (convict cichlid) were obtained from the Aquarium Fish Laboratory of Aquaculture Department, Faculty of Aquatic Sciences, İstanbul University.

Forty-eight convict cichlids with 0.54±0.27g mean weight (SD±) and 2.8±0.53 cm mean length were placed in four glass aquaria (A1, A2, B1, B2, each stocked with12 fish). 44 Rainbow cichlids with 0.14±0.09 g mean weight (SD±) and 1.4±0.36 cm mean length were placed in four glass aquaria (A3, A4, B3, B4, each stocked with 11 fish).

Aquariums

A total of eight aquariums were used during the experiment. 52.5 l (L70 cm x W30 cm x H30 cm) glass to glass aquariums which were part of a recirculating system with mechanic and biologic filtration were used. Water depth was 25 cm and water temperature was 22±2°C during the experiment. Aquariums were labeled as A1, A2, A3, A4, B1, B2, B3, B4. This study has been designed as 2 parallel for every group.

Table 1. Proximate composition of the commercial flake feed.

Commercial feed proximate composition	
Moisture	8% max
Crude Protein	47% min
Crude Lipids	4% min
Ash	7% max

Table 2. Feeding regime and fish distribution.

Species	Groups	09:00	12:30	16:00
Amatitlania nigrofasciata	A1 – A2	Nematod + Annelid	Flake feed	Nematod + Annelid
Amatitlania nigrofasciata	B1 – B2	Flake feed	Flake feed	Flake feed
Herotilapia multispinosa	A3 – A4	Nematod + Annelid	Flake feed	Nematod + Annelid
Herotilapia multispinosa	B3 – B4	Flake feed	Flake feed	Flake feed

Figure 2. Annelid culture media

Feeding Regime

A1-A2 and B1-B2 aquariums were stocked with convict cichlids, A3-A4 and B3-B4 aquariums were stocked with rainbow cichlids. A group aquariums were fed with live food twice a day (ad-libitum) at 09.00 and 16.00 and with flake feeds at 12:30. B group aquariums were fed only flake feeds at the same hours (Table 2).

Measurements

The experiment lasted for 3 months (90 days). Fish were measured for length by a ruler (mm) and weight (Digital scale, 0.01g) for every 30 day intervals. Data was analyzed using Student's T-test (Excel 2013, Microsoft).

Histological Examination

Randomly selected fish (3 from each group) were examined histologically. Histologic changes in liver and intestines were targeted. Fish were anaesthetized with 2-phenoxiethanol. After ventral incision, all fish was fixated in 10% formalin solution. Processed samples were placed in paraffin and cut into 4-5 µm cross-sections. Cross-sections were stained by Hematoxylin & Eosin method and examined under microscope (Culling, 1972).

RESULT AND DISCUSSION

Weight and Length Measurements

Weight Measurements

Mean weight gain results for live food fed groups are given in Figure 3.

Convict cichlids received live foods better than rainbow cichlids. Rainbow cichlids showed weight loss in the last period during the experiment.

Mean weight gain results for commercial flake feed fed groups are given in Figure 4.

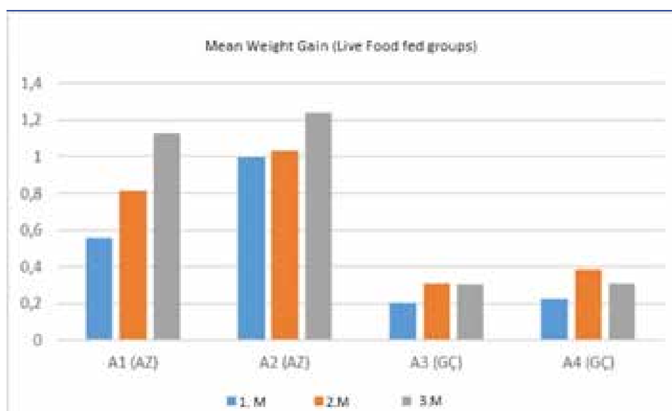

Figure 3. Mean weight gain comparison between live feed fed groups

Both fish species fed with commercial feeds had continuous growth in weight till the end of the experiment. Although A1-A2 groups have better results in terms of mean weight gain, live food feeding did not have statistically important differences with B1-B2 groups (p>0.05). Yet, when compared with A2-A3, B3-B4 groups have statistically better mean weight gain. Hoyland (2015) achieved continuous growth in *labrus bergylta* larvae with live feeds which was similar for convict cichlids response to live feeds in our study. We can reach to the conclusion that rainbow cichlids weight gain is inhibited with live food feeding. Mohseni et al. (2012) reported that *Huso huso* larvae, fed with live foods showed less growth in comparison to granulated pellets which is a similar result to our results with rainbow cichlids.

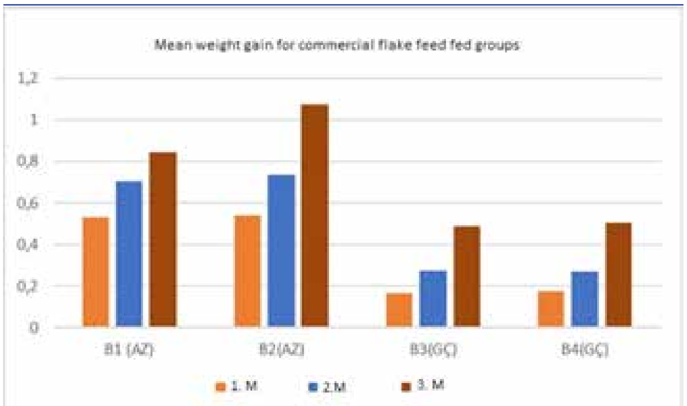

Figure 4. Mean weight gain results for commercial flake feed fed groups

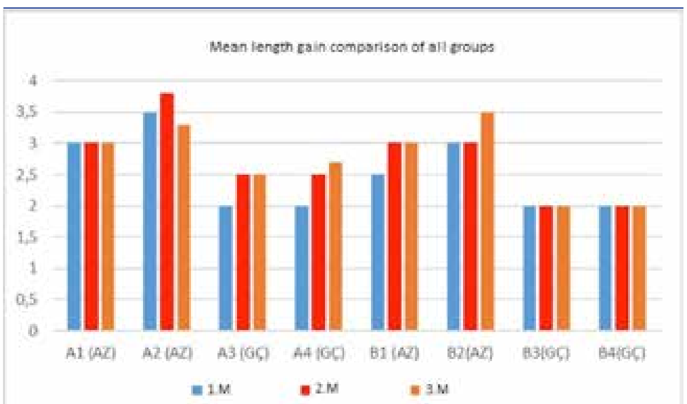

Figure 5. Mean length comparison of all groups
AZ: Convict cichlid; GÇ: Rainbow cichlid; M: month

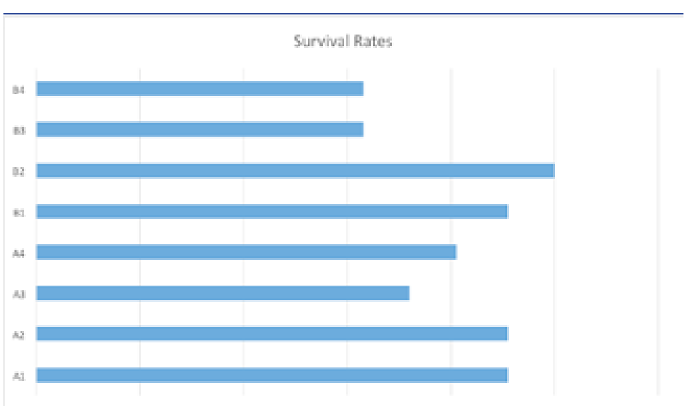

Figure 6. Survival rates (%) for the groups at the end of the trial

Length Measurements

Mean length measurements for all groups are given in Figure 5.

As it is evident in the given table, all groups except B3-B4 showed growth in length during the trial period. Commercial feed fed groups did not have significant differences in terms of growth in length compared to nematode and annelid fed groups.

Survival Rate

Calculated survival rates are given in Figure 6. Highest survival rates were calculated for A1, A2 (91%) and B1, B2 (91% and 100%) groups. Lowest survival rates were calculated for 63% for B3 and B4 (Figure 6).

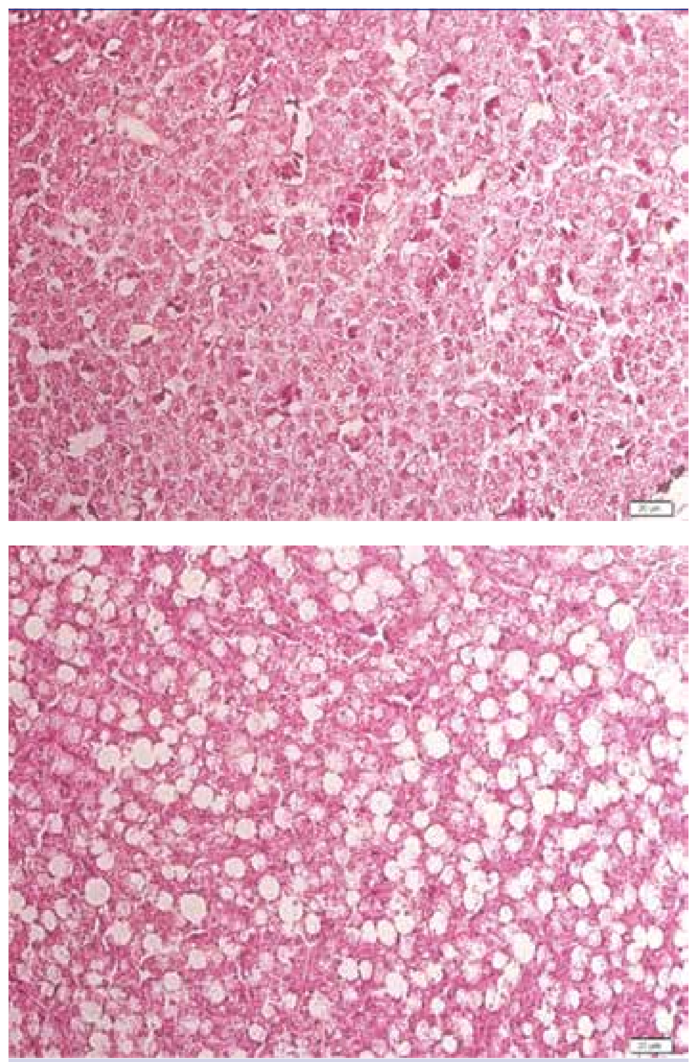

Figure 7. Lipid vacuoles in hepatocyte cells in cross sections of livers from live food fed groups Left; convict cichlid; right; rainbow cichlid

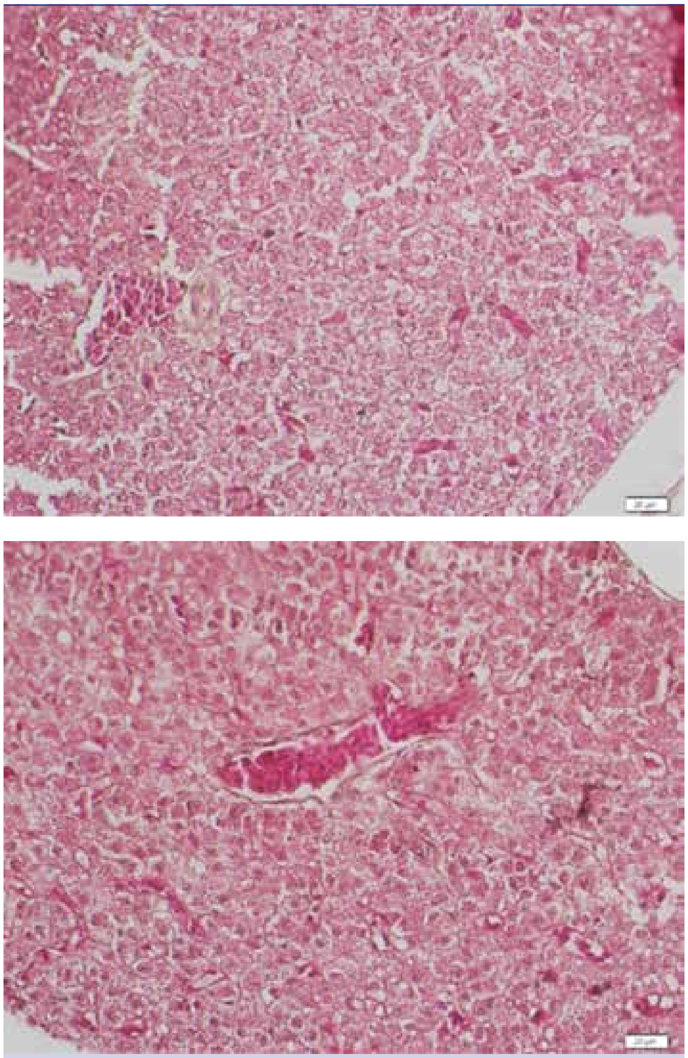

Figure 8. Healthy liver cross sections from commercial food fed groups without lipid accumulation
Left; convict cichlid; right; rainbow cichlid

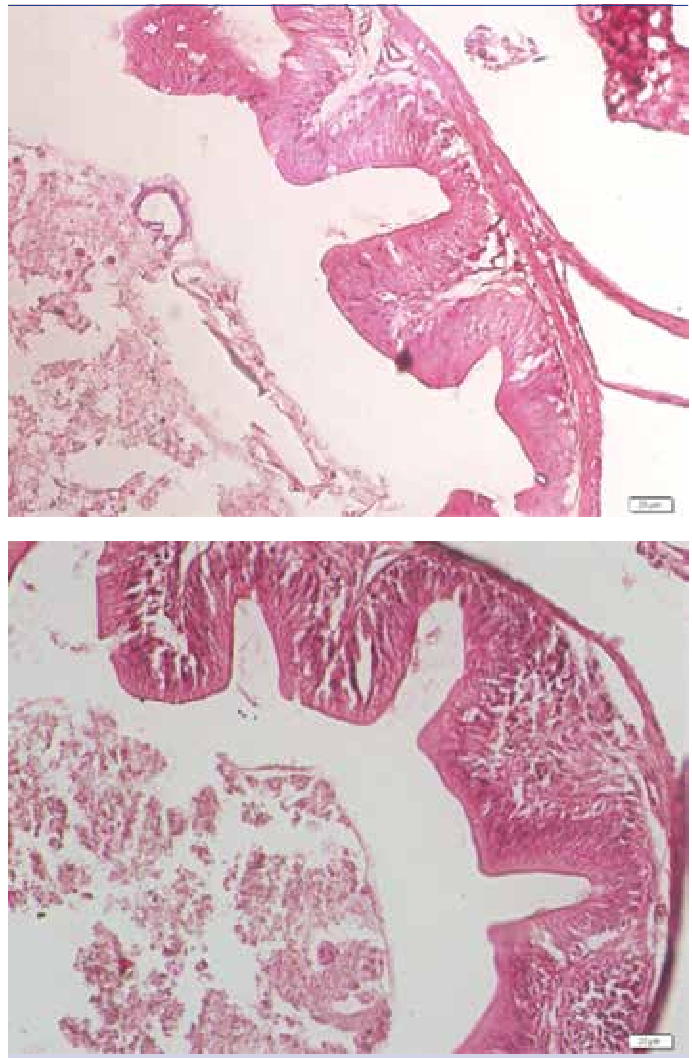

Figure 9. Cross sections of intestines from live food fed groups
Left; convict cichlid; right; rainbow cichlid

Figure 10. Healthy intestinal cross sections from commercial food fed groups
Left; convict cichlid; right; rainbow cichlid

Histological examination

A1, A2, A3 and A4 liver cross sections showed lipid vacuoles in hepatocyte cells. This showed that live food feeding resulted in lipids accumulation in liver cells for both convict cichlids and rainbow cichlids (Figure 7).

Contrary to the live food fed groups, commercial food fed groups (B1, B2, B3 and B4) had healthy liver cross sections (Figure 8).

Intestinal Examination

Cross sections from the intestines showed that live food fed groups (A1, A2, A3 and A4) had damaging effect. Villus were shortened with absorptive vacuoles on the tips, lamina propria were widened, and ruptures in serosa were found (Figure 9).

In cross sections from commercial food fed groups, intestines showed much healthier characteristics. Villus were longer, enterocytes had centered nucleus, lamina propria were not widened and goblet cells were present (Figure 10).

CONCLUSION

Live foods like nematodes and annelids which are widely used in aquarium fish maintenance and production are important alternatives with their ease of production and low costs. Alternative protein sources are gaining importance with increasing aquaculture production all around the world. *P. redivivus* is mainly important with its fast growth, easy mass production and ease of use as food. In addition, nutritional enhancement is possible. Brüggeman (2012), mentions that small size productions are easily adopted to industrial size production systems. Wilkenfeld (1984), points out that, production of these live foods can be more economical in comparison with artemia which presently is a very high priced commodity. As we demonstrated in our study, growth performances and survival rates of the fish fed with alternative live foods are not different compared with commercial feeds. This shows that, cheaper alternatives can be used for aquarium fish instead of expensive commercial products.

Our histological findings show that, amount of live feed used is important for healthy individuals. Raised amount of live feeds may result in internal damages to fish. Enhancing the live feeds or balancing the amount in the feeding regime should be evaluated in future studies. Culture mediums to produce suitable nematodes or annelids has to be formulated for better nutritional quality and healthy aquarium fish.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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