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Eisenia fetida as protein source for growth enhancement of *Heteropneustes fossilis*

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ABSTRACT

The present study was carried out to evaluate the efficacy of different species of earthworms (*Eisenia fetida*, *Eudrilus eugeniae* and *Pheretima posthuma*) as a protein source for the replacement fish meal. Different species of earthworms were used as fish feed. *E. fetida* proved best replacement for standard feed as it results in the significantly highest increase in fish body weight, length, Average Daily Weight Gain (ADG), Specific Growth Rate (SGR) (66.84 %, 23.49%, 0.22g/day, and 1.48 % per day respectively). It also had the best Feed conversion ratio (FCR) of 0.68. The aforementioned result is further confirmed by the increase in crude protein content in fish fed with *E. fetida*. Therefore, farmers can use it as an economical and sustainable protein-rich fish feed.

INTRODUCTION

Food production is increasing day by day to meet human demand and this has put pressure on aquaculture. Fisheries have become an important sector for demanding food supply. Therefore, nutritionally high-quality fish production also becomes paramount for sustainable development **(FAO, 2020; Chatla *et al.* 2021; Ngoc *et al.* 2021).** The nutritional value of any fish diet mainly depends on its protein content. Protein is the most expensive component in the diet of fish and is vital for the proper growth of fish. The use of fish meal is important for fish growth but is becoming a challenging task due to non-sustainable development. There is no doubt that fish meal is a good source of protein, but it is comparatively expensive and reduces fishing efficiency. Therefore, there is a need to search for the alternative cheap protein sources for fish growth **(Musyoka *et al.* 2019; Chakraborty *et al.* 2021)**. Several plant and animal protein sources are used to prepare fish diet. However, the main drawback of using plant-based protein sources is the anti-nutritional factors that affect the fish growth **(New, 1987; Rahate *et al.* 2021)**. Therefore animal-based protein sources are considered a better replacement for a fish meal due to better protein and amino acids quality than plant-based protein sources **(Robinson *et al.* 1998)**. Different animal-based proteins such as chicken offal, chicken liver, freshwater fish processing waste, surimi by-product, snail meat, squid, mussel, lean prawn **(Mohanta *et al.* 2013; Parolini *et al.* 2020),** whole earthworms, pelleted earthworm and earthworm custard etc. have been evaluated as a protein source **(Mohanta *et al.* 2016).**

Earthworms are cosmopolitan and under optimum conditions of temperature, moisture and feeding material, the earthworm multiply up to 256 earthworms by a single earthworm every 6 month. Being a highly rich in protein content, earthworm plays important role in the growth of fishes and can serve as protein supplement for fishes. Since the protein content in earthworm vary from 50-70%, therefore can play an important role in food web by transferring the energy to higher trophic level. Earthworms have previously been used as fish feed **(Sinha *et al.* 2010; Vital *et al.* 2016; Renu *et al.* 2020; Kumar *et al.* 2020).** Earthworms (*Hyperiodrilus euryaulos*) contain (on dry mass basis) 63.0% crude protein, 5.9% crude lipid, 1.9 % crude fiber, 8.9% ash, 11.8 % NFE, 0.53% Ca, 0.94% P, 0.43% Na, 0.62% K, 1638.20 KJ/100g digestible energy and 1476 kJ/ 100 g of metabolizable energy. In addition, they also have arginine 2.83 g/16gN, threonine 4.43 g/16gN, isoleucine 2.04 g/16gN, histidine 1.47 g/16gN, lysine 6.35 g/16gN, phenylalanine 6.26 g/16gN, leucine 4.11 g/16gN and valine 4.43 g/16gN on a protein basis in earthworm. Among different earthworm species, *E. fetida* could be used to replace the fish meal **(Sogbesan and Ugwumba, 2008)** as it has high protein content for the proper growth of fishes **(Musyoka *et al.* 2019; Mohanta *et al*. 2016).**

Commercial catfish play an important role in the upliftment of the socio-economic status of the farmers. *H. fossilis* (air-breathing catfish) commonly called Singhi, belongs to the family Heteropneustidae (air sac catfishes) confined to India, Bangladesh, Myanmar, Thailand and Pakistan. *H. fossilis* in India is prevalent in ponds, ditches, swamps, marshes and muddy rivers of fresh water, and brackish water with a pH range of 6.0 - 8.0. It is a bottom feeder fish **(Saha and Ratha, 2007; Ünlü *et al.* 2011; Chew *et al.* 2020)**, readily adapts to the local environment and can be cultured in relatively low oxygen. The local people prefer it due to its high nutritional value, delicious taste, high protein and low-fat content **(Khan and Abidi, 2014; Zafar and Khan, 2018; Goni *et al.* 2020; Roy *et al.* 2020).**

Very few studies have been reported on the effect of earthworms on the growth parameters of *H. fossilis*. *E. fetida, E. eugeniae* and *P. posthuma* were reproduced and adapted to the local environment much faster and more easily than other earthworm species. Therefore, the present study was aimed to evaluate several earthworm species for comparative effectiveness as a commercial feed for *H. fossilis*.

MATERIALS AND METHODS

H. fossilis were collected from local farmers from Haryana, India and earthworms from the vermiculture unit of the Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar, India. Fish were acclimatized for 15 days in the laboratory. All fishes were fed twice a day according to 3% of body weight for 15 days as described in Table 1. To examine the requirements of *H. fossilis* and to test the fulfillment of different diets, proximate analyses of commercial feed and different types of earthworms (*E. fetida, E. eugeniae* and *P. posthuma*) were determined as in shown Table 2. Bodyweight, length, ADG, FCR and SGR were determined at 5 days interval up to 15 days. Triplicate was maintained for each treatment. About two-thirds of water exchange was done to maintain the water quality. On day 15, proximate analysis of *H. fossilis* was also performed to the effects of different diets on fish. Data were presented as the mean ± standard error. A one-way analysis of variance analyzed the statistical significance of the data.

Table 1. Description of different diets fed to *H. fossilis*

Diets	Description of diets (3% of body weight)
D0	Commercial feed (as control)
D1	Whole live *E. fetida*
D2	Whole live *E. eugeniae*
D3	Whole live *P. posthuma*

Table 2. Proximate analysis of different types of diets

Chemical composition (%)**	**D0**	**D1**	**D2**	**D3**	**CD**
Dry Matter (DM)	88.52 ± 0.02	14.86 ±0.09	18.67 ±0.32	15.41 ±0.49	0.98*
Crude Protein (CP)	41.04 ± 0.01	57.82 ±0.22	50.05 ±0.93	44.05 ±0.58	1.85*
Crude Fat (Ether Extract; EE)	2.93 ± 0.02	8.40 ±0.32	5.10 ±0.32	5.30 ±0.40	1.01*
Crude Fiber (CF)	6.48 ± 0.01	3.82 ±0.27	5.34 ±0.13	5.36 ±0.60	1.10*
Total Ash (TA)	13.82 ± 0.01	14.81 ±0.27	20.88 ±0.30	21.92 ±0.43	0.97*
Nitrogen Free Extract (NFE)	35.74 ± 0.06	15.15 ±0.27	18.63 ±1.15	23.37 ±1.02	2.59*

*Values were significant at $p \leq 0.05$
**(% dry matter basis)

The growth performances were calculated according to the following formulas:

$$\text{Survivability} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$\text{Percent increment in body weight} = \frac{\text{Mean final weight- Mean initial weight (g)}}{\text{Mean initial weight}} \times 100$$

Average daily weight gain = mean final weight - mean initial weight (g) / Culture days

$$\text{Specific growth rate} = \frac{\text{Log final body weight – log initial body weight}}{\text{Number of days}} \times 100$$

$$\text{Percentage increment in length} = \frac{\text{Mean final length – Mean initial length (cm)}}{\text{Mean initial length}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Amount of dry weight consumed}}{\text{Live weight gain}}$$

RESULTS AND DISCUSSION

Earthworms as a protein source for *H. fossilis* growth and the effect of different earthworms species on growth parameters of *H. fossilis* was investigated based on the increase in body weight, length, ADG, FCR and SGR. *H. fossilis* soon became accustomed to feeding in all diets and actively fed during the whole study period. All the fish showed progressive increase in their weight. The results showed that the fish fed with whole live earthworms (D1-D3) (*E. fetida*, *E. eugeniae* and *P. posthuma*) showed a significant increase in all growth parameters compared to D0 (Fig. 1-4). But it increased more significantly when *E. fetida* was used as a diet (D1). There was 100 % survivability in all diets (D0-D3). No major changes were noticed in the water quality parameters during the experiment and all the parameters were within the normal range, as shown in Table 3.

Body weights of fish fed with D0–D3 on day zero were almost the same, but significantly highest mean body weight was 8.12 ± 0.15 (g) in D1 on day 15, compared to 6.98± 0.18 (g) on day D0. The highest increment of 66.84% in body weight was observed in D1, whereas the lowest increment of 41.53 % was found in control D0 (Fig. 1). Significantly highest ADG (0.22 g/day) was observed in D1 as compared to all other diets, while the lowest ADG (0.14 g/day) was found in control D0. But, during the entire experimental period, the highest ADG (0.24 g/day) was calculated in D1 from day 10-15 and the lowest (0.13 g/day) from day 0-5 (Fig. 2). Decrement in SGR was calculated as days increased (Fig. 3). On the 15th day of feeding trials, significantly highest SGR (1.48

%/day) was calculated in D1 compared to all other diets while the lowest SGR (1.00 %/day) was calculated in control D0. But, during the entire experimental period, the highest SGR (1.54 %/day) was calculated in D1 from day 0-5 and lowest (0.93%/day) from day 10-15. As for bodyweight, a similar trend was observed in the percentage increase in mean body length, where the highest increase in body length (23.49 %) was observed in D1, while the lowest body length (11.19 %) was observed in D0 (Fig. 4). Increment in FCR was calculated as days increased (Fig 5). Significantly highest FCR (1.09) was calculated in control D0 compared to all the other diets while lowest (better) FCR (0.68) was calculated in D1. But, during the entire experimental period, the highest FCR (1.34) was calculated in control D0 from 10-15 days and lowest (0.77) from 0-5 days.

Table 3. Water quality parameters during the experiment

Characteristics	**Fresh water on zero-day**	**D0 (on 15th day)**	**D1(on 15th day)**	**D2(on 15th day)**	**D3(on 15th day)**	**CD**
Temperature (ºC)	25.83 ± 0.17	26.37 ± 0.19	26.53 ± 0.03	26.50 ± 0.29	27.10 ± 0.32	0.71*
Dissolved Oxygen (ppm)	5.97 ± 0.03	5.67 ± 0.33	6.00 ± 0.58	5.67 ± 0.33	5.33 ± 0.33	NS
pH	7.47 ± 0.03	7.07 ± 0.09	7.00 ± 0.06	6.93 ± 0.07	6.93 ± 0.12	0.25*
Total alkalinity (ppm)	99.33 ± 0.67	113.33 ± 3.33	115.00 ± 2.89	113.67 ± 2.03	118.33 ± 1.76	7.43*
Total Hardness (ppm)	180.00 ± 0.00	206.67 ± 0.33	210.00 ± 0.58	213.67 ± 2.40	208.67 ± 0.88	3.78*
Salinity (ppt)	0.15 ± 0.01	0.15 ± 0.01	0.21 ± 0.01	0.19 ± 0.03	0.24 ± 0.02	0.05*
EC (µS/cm)	281 ±12.29	361.00 ± 4.16	358.67 ± 2.96	361.00 ± 4.16	361.00 ± 3.22	20.43*
TDS (ppm)	210.00 ± 0.00	242.33 ± 1.33	228.33 ± 2.33	226.33 ± 3.93	229.33 ± 3.18	8.17*
Ammonia (ppm)	0.00 ± 0.00	0.83 ± 0.17	1.33 ± 0.33	1.33 ± 0.33	1.17 ± 0.44	0.95*
Nitrite (ppm)	0.00 ± 0.00	0.67 ± 0.17	0.50 ±0.00	0.83±0.17	0.87 ± 0.19	0.43*
Nitrate(ppm)	0.00 ± 0.00	1.33 ± 0.33	1.17 ±0.44	1.67±0.33	1.33 ± 0.33	1.04*
Chlorides (ppm)	39.67 ± 0.07	41.77 ± 1.43	41.97 ±0.86	41.67±1.20	45.00 ± 2.31	NS
Free CO2 (ppm)	0.58± 0.08	0.92 ± 0.08	1.17 ± 0.17	1.17±0.17	1.10 ± 0.10	0.40*

*Values were significant at $p \leq 0.05$

Where; NS = non-significant

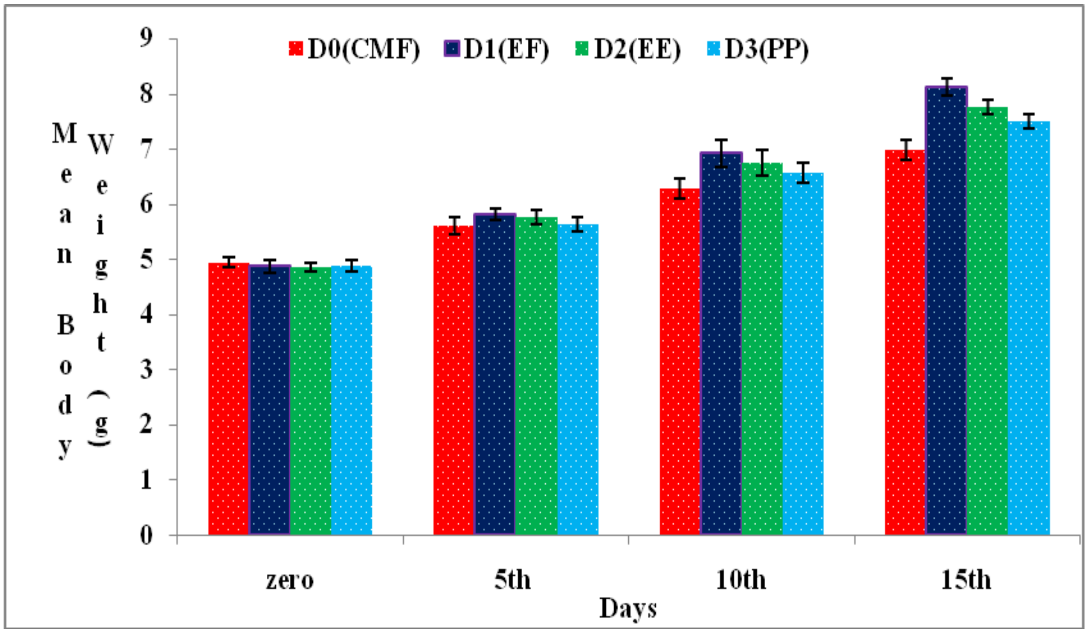

Fig. 1. Effect of different diets on the body weight (g) of *H. fossilis*.

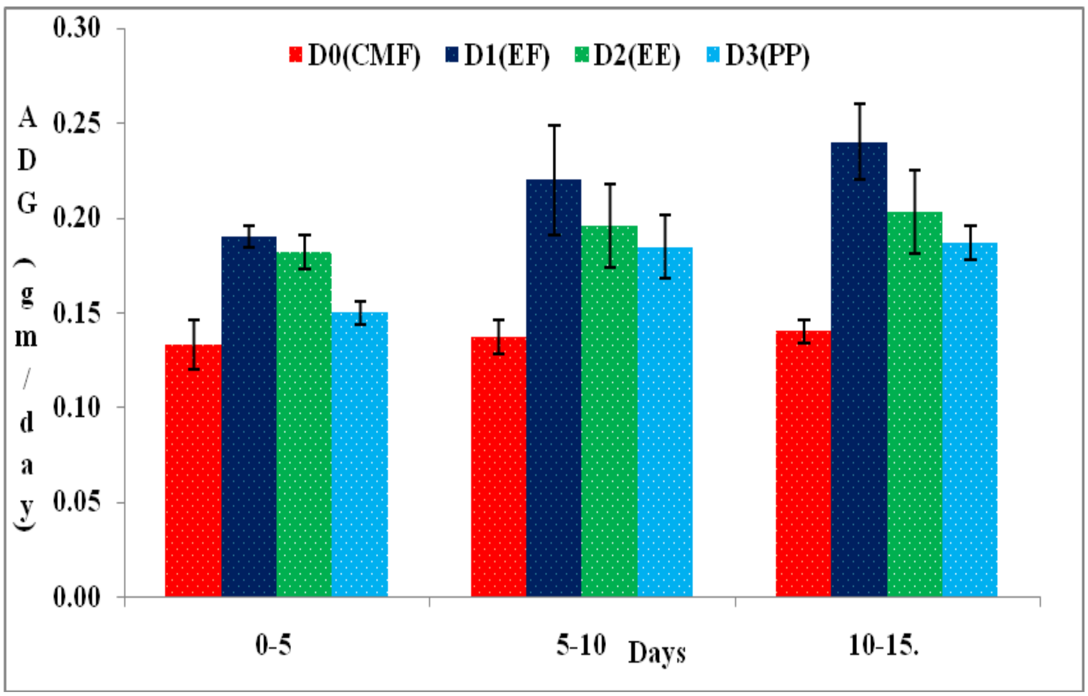

Fig. 2. Effect of different diets on the ADG (g/day) of *H. fossilis*.

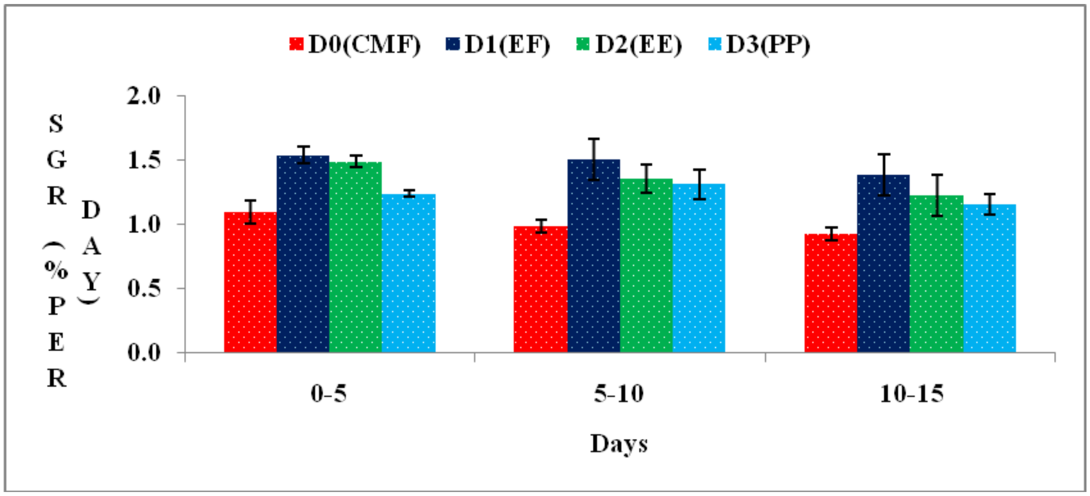

Fig. 3. Effect of different diets on the SGR (% per day) of *H. fossilis*.

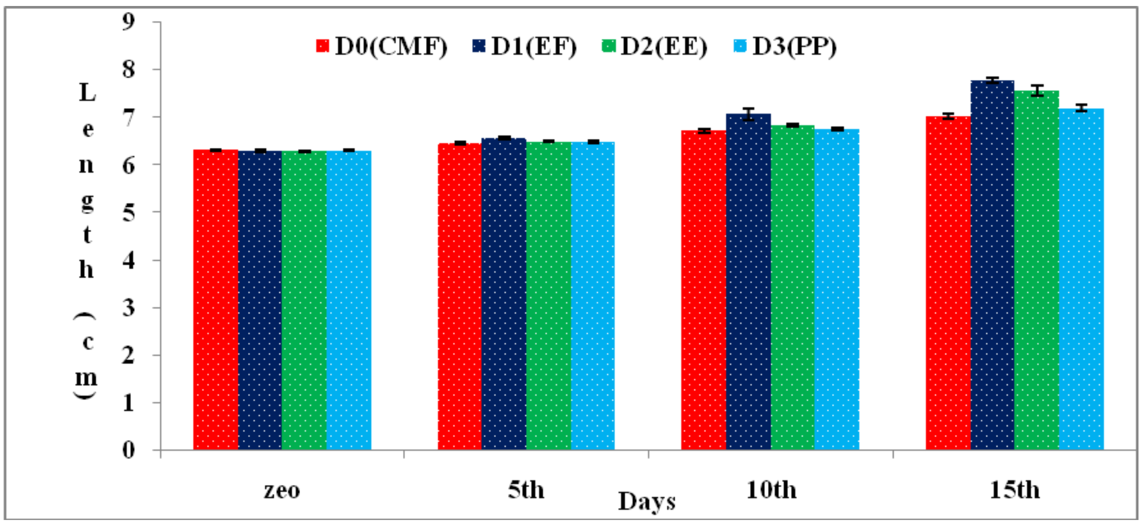

Fig. 4. Effect of different diets on the length (cm) of *H. fossilis*.

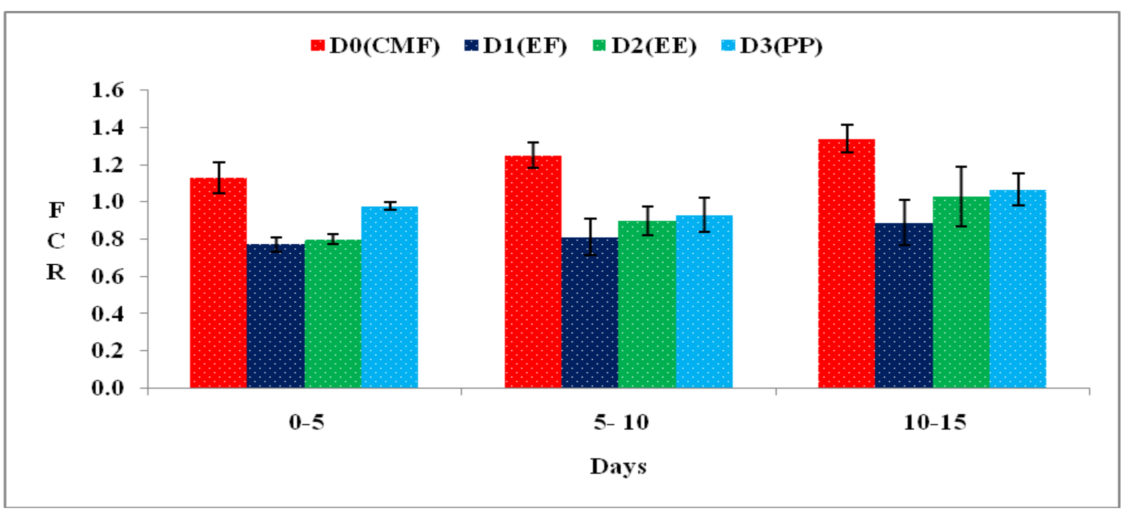

Fig. 5. Effect of different diets on the FCR of *H. fossilis*

The proximate analysis of *H. fossilis* fed with different diets was shown in Table 4. D1 counted the highest crude protein and crude fat (61.64 and 12.41%) while D3 had the lowest (58.24 and 6.20% respectively) counts. The highest crude fiber (2.63%) and NFE (19.40%) were found in D0 and D3 while the lowest (1.64 and 10.31%) were in D1, respectively. Inorganic constituents (total ash) were highest (15.00%) in D2 while lowest (13.63%) in D3.

Table 4. Whole-body proximate analysis of *H. fossilis* fed with different diets

Chemical composition**	D0	D1	D2	D3	CD
DM	21.68 ±0.50	22.65 ±0.43	22.46±0.35	26.26±0.71	1.70*
CP	59.37 ±0.75	61.64 ±0.17	60.31±1.02	58.24±0.48	2.26*
EE	6.87 ±0.19	12.41 ±0.82	11.25±0.14	6.20±0.25	1.48*
CF	2.63 ±0.33	1.64 ±0.27	1.69±0.29	2.53±0.09	0.86*
TA	13.67 ±0.33	14.00 ±0.58	15.00 ± 0.58	13.63±0.38	2.18*
NFE	17.47±0.66	10.31±0.70	11.74 ±0.55	19.40±0.72	NS

Where; *Values were significant at $p \leq 0.05$
**(% dry matter basis)
NS = non significant

In aquaculture, research on alternative protein sources compared to fish meal is a priority due to the consistency in global fish meal production. Plant-based feeds have limited consequences on fish growth due to protease inhibitors, oxalic acids, alkaloids, phytates, mimosine, saponegin, heamatoglutinins, linamarin, glycosides and cyanoglycosides **(New, 1987; Rahate *et al.* 2021)**. Hence, animal-based feed is preferred. Fish meal meets the growing needs of fish, but frequent use may threaten sustainable development in fisheries. Another limiting factor for the use of fish meal is that it is comparatively more expensive for farmers and affects the cost-benefit for framers. Therefore the experiment was conducted to evaluate the efficacy of different species of earthworms as a protein source compared to fish meal. The use of earthworms as a protein substitute for fish diets is an opportunity to provide sustainable development with clean technologies. The recommended level of earthworm meal had protein levels, amino acids and lipid content similar to or somewhat higher than a fish meal for fish growth (**Vital *et al.* 2016)**. Earthworms contain various essential minerals (Ca, Na, P, K, etc.) for the optimum growth of fish **(Sogbesan and Ugwumba 2008)**. Proximate analysis of different species of earthworms used as fish diet (Table 2) confirmed that they contain essential nutrients necessary for the growth of *H. fossilis*.

The present 15-day study showed that when whole live earthworms were used as a diet, the mean body weight increased from 4.87 to 8.10 (g). The FCR and SGR at 15 days were 0.68 and 1.47 %/day in D1, while those at D0 were 1.08 and 1.01 %, respectively. A similar finding was observed by **Rawling *et al.* (2012)** that the earthworm (*Perionyx escavatus*) diet had a significant increase in fish growth parameters compared to fish meal and soybean-based fish diet. They observed that after 60 days, body weight increased from 11.73 to 83.01 (gm) in the earthworm meal-based fish diet, and FCR (1.08) and SGR (3.26 %) also showed significant positive effects compared to fish meal-based fish diet. A significant effect of *E. fetida* as a protein diet on the growth of *Labeo rohita* was observed by **Mohanta *et al.* (2016)**. A similar increase in weight gain was also observed in *Clarias batrachus* when *E. fetida* was used as feed **(Ghosh, 2004)**. For proper growth and nutrient utilization of hybrid mud catfish (*Heteroclarias*) fingerlings, replacement of fish meal with 50 - 70 % earthworm (*E. eugeniae*) meal was suitable **(Monebi and Ugwumba, 2013)**. If a feed has a low FCR, it is considered preferable. As the amount of protein in the diet increases, the FCR decreases **(New, 1987; USAID 2011)**. In our study, a lower (better) FCR (0.68) was recorded in D1 as compared to D0 (1.08), which is fully justified by the studies conducted by **Rani and Rani (2016)**. Thus our study showed that *H. fossilis* prefers live earthworms as food.

It is clear from the study of growth parameters that different earthworm species meet the requirement for the growth of *H. fossilis* and are supported by proximate analysis of different earthworm species (table 2). When *E. fetida* was used as the diet (D1), crude protein was increased by 61.64 %, compared to control diet (D0) (59.37 %) as shown in table 4. A similar finding was reported by **Mohanta *et al.* (2016),** who used the whole *E. fetida* as a protein diet for the growth of *L. rohita*. *P. escavatus* had a significant effect on body weight gain, SGR, FCR, protein efficiency ratio (PER) as compared to soybean and fish meal based fish diet on mirror carp (*Cyprinus carpio*) **(Rawling *et al.* 2012)**. **Kostecka and Pączka (2006)** showed that *E. fetida* could be used to reproduce and survive aquarium fish (*Poecilia reticulate*).

The present study showed that *H. fossilis* quickly ate earthworms. The present study of growth parameters (Fig. 1-4) showed that earthworms as the main protein source are similar to or even better than various non-traditional protein sources used by earlier researchers, such as fermented fish offal in *Labeo bata* **(Mondal *et al.* 2011)**, silkworm pupae in carps **(Nandeesha *et al.* 2000)**, slaughterhouse waste in *L. rohita* **(Singh *et al.* 2005)**, garden snail meal in *Clarias gariepinus* **(Sogbesan *et al.* 2006)**, tuna-by product meal in rainbow trout, *Oncorhynchus mykiss* (**Tekinay *et al.* 2009)**, poultry by-product meal in *Morone chrysops* × *M. saxatilis* **(Rawles *et al.* 2006)** and fermented fish offal in *H. fossilis* **(Mondal *et al*. 2008)**. The above literature and our finding confirm that earthworms (mainly *E. fetida*) can be used as protein sources for the growth of *H. Fossils*.

CONCLUSION

Earthworms can replace a fish meal for the better growth of *H. fossilis* and are enthusiastically eaten by experimental fish. *E. fetida* was found highly beneficial food for the growth of *H. fossilis*, which has also increased the protein content in fish. The research results indicate the possibility of reducing the cost of fish farming and leading to better outcomes for aquaculture.

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