ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(3): 357-361

www.fisheriesjournal.com
Received: 05-03-2016
Accepted: 06-04-2016

MM Beg
Department of Aquaculture Management and Technology, Vidyasagar University, Midnapore - 721102.West Bengal, India.

B Mandal
Department of Aquaculture Management and Technology, Vidyasagar University, Midnapore - 721102.West Bengal, India.

S Moulick
School of Civil Engineering, KIIT University, Bhubaneswar 751024, Odisha, India.

Correspondence
MM Beg
Department of Aquaculture Management and Technology, Vidyasagar University, Midnapore - 721102.West Bengal, India.

Potential of earthworm meal as a replacement of fish meal for Indian major carps

MM Beg, B Mandal, S Moulick

Abstract
Fish meal is considered as the best protein source in fish and shrimp feed. However, due to its increasing cost and high demand in animal husbandry and aquaculture industry, there is a need for replacement of fish meal with ideal alternative protein source. Recent research shows the potential of earthworm meal as a replacement of fish meal. In the present study, the potential of earthworm meal (*Eisenia fetida*) as a replacement of fish meal for culture of three Indian major carps, catla (*Catla catla,* Hamilton), rohu (*Labeo rohita*, Hamilton,) and mrigal (*Cirrhinus mrigala*, Hamilton) with stocking ratio 1:1:1 was evaluated in 12 cement concrete tanks with size of 2.5 m ×1.5 m ×1.5 m for a culture period of 90 days. Four experimental iso-nitrogenous (35% crude protein) and iso-caloric (15MJ kg^{-1}) diets were prepared with (i) no replacement of fish meal (Diet A), (ii) 20% replacement of fish meal with earthworm meal (Diet B), (iii) 50% replacement of fish meal with earthworm meal (Diet C) and 100% replacement of fish meal with earthworm meal (Diet D). These diets were fed to the fishes twice a day at 3% body weight using broadcasting methods. Diet C treatment produced higher growth of fishes in comparison to the other treatments. In case of Diet C treatment, total fish production was found to be 63% higher than Diet A, 45% higher than Diet B and 16% higher than Diet D treatment. Based on the results obtained from the present study, it can be concluded that 50% replacement of fish meal with earthworm meal in the diet may be used as an ideal alternate protein source for better growth performance of Indian major carps.

Keywords: *Eisenia fetida,* earthworm meal, Indian major carps, replacement of fish meal

1. Introduction
Fish meal is considered as the best protein source in fish and shrimp feed because of its balanced amino acids, vitamin contents, palatability, growth factor, and attractant properties [1, 2]. But fish meal, being a restricted fishery resource, will not be able to supply the aquaculture industry with a continuous source of inexpensive protein indefinitely [3]. Moreover, the increasing cost of high-quality fish meal used in production of aqua-feeds compelled the fish nutritionists around the globe to investigate and identify the novel and renewable alternative non-conventional protein source for the continued expansion and sustainability of aquaculture industry [4, 5].
Various researchers are focusing on other locally available cheap protein sources (e.g. plant protein, agricultural by-products, fishery by-products, terrestrial animal by-products, grain legumes, oil seed plants etc.) in animal feeds. Plant protein sources have been tried as alternatives in the diets of fish [6]. A major drawback in the use of plant proteins, however, is the presence of anti-nutritional factors in organic fish culture [7]. Some researchers used other animal sources such as poultry by-product [8], meat and bone meal [9] with favorable results in some cases.
Several researchers reported the use of earthworm as dietary protein source either alone or in combination with other feed ingredients in formulating the fish diets [10-14]. Earthworms possess amino acid profiles similar to fish and have been used as protein supplements in the diets of fish [11]. Earthworm contains variable lipid contents naturally high in ω-3 fatty acid which enhances immunity in animals [15-17]. Extensive analysis of the lipid fraction of earthworms have revealed a high proportion of polyunsaturated fatty acids (linolenic; ω-3 fatty acids), which is essential for formulating fish feed of many species [18]. The protein and lipid contents of earthworm have been reported to vary from 50 to 70% and 5 to 10%, respectively [15-17]. The quantity of sodium, calcium and potassium available in the earthworm is sufficient in meeting the requirement levels for cat fish and all tropical fish [5].

Dried earthworm meal was used as a replacement for fish meal in a culture trial with common carp [12]. It was found that fish meal partially replaced by earthworm meal and enriched with 5% sardine oil resulted in the best growth of fish. Three forms of earthworm viz. (i) whole earthworm (ii) earthworm custard, and (iii) pelleted earthworm diet were used to prepare three iso-nitrogenous (500 g protein kg^{-1} diet) and iso-caloric (17.0 MJ kg^{-1} diet) experimental diets for rohu advanced fry [19]. It was found that maximum growth of advanced fry occurred in pelleted earthworm diet.

It can be observed from the previous literatures that a lot of research has already being conducted to evaluate the potential of earthworm meal as a replacement of fish meal. However, till date no work is reported on utilization of earthworm meal as a replacement of fishmeal in the culture of Indian major carps.

In the present study, the efficacy of earthworm meal as a replacement of fish meal for culture of three Indian major carps, catla (*Catla catla,* Hamilton), rohu (*Labeo rohita*, Hamilton,) and mrigal (*Cirrhinus mrigala*, Hamilton) was evaluated. Four experimental iso-nitrogenous (35% crude protein) and iso-caloric (15MJ kg^{-1}) diets were prepared with (i) no replacement of fish meal (Diet A), (ii) 20% replacement of fish meal (Diet B), (iii) 50% replacement of fish meal (Diet C) and 100% replacement of fish meal (Diet D).

2. Materials and methods

Indian major carps comprising catla (*Catla catla,* Hamilton), rohu (*Labeo rohita*, Hamilton) and mrigal (*Cirrhinus mrigala*, Hamilton), collected from Naihati Fish Hatchery were cultured in 12 cement concrete tanks with size of 2.5 m ×1.5 m ×1.5 m. The effective water volume in each tank was kept at 3.75 m^3. 12 fishes per experimental tank were stocked at a stocking ratio of 1:1:1 (i.e., 4 nos. catla, 4 nos. rohu and 4 nos. mrigal). Prior to tank culture, the fishes were acclimatized in a wet laboratory for 15 days in a cement concrete tank with size 3.6 m ×2.5 m ×1.5 m with supplemental aeration using an air pump (Medini pump). During acclimatization, the fish were fed with a local (EPIC, Govt. West Bengal) fish feed containing 26% crude protein based sinking peletted feed. After acclimatization, the fish seeds were kept in a bath in 2% NaCl solution for 1-2 minute and were stocked in the experimental tanks at a density of 3 numbers per m^3. The initial stocked size of fingerlings for rohu, catla and mrigal were 8.03 ± 0.02 g; 8.18±0.05 g, and 7.85± 0.03 g respectively. Stocking was done in the early mornings until 9:00 h. Best management practices was followed in the culture experiments which included (i) aeration to maintain dissolved oxygen level above 5.0 mg L^{-1} and (ii) Removal of fecal matter and uneaten feed though siphoning and 30% water exchange on a daily basis to maintain optimum water quality parameters.

Four experimental iso-nitrogenous (35% crude protein) and iso-caloric (15MJ kg^{-1}) diets were prepared with (i) no replacement of fish meal (Diet A), (ii) 20% replacement of fish meal with earthworm meal (Diet B), (iii) 50% replacement of fish meal with earthworm meal (Diet C) and 100% replacement of fish meal with earthworm meal (Diet D) and were fed to fishes for a culture period of 90 days. Each of the above four treatments were replicated thrice. The fish were fed twice a day at 3% body weight by broadcasting methods at 8.30 to 9.00 and 16.30 to 17.00 h [20].

2.1 Proximate composition of experimental diets

The proximate composition of earthworm (*Eisenia fetida*) and formulated diets was evaluated [21]. Moisture content was determined by differential weighing of dried and fresh samples. Crude protein was determined by the macro-kjeldahl method. Ash content was measured by weighing after drying and ashing at 500°C in a muffle furnace. Ether extract or crude fat was estimated by ether extraction method. Crude fiber was found out by acid digestion of residues from the ether extraction and loss in weight on ignition. Gross energy was determined using bomb calorimeter (Parr 6300 Calorimeter, Moline, IL, USA), using benzoic acid as a standard. The proportions of different ingredients in the experimental diets are presented in Table 1. The proximate composition of feed ingredients is shown in Table 2. The proximate compositions of the experimental diets are presented in Table 3. It can be seen from the table that all the experimental diets were iso-nitrogenous (35% crude protein) and iso-caloric (15MJ kg^{-1}).

Table 1: Proportions (%) of ingredients of different feed treatments in Indian major carp

Ingredients	Diet A	Diet B	Diet C	Diet D
Earthworm meal	-	5.12	12.80	25.60
Fish meal	25.60	20.48	12.80	-
Soybean meal	35.00	35.00	35.00	35.00
Mustard oil cake	24.40	24.40	24.40	24.40
Wheat floor	13.00	13.00	13.00	13.00
Vitamin	1	1	1	1
Mineral mixture	1	1	1	1

Table 2: Proximate composition of feed ingredients

Ingredients	Crude protein	Crude fat	Ash	Moisture
Earthworm meal	54	9.13	8.44	10.14
Fish meal	54	8.5	17	8
Soybean meal	40	6	9	8
Mustard oil cake	23	7	11	10
Wheat floor	11	4	8	8
Vitamin and Mineral mixture	-	-	-	

Table 3: Proximate composition of experimental diets

	Diet-A	Diet-B	Diet-C	Diet-D
Crude protein (%)	34.86	34.85	34.85	34.86
Crude fat (%)	6.50	6.53	6.55	6.65
Carbohydrate (%)	27.07	27.83	28.52	29.77
Crude fiber (%)	10.50	10.9	10.12	10.15
Ash (%)	11.22	10.78	10.16	9.07
Moisture (%)	10.11	9.11	9.8	9.5
Energy (MJ kg^{-1})	15.66	15.65	15.66	15.70

2.2 Preparation of earthworm meal

Mature earthworms (*Eisenia fetida*) were collected from an existing vermicomposting farm. The earthworms were washed using water to separate manure from outside skin and fecal mud and then kept in cold water (-14° C) for 24 hours [22]. The earthworms were grinded and dried using vacuum oven at 50° C for 12 hours and sieved to obtain earthworm meal powder in homogenized form.

2.3 Water quality parameters

Water samples were collected at 9.00 h and 3.00 h at alternate day for analyzing temperature, pH and dissolved oxygen (DO). Total ammonia nitrogen (TAN), orthophosphate and BOD were analyzed weekly once [23].

The water temperature, pH and DO were recorded with the help of a portable digital YSI Dissolved Oxygen Meter. TAN and Orthophosphate level of tank water was estimated by spectrophotometric method [23]. For BOD measurement, water sample was collected in BOD bottle and kept in the BOD incubator for 5 days. The change in DO concentration in the water sample was measured over 5 day incubation period at 20 °C.

2.4 Determination of fish growth parameters

More than 50% of the fish of all the tanks were sampled weekly and individual measurements were taken to determine the fish yield parameters. The major fish growth parameters included: i) Net weight gain, ii) specific growth rate (SGR), iii) feed conversion ratio (FCR). These parameters were calculated using the following equations:

Net weight gain= Average final weight (g) - Average initial weight (g)

$$SGR\ (\%\ body\ weight\ /\ day) = 100 \times \frac{\ln(\ final\ weight\ /\ initial\ weight\ )}{culture\ period\ (days)}$$

$$FCR = \frac{amount\ of\ feed\ fed\ (dry\ weight\ basis\ )}{net\ weight\ gain\ (wet\ weight\ basis\ )}$$

2.5 Statistical analysis

Data of different water quality and fish growth parameters were analyzed by one-way ANOVA with different diet treatments as the factor. Post-hoc comparisons were made using Duncan's new multiple range test [24] to detail the significant differences among the treatments ($p<0.05$). All statistical analysis was performed using SPSS version 17.

3. Results and Discussion

The values of temperature, DO, pH, Total Ammonia Nitrogen (TAN), orthophosphate and BOD ranged from 28.1 to 28.3 ºC; 3 to 5 mg L^{-1}; 7.93 to 8.14; 0.41 to 0.48 mg L^{-1}; 0.76 to 0.85; 9.12 to 9.22 mg L^{-1} respectively. It can be noticed from the above data that all the values of the measured water quality parameters are within the normal range of carp rearing [25]. Mean values (± SD) of water quality parameters recorded in different feeding trails are presented in Table 4.

Table 4: Mean values (± SD) of water quality parameters recorded in different feeding trials

Parameters	Diet A	Diet B	Diet C	Diet D
Temperature(º C)	28.31 ± 0.09^{a}	28.45 ± 0.74^{a}	28.35 ± 0.08^{a}	28.37 ± 0.06^{a}
DO (mg L^{-1})	6.81 ± 0.11^{b}	6.50 ± 0.10^{ab}	6.16 ± 0.02^{a}	6.39 ± 0.22^{a}
pH	7.93 ± 0.02^{a}	8.05 ± 0.01^{b}	8.14 ± 0.03^{c}	7.98 ± 0.02^{ab}
TAN (mg L^{-1})	0.41 ± 0.01^{a}	0.47 ± 0.004^{b}	0.47 ± 0.01^{b}	0.48 ± 0.003^{b}
Orthophosphate (mg L^{-1})	0.76 ± 0.006^{a}	0.84 ± 0.014^{b}	0.85 ± 0.005^{b}	0.83 ± 0.50^{c}
BOD (mg L^{-1})	9.12 ± 0.06^{a}	9.18 ± 0.09^{a}	9.22 ± 1.01^{a}	9.14 ± 1.05^{a}

Note: Mean values followed by different letters in the same row are different by *Tukey HSD[a] test, TukeyB[a]* ($p<0.05$)

In the present study, there was no mortality rate in all the different dietary treatments. Growth performances of rohu, catla and mrigal fingerlings are presented in Table 5, 6 and 7 respectively. Weekly growth performances of rohu, catla and mrigal fingerlings are shown in Fig. 1, 2 and 3 respectively. It can be seen from Table 5, 6 and 7 and Fig. 1, 2 and 3 that Diet C treatment contributed greater individual weight gain, and net fish production followed by Diet D, Diet B and Diet A treatments. Weekly SGR values of rohu, catla and mirgal are presented in Fig. 4, 5 and 6. It can be noticed from the figures that in 2nd and 3rd week of culture, maximum SGR was recorded in Diet C followed by Diet D, Diet B and Diet A treatment for all fish species. However, as per Table 5, 6 and 7, maximum overall SGR values for rohu, catla and mrigal were recorded as 3.75 in Diet C; 3.62 in Diet C and 3.59 in Diet D respectively. Minimum FCR values for rohu, catla and mrigal were found to be 1.50, 1.54 and 1.62 respectively in Diet C treatment.

Table 5: Growth performance of Rohu in different feed trial systems

Parameter	Diet A	Diet B	Diet C	Diet D
Initial weight(g)	8.03^{a}	8.04^{a}	8.01^{a}	8.04^{a}
Final weight(g)	69.86^{a}	99.64^{b}	187.59^{d}	154.36^{c}
Net weight gain(g)	61.83^{a}	91.62^{b}	179.58^{d}	146.32^{c}
RGR	24.32^{a}	24.32^{a}	51.52^{d}	30.59^{c}
SGR	2.58^{a}	3.00^{b}	3.75^{d}	3.49^{c}
FCR	1.76^{d}	1.70^{c}	1.50^{a}	1.53^{b}

Note: Mean values in same row with different superscripts are significantly different by *Tukey HSD[a] test, TukeyB[a]* ($p<0.05$)

Table 6: Growth performance of Catla in different feed trial systems

Parameter	Diet A	Diet B	Diet C	Diet D
Initial weight(g)	8.18^{a}	8.12^{a}	8.19^{a}	8.20^{a}
Final weight(g)	67.40^{a}	97.64^{b}	180.93^{d}	145.36^{c}
Net weight gain(g)	59.22^{a}	89.46^{b}	172.74^{d}	137.16^{c}
RGR	19.57^{a}	23.78^{b}	35.22^{d}	29.65^{c}
SGR	2.51^{a}	2.96^{b}	3.62^{d}	3.42^{c}
FCR	1.81^{d}	1.73^{c}	1.54^{a}	1.58^{b}

Note: Mean values in same row with different superscripts are significantly different by *Tukey HSD[a] test, TukeyB[a]* ($p<0.05$)

Table 7: Growth performance of Mrigal in different feed trial systems

Parameters	Diet A	Diet B	Diet C	Diet D
Initial weight(g)	7.66^{a}	7.85^{b}	8.03^{c}	7.95^{b}
Final weight(g)	65.40^{a}	90.64^{b}	134.26^{d}	125.36^{c}
Net weight gain(g)	57.74^{a}	82.79^{b}	126.23^{d}	117.41^{c}
RGR	19.95^{a}	23.48^{a}	30.66^{c}	28.10^{b}
SGR	2.55a	2.91^{b}	3.35^{c}	3.59^{d}
FCR	1.84^{d}	1.77^{c}	1.62^{a}	1.65^{b}

Note: Mean values in same row with different superscripts are significantly different by *Tukey HSD[a test], TukeyB[a]* ($p<0.05$)

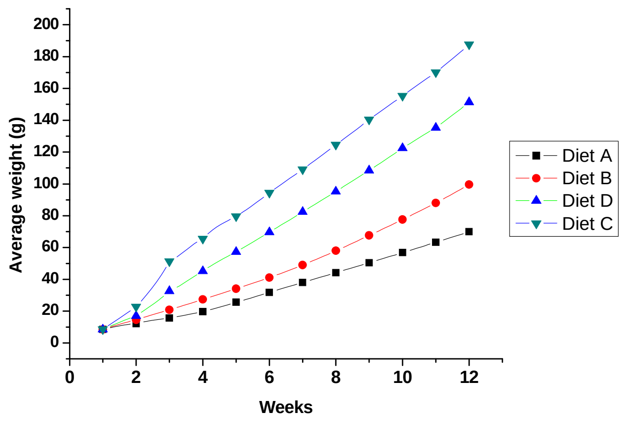

Fig 1: Growth performance of rohu in different treatments

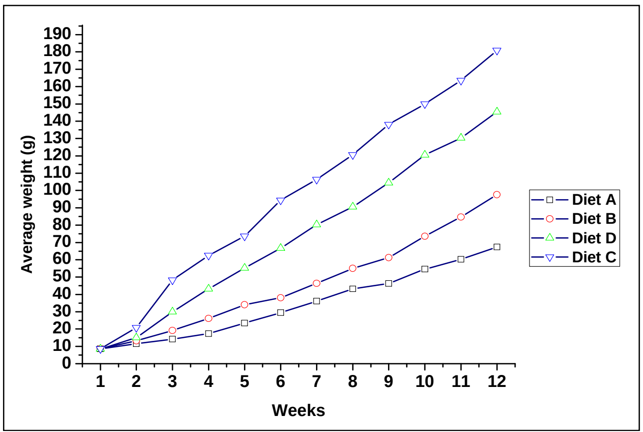

Fig 2: Growth performance of catla in different treatments

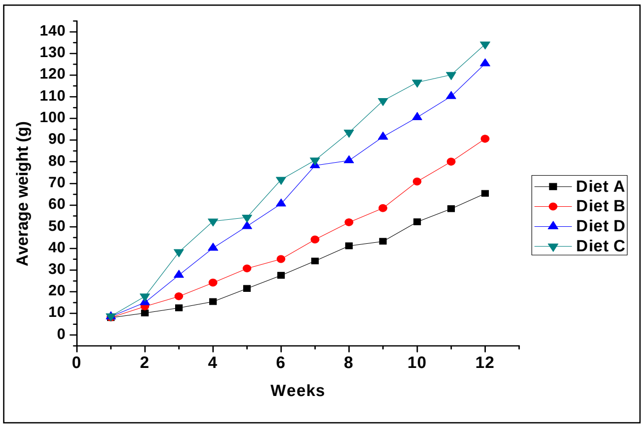

Fig 3: Growth performance of mrigal in different treatments

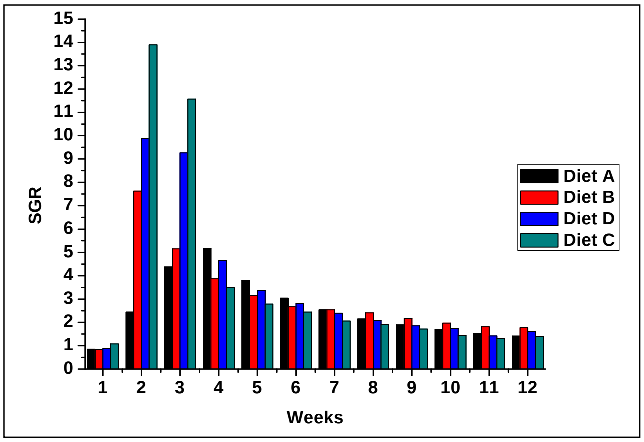

Fig 4: Weekly SGR of rohu in different treatments

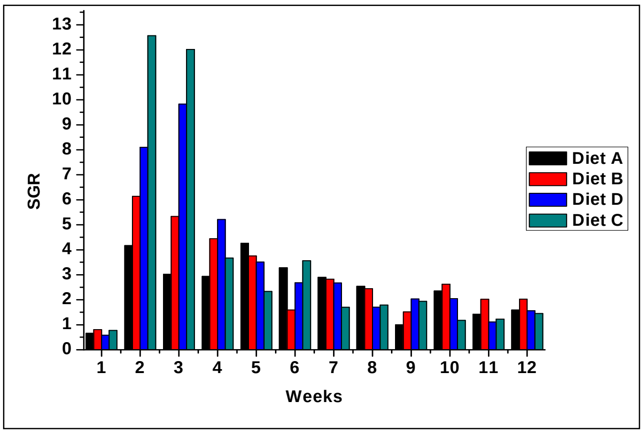

Fig 5: Weekly SGR of catla in different treatments

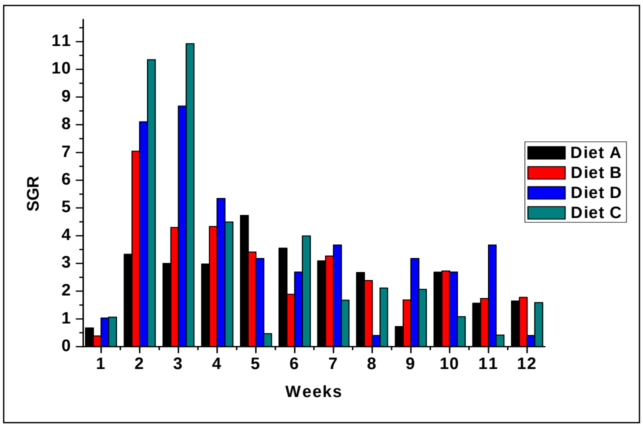

Fig 6: Weekly SGR of mrigal in different treatment

It can be observed from the above findings that, Diet C treatment produced higher growth in comparison to the other treatments. In case of Diet C treatment, total fish production was found to be 63% higher than Diet A, 45% higher than Diet B and 16% higher than Diet D treatment.

The hard cuticle in the earthworm contains the chitin. The growth and dietary performance of fish in Diet D treatment (100% replacement of fish meal with earthworm meal) was slightly less than that in Diet C treatment. It may be due to the presence of more chitin in Diet D treatment than Diet C treatment. Chitin is a polymer of glucosamine and is insoluble in common solvents. Various researchers reported that this chitin depresses growth in fishes. Depressed growth in tilapia *Oreochromis niloticus* × *O. aureaus*, fed with diets containing chitin as low as 2% was observed [26]. Depression in growth in catfish fed with high levels of meal worms occurred because of presence of high levels of chitin [3]. Similar results of depressed growth were also observed while feeding rohu advanced fry with whole earthworm diet and earthworm custard diet containing high levels of chitin [19]. Further, earthworm diets has deficiency in certain amino acids like lysine, methionine and cystine [27, 28] which might have resulted in low growth and feed utilization in Diet D treatment in comparison to Diet C treatment.

4. Conclusion

The main aim of the present study was to evaluate the potential of earthworm meal (*Eisenia fetida*) as a replacement of fish meal for culture of three Indian major carps, catla (*Catla catla*, Hamilton), rohu (*Labeo rohita*, Hamilton,) and mrigal (*Cirrhinus mrigala*, Hamilton). Four experimental iso-nitrogenous (35% crude protein) and iso-caloric (15MJ kg^{-1})

diets were prepared with (i) no replacement of fish meal (Diet A), (ii) 20% replacement of fish meal with earthworm meal (Diet B), (iii) 50% replacement of fish meal with earthworm meal (Diet C) and 100% replacement of fish meal with earthworm meal (Diet D). Based on the results obtained from the study, it can be concluded that 50% replacement of fish meal with earthworm meal in the diet may be used as an ideal alternate protein source for better growth performance of Indian major carps.

5. References

1. Goddard S, Al-Shagaa G, Ali A. Fisheries by-catch and processing waste meals as ingredients in diets for Nile tilapia, Oreochromis niloticus. Aquaculture Research 2008; 39:518-525.
2. Hardy RW, Barrows FT. Diet formulation and manufacturing. In J. E. Halver and R. W. Hardy (Eds.), Fish Nutr. New York, NY: Academic Press. 2002; 3:505-600.
3. Ng WK, Liew FL, Ang LP, Wong KW. Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, Clarias gariepinus. Aquac. Res. 2001; 32:273-280.
4. Sales J, Janssens PJG. Nutrient requirements of ornamental fish. Aqua. Liv. Res. 2003; 16:533-540.
5. Sogbesan AO, Ugwumba AAA. Nutritional values of some non-conventional animal protein feedstuffs used as fishmeal supplement in aquaculture practices in Nigeria. Turk. J of Fish. and Aqua. Sc. 2008; 8:159-164.
6. Zhou F, Song W, Shao Q, Peng X, Xiao J, Hua Y, *et al*. Partial replacement of fish meal by fermented soybean meal in diets for black seabream, *Acanthopagrus schlegelii*, Juveniles. J World Aquacult. Soc 2011; 42: 184-197.
7. EU, Council Regulation (EC) No. 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No. 2092/91. Official Journal of the European Union, Legislation 2007; 189(50):1-23.
8. Shapawi R, Ng WK, Mustafa S. Replacement of fish meal with poultry by- product meal in diets formulated for the humpback grouper, *Cromileptes altivelis*. Aquac. 2007; 273:118-126.
9. Forster IP, Dominy W, Obaldo L, Tacon AGJ. Rendered meat and bone meals as ingredients of diets for shrimp *Litopenaeus vannamei* (Boone, 1931). Aqua; 2003; 219:655-670.
10. Edwards CA, Niederer A. The production and processing of earthworm protein. In C. A. Edwards and E. F. Neuhauser (Eds.), Earthworms in waste and environmental management Hague: Academic Publishing. 1988, 169-180.
11. Hilton JW. Potential of freeze-dried worm meal as a replacement for fish meal in trout diet formulations. Aquaculture 1983; 32:277-283.
12. Nandeesha MC, Srikanth GK, Basavaraja N, Keshavanath P, Varghese TJ, Bano K, *et al*. Influence of earthworm meal on the growth and flesh quality of common carp. Biological Wastes 1988; 26:189-198.
13. Sogbesan OA, Madu CT. Evaluation of earthworm (*Hyperiodrilus euryaulos*; Clausen, 1914; Oligocheata: Eudrilidae) meal as protein feedstuff in diets for *Heterobranchus longifilis*, Valenciennes 1840 (Teleostei Clariidae) fingerlings under laboratory condition. Res. Jour. of Envi. Sci. 2008; 2:23-31.
14. Tacon AGJ, Stafford EA, Edwards CA. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture 1983; 35:187-199.
15. Dynes RA. Earthworms–Technology information to enable the development of earthworm production. (Publication No.03/085,).Kingston: RIRDC. 2003; 1-33.
16. Guerrero RD. The culture and use of *Perionxy excavatus* as protein resource in the Philippines. In J. E. Satchell (Ed.), Earthworm ecology London: Chapman and Hall. 1983, 309-313.
17. Tacon AGJ. Feed ingredients for carnivorous fish species. Alternatives to fishmeal and other fishery resources (FAO of United Nations, Fisheries Circular No.881. FIRI/C881,), Rome. 1994, 35.
18. Hansen RP, Czochanska Z. The fatty acid composition of the lipids of earthworms. J of the Sc. of Food and Agri. 1975; 26:961-971.
19. Mohanta KN, Subramanian S, Korikanthimath VS. Potential of earthworm (*Eisenia foetida*) as dietary protein source for rohu (*Labeo rohita*) advanced fry. Cogent Food & Agri. 2016; 2:1138594.
20. Javed M, Sial MB. Fish pond fertilization. Effect of layer manure fertilization on the growth performance of fish viz., *Catla catla, Labeo rohita* and *Cirrhinus mrigala*. Pak. J Agri. Sci. 1991; 28:115-120.
21. AOAC (Association of Official a nalytical Chemist). Official Methods of Analysis of the A.O.A.C. International 17th edn, Washington, D.C., USA. 2003.
22. Edwards CA. Production of feed protein from animal waste by earthworms. Phil. Trans. R. Soc. Lond. 1985; 310:299-307.
23. APHA. Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, USA. 2012.
24. Duncan DB. Multiple range and multiple F tests. Biometrics. 1955; 11:1-42.
25. Renukaradhya KM, Varghese TG. Protein requirement of carps, *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton). Proceedings: Anim. Sc. 1986; 95:103-107.
26. Shiau SY, Yu YP. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus O. aureus. Aquac*. 1999; 179:439-446.
27. Tacon AGJ, Stafford EA, Edwards CA. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture. 1983; 35:187-199.
28. Pereira JO, Gomes EF. Growth of rainbow trout fed a diet supplemented with earthworms, after chemical treatment. Aquac. Int. 1995; 3:36-42.