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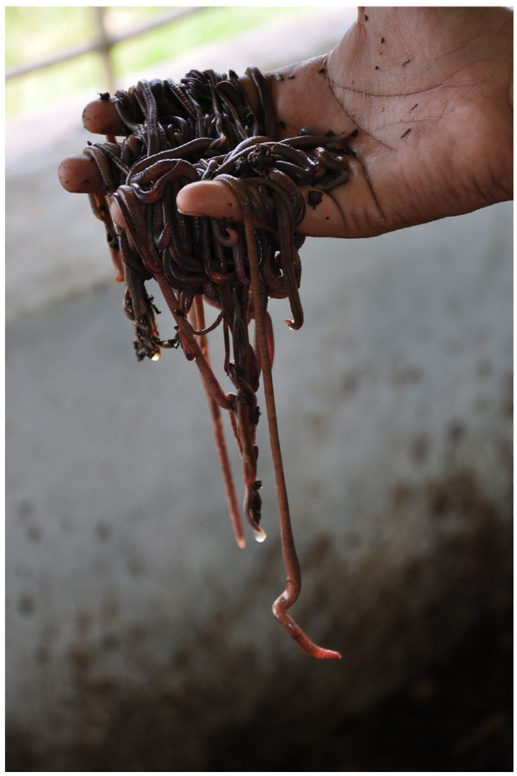

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Abstract: Earthworm, a non-conventional protein source with 520–530 g protein kg^{-1} on dry matter basis was used in three forms viz. (i) whole earthworm (ii) earthworm custard, and (iii) pelleted earthworm diet to prepare three iso-nitrogenous (500 g protein kg^{-1} diet) and iso-caloric (17.0 MJ kg^{-1} diet) experimental diets. The formulated diets were fed ad libitum twice daily to *Labeo rohita* advanced fry (0.71 ± 0.04 g) in triplicates for a period of 35 days. In each replicate, 10 fish were stocked. The 300 L fiberglass-reinforced plastic tanks containing 100 L of water with the provision of continuous aeration were used for rearing the fish. At the end of experiment, the weight gain (g), food conversion ratio, specific growth rate, protein efficiency ratio, protein retention efficiency (%), and energy retention efficiency (%) of fish fed pelleted earthworm diet (2.19, 1.58, 4.21, 1.26, 23.0, and 18.6, respectively) was significantly better ($p < 0.05$) than the corresponding values of whole earthworm (1.53, 2.30, 3.38, 0.84, 14.34, and 11.93, respectively) and earthworm custard (0.94, 3.18, 2.42, 0.62, 10.50, and 8.21, respectively) fed diets. The experiment results indicated that the pelleted earthworm diet could be used in rearing of rohu advanced fry.

Subjects: Bioscience; Environment & Agriculture; Food Science & Technology

Keywords: earthworm; non-conventional feeding; protein; diet; rohu; *Labeo rohita*

Kedar Nath Mohanta

ABOUT THE AUTHORS

The research group of ICAR Research Complex for Goa (ICAR RCG), Goa, India is involved in both strategic and applied research to develop nutritionally balanced cost-effective diet for food and ornamental fish. The first author, Dr. Kedar Nath Mohanta was a Senior Scientist in ICAR RCG, Goa where the present work was carried out. Presently, he is working as Principal Scientist in Fish Nutrition and Physiology Division of Central Institute of Freshwater Aquaculture, Bhubaneswar, India. Dr. Mohanta is mainly involved in study on nutrient requirement and cost-effective diet development of carp and freshwater ornamental fish for nursery and grow-out stages. Currently he has two research projects, one on development of appropriate feeding strategies based on compensatory growth of fish and the other on improvement in protein use efficiency of carp diet. The present work is an attempt to utilize earthworm, a waste product of vermicompost in formulating the low-cost diet for carps.

PUBLIC INTEREST STATEMENT

There is an acute shortage of conventional protein sources particularly the fish meal and oilcakes used in fish feed due to their extensive use and stiff competition from other animal production sectors like dairy and poultry. Therefore, an intensive research efforts have been made by fish nutritionists around the globe to identify and use the suitable non-conventional alternate protein sources. Earthworm, the by-product of waste management process or organic farming is very rich in protein and polyunsaturated fatty acid, could be used as potential fish feed ingredient for nursery rearing of fish. So far, very limited research has been carried out on use the earthworm in fish diet. In the present study we have evaluated the earthworm (*Eisenia foetida*) as alternate dietary protein source in *Labeo rohita* advanced fry. The study results would be helpful in formulating nutritionally balanced low-cost feed for rohu fry.

1. Introduction

Vermiculture, a century old practice is now being revived worldwide for waste management, sustainable organic agriculture, and aquaculture (Chakrabarty, Das, & Das, 2009). Detritivorous terrestrial and aquatic Oligocheate worms are well known for their capacity to break down and utilize human and animal wastes (Guerrero, 1981). Among the various terrestrial worms, earthworm (*Eisenia foetida*) has been proven the most promising and successful till date for vermiculture (Hartenstein, Neuhauser, & Kaplan, 1979; Watanabe & Tsukamoto, 1976). Earthworm effectively converts the waste materials to loose friable compost with potential value as a plant growth medium. Although the earthworm is considered as a by-product of waste management process or vermiculture unit, it can be used as a potential non-conventional animal protein source for formulating the fish feed (Pereira & Gomes, 1995). Earthworm has been found to be a good source of protein with favorable amino acids (Guerrero, 1981; Istiqomah, Sofyan, Damayanti, & Julendra, 2009; Lieberman, 2002; Medina, Cova, Vidna, Pujic, Carlos, & Toress, 2003; Tacon, Stafford, & Edwards, 1982; Reinecke & Alberts, 1987; Tacon, 1994) and it contains variable lipid contents naturally high in ω-3 fatty acids (Dynes, 2003; Guerrero, 1983; Reinecke & Alberts, 1987; Tacon, 1994). Extensive analysis of the lipid fraction of earthworms conducted by Hansen and Czchanska (1975) revealed a high proportion of polyunsaturated fatty acids (linolenic; ω-3 fatty acids), which is essential for formulating fish feed of many species. The protein and lipid contents of earthworm is varying from 50 to 70% and 5 to 10%, respectively, as reported by several earlier researchers (Dynes, 2003; Guerrero, 1983; Hansen & Czochanska,1975; Medina et al., 2003; Pereira & Gomes, 1995; Reinecke & Alberts, 1987; Sogbesan & Madu, 2008; Tacon, 1994). Sogbesan and Ugwumba (2008a) reported that 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 (NRC, 1993). Several researchers have reported the use of earthworm as dietary protein source either alone or in combination with other feed ingredients in formulating the fish diets (Edwards & Niederer, 1988; Guerrero, 1981; Hartenstein et al., 1979; Hilton, 1983; Keshavappa, Devaraj, & Seenappa, 1989; Kostecka and Paczka, 2006; Nandeesha, Srikanth, Basavaraja, et al., 1988; Pereira & Gomes, 1995; Sogbesan & Madu, 2008; Stafford & Tacon, 1984, 1985, 1988; Tacon, Stafford, & Edwards, 1983; Velasquez, Ibañez, Herrera, & Oyarzun, 1991; Watanabe & Tsukamoto, 1976). But, so far the available literature on use of earthworm in formulating the carp diet is very limited. Considering the high nutrient value of earthworm and its huge production potential in the coming years as by-product of vermiculture and waste management processes, a study was undertaken to evaluate the possibility for use of different forms of earthworm in formulating the diets of rohu, *Labeo rohita* advanced fry.

2. Materials and methods

2.1. Experimental diets

Except earthworm, all the other required fish feed ingredients viz. fish meal (sun-dried miscellaneous marine trash fish, mainly the lesser sardines of family Engrolidae and ribbon fish of family Trichuridae available in the locality, neither solvent extracted nor dehulled), groundnut oil cake (de-oiled groundnut; mechanical extraction of oil), lean prawn (Acetes indicus) meal, gelatin (Himedia, Mumbai, India), mineral and vitamins (EMIX PLUS, Mumbai, India), vegetable oil (Marico Industries Limited, Mumbai, India), skimmed milk powder (Amul India, Anand, Gujarat, India), and hen's egg (Premium meat supplier, Panaji, India) were procured from the local market. The earthworm (*E. foetida*) was procured from a local vermicompost unit (Margao, Goa, India). The earthworm was kept unfed for 48 h so as to eliminate all the feed contents that remained earlier in the gut. The fish meal, groundnut oil cake, lean prawn, and earthworm were oven-dried for 24 h at 105°C and then finely powdered using a mixer grinder and sieved through a fine-meshed screen (0.5 mm diameter). The proximate composition of the ingredients was determined (Association of Official Analytical Chemists, 1990), the details of which are presented in Table 1.

Three experimental diets were prepared in which the earthworm was used in three forms as described below:

Table 1. Proximate composition (g kg^{-1} dry matter basis) of feed ingredients used for formulating the experimental diets

Ingredients	Dry matter	Crude protein	Ether extract	Crude fiber	Total ash
Earthworms	170	520	180	78	126.5
Fish meal	922	562.5	81.6	18.0	153.2
Groundnut oil cake	935	445	75.0	69.0	76.0
Prawn meal	926	481	45.0	86.0	182.3
Hen's egg	260	480	456	12	35
Skimmed milk powder	920	370	15	8	3
Gelatin	950	910	5	7	68

2.1.1. Whole earthworm diet (D-1)
The whole earthworm (after 48 h of fasting) were boiled (with a pinch of common salt) in water, thoroughly washed with clean water and chopped into pieces (0.5 mm size) using a sharp knife and stored in a refrigerator at 4°C until use.

2.1.2. Earthworm custard (D-2)
For preparation of earthworm custard, first the earthworm was boiled for 45 min in water adding a pinch of common salt, washed thoroughly in clean water, and then ground using a kitchen mixer grinder (Philips India Limited, Mumbai). The required quantities of skimmed milk powder and hen's egg (yolk + albumin) were added to the ground earthworm and mixed thoroughly. A desired quantity of water was heated in a 1-L beaker to 80°C by using an electric heater and the required amount of gelatin was dissolved in to it with slow stirring. After the gelatin is dissolved properly, it was added to the feed mix. Then the mineral and vitamin mix was added and the whole feed mix was evenly mixed using the kitchen mixer grinder. The required quantity of lukewarm water was added to the feed mix, blended properly, and a dough of feed mix was prepared. The dough was put in to an aluminum container and steam cooked for 20 min using a domestic pressure cooker (Hawkins Make, Mumbai, India) to obtain the earthworm custard. The earthworm custard was cooled at room temperature and stored in a refrigerator at 4°C until use. For daily feeding of the earthworm custard, a piece of earthworm custard was taken and cut in to small pieces (0.5 mm) with a kitchen knife and fed to the experimental fish.

2.1.3. Pelleted earthworm diet (D-3)
For preparation of pelleted earthworm diet, at first the earthworm was boiled in water for 45 min adding a pinch of common salt and washed thoroughly in clear water as described above. Then it was oven-dried for 24 h and powdered with a kitchen grinder (Philips India Limited, Mumbai) and the earthworm meal was prepared. The required quantities of dried earthworm meal, fish meal, groundnut oil cake, prawn meal, mineral and vitamin mix and vegetable oil were mixed thoroughly using a kitchen mixer (Philips India Limited, Mumbai). A desired quantity of water was heated in a 1 L beaker to 80°C by using an electric heater and the required amount of gelatin was dissolved into it with slow stirring. After the gelatin is dissolved properly, it was added to the feed mix. The required quantity of lukewarm water was added to the feed mix, blended properly, and a dough of feed mix was prepared. The dough was passed through a hand pelletizer to obtain 2 mm diameter feed pellets. The pellets were dried at 60°C and stored in a refrigerator at 4°C until use.

All the experimental diets used were iso-nitrogenous (500 g protein kg^{-1} diet) and iso-caloric (17.0 MJ kg^{-1} diet). The ingredient and chemical composition of the experimental diets are presented in Tables 2 and 3, respectively.

Table 2. Ingredient composition (g kg^{-1}) of the experimental diets

Ingredient	D-1 (Whole earthworm diet)	D-2 (Earthworm custard diet)	D-3 (Pelleted earthworm diet)
Earthworm	1000	600	400
Fish meal	–	–	100
Groundnut oil cake	–	–	300
Prawn meal	–	–	100
Skimmed milk powder	–	100	–
Hen's egg	–	220	–
Gelatin	–	60	60
Vitamin & mineral mixture*	–	20	20
Vegetable oil	–	–	20

*Composition of Vitamin and Mineral mix (EMIX PLUS, Mumbai, India) (Quantity/kg): Vitamin A: 22,00,000 IU; Vitamin D3: 4,40,000 IU; Vitamin B2: 800 mg; Vitamin E: 300 mg; Vitamin K: 400 mg; Vitamin B6: 400 mg; Vitamin B12: 2.4 mg; Calcium Pantothenate: 1,000 mg; Nicotinamide: 4 g; Choline Chloride: 60 g; Mn: 10,800 mg; I: 400 mg; Fe: 3,000 mg; Zn: 2,000 mg; Cu: 800 mg; Co: 180 mg; Ca: 200 g; P: 120 g; L-lysine: 4 g; DL-Methionine: 4 g; Selenium: 20 ppm.

Table 3. Proximate composition (g kg^{-1} dry matter basis) of the experimental diets

Parameters	D-1 (Whole earthworm diet)	D-2 (Earthworm custard diet)	D-3 (Pelleted earthworm diet)
Dry matter	182	450	923
Crude protein	520	508	501
Ether extract	160	150	180
Crude fiber	35	18	85
Ash	124	79	126
Gross energy (MJ kg^{-1})	17.56	17.97	17.14

2.2. Experimental design and fish maintenance

Four hundred rohu advanced fry (average weight 0.48 g) were procured from the Anjunem hatchery of the State Fisheries Department, Government of Goa (India) and acclimatized to the laboratory condition for 15 days using two 1000 L fiber-reinforced plastic (FRP) tanks under the provision of continuous aeration using an electrically operated air-blower (Roots Air Blower, Kolkata, India) with a generator back up (Kirloskar, Mumbai, India). During acclimatization, the fish were fed with a fish meal-oil cake-rice bran-based prepared diet (crude protein: 400 g protein kg^{-1} diet; ether extract: 120 g kg^{-1} diet; energy: 16.72 MJ kg^{-1} diet).

After 15 days of acclimatization, groups of 10 healthy and uniformly sized advanced rohu fry (0.71 ± 0.04 g; average weight ± SE) were stocked randomly in triplicate tanks for each dietary treatment following a complete randomized design.

The experimental fish were fed ad libitum twice daily (0830 and 1630 h) to their respective diets for a period of 35 days. Three hundred liter FRP tanks containing 100-L water (static water system) were used for rearing the fish. The unconsumed feed and excreta were removed everyday by siphoning at 0730 and 1530 h and the unconsumed feed with respect to each tank was oven-dried overnight at 105°C and stored separately in the refrigerator at 4°C. The tank wise pooled unconsumed feed sample collected over a period of 35 days was used for calculating the total feed consumption and feed conversion ratio (FCR) after the experiment was completed. The water lost during the removal of unconsumed feed and excreta by siphoning was replenished with the seasoned freshwater every day. In addition to this, about 50% of the water was exchanged in every three days interval.

The experimental tanks were supplied with continuous aeration throughout the experiment period by using an electrically operated air-blower as mentioned above. The final weight was recorded at the end of the experimental period by batch weighing of the fish. The seasoned and aerated groundwater was used for rearing the fish. The natural light cycle was 12 h light/12 h darkness for the experiment.

2.3. Chemical analyses

The proximate composition of experimental diets and whole body was analyzed in triplicates (Association of Official Analytical Chemists, 1990). Dry matter was estimated by oven-drying the samples at 105°C till a constant weight and crude protein percent was calculated by estimating nitrogen content by micro-Kjeldahl method and multiplying with a factor 6.25. Ether extract was determined by solvent extraction with petroleum ether, boiling point 40–60°C, for 10–12 h. Total ash content was determined by incinerating the sample at 650°C for 6 h and crude fiber by acid digestion (1.25%) followed by alkali digestion (1.25%). Gross energy in diets and fish body was calculated by using Bomb Calorimeter (Parr, model 1341; Parr Instrument Company, Moline, IL, USA).

2.4. Water analyses

Except water temperature which was recorded twice daily (0830 and 1430 h), the other water quality parameters such as temperature, dissolved oxygen, total alkalinity, total hardness, ammonia nitrogen (NH_3-N), nitrate nitrogen (NO_3-N), and nitrite nitrogen (NO_2-N) of the experimental tanks were analyzed every week (American Public Health Association, 1989). The water quality parameters in experimental tanks were in the range of Temperature (°C): 25.6–26.7, Dissolved Oxygen (mg L^{-1}): 6.8–7.5, pH: 7.2–7.5, Ammonia Nitrogen (mg L^{-1}): 0.06–0.09; Nitrite Nitrogen (mg L^{-1}): 0.04–0.08, Nitrate Nitrogen (mg L^{-1}): 13.0–18.0 and Total Alkalinity (mg $CaCO_3$ L^{-1}): 95.2–116.3 which were found to be in the normal range of carp rearing (Renukaradhya & Varghese, 1986).

2.5. Calculation of nutritional indices

The different nutritional indices were determined as follows:

$$\text{Specific growth rate (SGR)} = \frac{\text{ln final weight} - \text{ln initial weight}}{\text{Experimental duration(days)}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed intake(dry weight)}}{\text{Total live weight gain}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Total live weight gain}}{\text{Total protein intake}}$$

$$\text{Protein/Energy retention efficiency (PRE/ERE;\%)} = \frac{\text{Protein/Energy gain in body}}{\text{Protein/Energy intake}} \times 100$$

For calculating the FCR of the fish fed whole earthworm diet (D-1) and the earthworm custard diet (D-2), the total feed consumed in wet weight was converted to dry weight (total feed consumed in wet weight × % of dry matter content of the respective diets) and it was divided by weight gain of fish (wet weight). Similarly, for calculating the other nutritional parameters (PER, PRE, and ERE), the feed and nutrient consumption with respect to each dietary treatment were calculated on dry matter basis as the dry matter contents of three experimental diets used were varied widely among them.

2.6. Statistical analysis

The difference among the treatments was tested by one-way analysis of variance (ANOVA) and comparison between the treatments was done by Duncan's multiple range test at $p < 0.05$. The

Table 4. The growth performance of *L. rohita* advanced fry fed different experimental diets after 35 days of culture

Parameters	D-1 (Whole earthworm diet)	D-2 (Earthworm custard diet)	D-3 (Pelleted earthworm diet)
Initial weight (g)	0.66 ± 0.03^a	0.71 ± 0.03^a	0.75 ± 0.03^a
Final weight (g)	2.17 ± 0.08^b	1.65 ± 0.05^c	2.85 ± 0.07^a
Weight gain (g)	1.53 ± 0.05^b	0.94 ± 0.03^c	2.19 ± 0.04^a
Feed conversion ratio (FCR)	2.30 ± 0.05^b	3.18 ± 0.03^a	1.58 ± 0.05^c
Specific growth rate (SGR, % day^{-1})	3.38 ± 0.07^b	2.42 ± 0.06^c	4.21 ± 0.08^a
Protein efficiency ratio (PER)	0.84 ± 0.01^b	0.62 ± 0.01^c	1.26 ± 0.04^a
Protein retention efficiency (PRE)	14.34 ± 0.15^b	10.50 ± 0.16^c	23.0 ± 0.68^a
Energy retention efficiency (ERE)	11.93 ± 0.12^b	8.21 ± 0.09^c	18.56 ± 0.69^a

Note: Mean values with the same superscripts in each row are not significantly different ($p > 0.05$). Values are the means of three replicates ± standard error (SE) for each experimental diet.

statistical package used for the analysis of data was PC-SAS programme for Windows, release v6.12 (SAS, 1996).

3. Results

In the present study, there was no mortality or abnormality of fish found in any of the dietary treatments. The fish fed pelleted earthworm diet (D-3) had significantly higher ($p < 0.05$) weight gain, specific growth rate, protein efficiency ratio, and protein and energy retention and lowest food conversion ratio ($p < 0.05$) compared to whole earthworm (D-1) and earthworm custard (D-2) fed groups (Table 4). However, between the whole earthworm diet and earthworm custard diet fed groups, the growth and dietary indices as mentioned above of the former group was better than the later group.

The whole body composition of fish (Table 5) indicated that there was no variation ($p > 0.05$) in moisture and energy contents among the three dietary treatment groups. But the whole body protein content of fish was significantly higher ($p < 0.05$) for pelleted earthworm diet groups followed by whole earthworm and earthworm custard diet fed groups. However, in contrary to the protein, the whole body ether extract content of fish fed pelleted earthworm diet was significantly lower ($p < 0.05$) than the other two diet fed groups. The total ash content was significantly lower ($p < 0.05$) in fish fed pelleted earthworm diet followed by whole earthworm and earthworm custard diets.

Table 5. Whole body composition of *L. rohita* (g kg^{-1} dry matter basis) advanced fry before and after the experiment

Parameters	Initial	Final		
		D-1 (Whole earthworm diet)	D-2 (Earthworm custard diet)	D-3 (Pelleted earthworm diet)
Moisture	756 ± 5.2^a	743 ± 6.3^a	748 ± 8.0^a	736 ± 4.6^a
Crude protein	652.3 ± 0.5^c	662.6 ± 0.8^b	654.8 ± 1.0^c	668.6 ± 0.7^a
Ether extract	141.3 ± 0.6^a	139.8 ± 0.5^a	140.6 ± 0.5^a	135.2 ± 0.3^b
Total ash	102.6 ± 0.5^a	98.8 ± 0.4^c	100.6 ± 0.5^b	92.8 ± 0.3^d
Gross energy (kcal/g)	5.35 ± 0.03^a	5.40 ± 0.03^a	5.38 ± 0.04^a	5.42 ± 0.02^a

Note: Mean values with the same superscripts in each row are not significantly different ($p > 0.05$). Values are the means of three samples ± standard error (SE) for each experimental diet.

4. Discussion

Fish meal is considered as the best protein source due to its balanced amino acids, vitamins contents, palatability, growth factor, and attractant properties and therefore, most widely used protein ingredients in aquafeed (Goddard et al., 2008; Hardy & Barrows, 2002). But fish meal represents a finite fishery resource and will not be able to supply the aquaculture industry with a continuous source of cheap protein indefinitely (Ng, Liew, Ang, & Wong, 2001). Moreover, the increasing cost of high-quality fish meal required for aquafeed and the declining stocks of fish from capture fishery and competition for feed in animal husbandry 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 (Sales & Janssens, 2003; Sogbesan & Ugwumba, 2008a). The utilization of non-conventional feed stuffs of plant origin had been limited as a results of the presence of alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, heamatoglutinins, saponegin, mimosine, cyanoglycosides, and linamarin to mention a few despite their nutrient values and low cost implications (New, 1987) and therefore, the animal protein source is preferred over the plant protein in formulating the fish feed. Many researchers have reported the use of several non-conventional animal protein sources such as earthworm (Kostecka and Paczka, 2006; Pereira & Gomes, 1995; Sogbesan & Madu, 2008; Stafford & Tacon, 1984; Tacon et al., 1983), worm meal (Ng et al., 2001), silkworm pupae (Edwards & Niederer, 1988a; Nandeesha, Srikanth, Varghese, Keshavanath, & Shethy, 1988; Nandeesha et al., 1990), fermented fish silage (Fagbenro & Jauncey, 1995), maggot meal (Sogbesan, Ajuonu, Ugwumba, & Madu, 2005; Ugwumba, Ugwumba, & Okunola, 2001), garden snail meal (Sogbesan, Ugwumba, & Madu, 2006), termite meal (Sogbesan & Ugwumba, 2008b), poultry by-product (Yigit et al., 2006; Shapawi, Ng, & Mustafa, 2007), fisheries by-catch and fish processing by-products (Goddard et al., 2008; Hardy, Sealey, & Gatlin III, 2005; Li, Wang, Hardy, & Gatlin III, 2004; Rathbone, Babbitt, Dong, & Hardy, 2001; Whiteman & Gatlin, 2005), co-dried fish silage (Goddard & Perret, 2005), slaughter house waste meal (Singh, Gaur, Barik, Sulochona, & Singh, 2005), turkey meal (Muzinic, Thompson, Metts, Dasgupta, & Webster, 2006; Thompson et al., 2007), tuna by-product meal (Tekinay, Deveciler, & Guroy, 2009), fermented fish offal meal (Mondal, Kaviraj, & Mukhopadhyay, 2008, 2011), and surimi by-product meal (Mohanta, Subramanian, & Korikanthimath, 2013). In the present study, earthworm was used in formulating experimental diets and the diets were fed to rohu advanced fry to elucidate the potentiality of earthworm as a dietary protein source.

The earthworm (*E. foetida*) meal has high protein content and the amino acid compositions are close to fish meal and hen egg (Medina et al., 2003; Istiqomah et al., 2009; Zhenjun, Xianchun, Lihui, & Chunyang, 1997). The whole body protein and lipid contents of earthworm used in the present study were 52% and 18%, respectively. The crude protein content of earthworm was lower than many of the earlier reported values (Guerrero, 1983; Medina et al., 2003; Reinecke & Alberts, 1987; Sogbesan & Madu, 2008; Zhenjun et al., 1997). But the whole body lipid content of 18% is found to be significantly higher than the earlier reported values (Dynes, 2003; Guerrero, 1983; Reinecke & Alberts, 1987; Sogbesan & Madu, 2008; Tacon, 1994). The higher body lipid content might have resulted in lower body protein content as there is always an inverse relationship between the protein and lipid contents in animal tissue. Moreover, the protein and lipid contents of the earthworm are species specific. Again, the culture environment (nutrient/medium) plays a great role in determining the nutrient composition of earthworm. As we do not know the culture environment of the earthworm as reported by the earlier researchers, it is difficult to compare the nutrient composition of the earthworm found in the present study and the earlier reports.

The hard cuticle in the earthworm contains the chitin. The growth and dietary performance of fish fed whole frozen earthworm (D-1) and earthworm-based custard (D-2) was not encouraging; it may be due to the presence of more chitin that is derived from cuticle when earthworm was incorporated at higher levels (100% in D-1 and 60% in D-2) in the diets. Chitin is a polymer of glucosamine and is insoluble in common solvents. Although we have not measured the chitin contents in the diets, the earthworm used in the present study contains 7.8% fiber (on dry weight basis), which mostly represent its chitin content. Similar to our observations, Ng et al. (2001) reported the growth depression and poor feed and protein utilization in cat fish fed high levels of meal worm or solely on meal worms due to presence of chitin in the meal worm. Shiau and Yu (1999) reported that tilapia *Oreochromis niloticus* × *O. aureaus* fed diets with chitin as low as 2% exhibited depressed growth and feed efficiency. Another reason for low growth and nutrient utilization in fish fed solely (D-1) or higher levels (D-2) of earthworm in the diets may be due to deficiency in certain amino acid like lysine, methionine, and cystine in earthworm as reported by Tacon and Jackson (1985) and Pereira and Gomes (1995).

Tacon et al. (1983) reported that the rainbow trout fed 100% worm meal (*E. foetida*) protein in the form of frozen slices did not yield any encouraging growth and dietary performance which is in agreement with our study. Similar to our results, Pereira and Gomes (1995) also reported that the growth rate and feed utilization efficiency affected adversely when the diets containing high level of frozen worms were fed to rainbow trout. According to Edwards and Niederer (1988), worm meal is able to substitute fish meal for monogastric animals and fish, with 25–50% of dietary protein could be supplied from worm meal. In the present study, the diet D-3, for which the growth is significantly better ($p < 0.05$) than other two diets, about 25% of the dietary protein had supplied by earthworm meal. Stafford and Tacon (1985) evaluated the dried earthworm meal derived from *E. foetida* in the diet of rainbow trout *Salmo gairdneri* and reported that there was no adverse effect on the growth performance or feed utilization efficiency of fish fed diets containing low levels of earthworm meal. Ganesh et al. (2003) observed better growth and nutrient utilization in carp (*Cirrhinus mrigala*) fed diet containing *E. foetida* worm meal than the fish meal-based diet. Experimental results of Keshavappa et al. (1989) showed no difference in weight gains in carp (*Catla catla*) fry fed 30% inclusion of earthworm meal compared to the 30% inclusion of fish meal. Further, survival was higher in former (75.75%) than the later (66.66%). Kostecka and Paczka (2006) reported that the aquarium fish *Poecilia reticulata* fed earthworm (*E. foetida*) biomass resulted in significantly increased brood number and also produced twice the off-spring than the fish fed a diet without earthworm.

Worm meal obtained from *E. foetida*, when used to replace 25 and 50% of the fish meal component in the diets for rainbow trout, gave higher growth rates in fish fed these diets compared to control diet without any worm meal (Velasquez et al., 1991). Nandeesha, Srikanth, Basavaraja, et al. (1988) tried to replace fish meal partially or completely with dried worm meal (*Eudrilus eugeniae*) in the culture of common carp and found that the diet where the fish meal is partially replaced by earthworm meal (incorporating 5% sardine oil) gave best results than the diets where the fish meal is completely replaced by earthworm meal or a diet prepared by using fish meal as sole protein source without any worm meal (control). In contrast, Tacon et al. (1983) reported a slightly lower growth response in rainbow trout when worm meal (*E. foetida*) was used to replace 50% dietary herring meal protein compared to fish fed control diet, in which herring meal was used as the sole source of dietary protein. Stafford and Tacon (1984) reported that the growth and feed utilization efficiency of rainbow trout was not affected when the herring meal protein is replaced by earthworm (*Dendrodrilus subrubicundus*) meal at 10%, but there was decline in fish performance at higher replacement levels of 50 and 100%. Akiyama et al. (1984) reported that the chum salmon *Oncorhynchus keta* swim up fry fed fish meal diets supplemented with earthworm powder (5%) showed the best growth performance and feed efficiency than fish fed fish meal diets supplemented with silkworm pupae powder (5%), dried beef liver (5%), and krill meal (5%). In the present study, we observed best growth and nutrient utilization in fish fed pelleted earthworm diet (D-3) containing 40% earthworm as major protein source.

The FCR of 1.58 obtained in the present study in fish fed diets D-3 containing dried earthworm as main protein source is similar or even better to the earlier researchers who used different non-conventional dietary protein sources such as fisheries by-catch and processing waste for tilapia (1.34–1.55, Goddard et al., 2008), garden snail meal in *Clarias gariepinus* (1.21–1.44, Sogbesan et al., 2006), silkworm pupae in carps (1.52–3.35, Edwards & Niederer, 1988a; Mahata, Bhuiyan, Zaher, Hossain, & Hasan, 1994; Nandeesha, Srikanth, Varghese, et al., 1988; Nandeesha et al., 1990; Nandeesha, Gangadhara, Varghese, & Keshavanath, 2000), slaughter house waste in *L. rohita* (1.89–2.50, Singh et al., 2005), poultry by-product meal in *Morone chrysops* × *M. saxatilis* (1.58–1.72, Rawles et al., 2006); turkey meal in *M. Chrysops* × *M. saxatilis* (1.70–2.65, Muzinic et al., 2006; Thompson et al., 2007), blood meal in *L. rohita* (1.86–3.1, Saha & Ray, 1998), fermented fish offal in *Heteropneustes fossilis* (1.54–1.87, Mondal et al., 2008), tuna-by product meal in rainbow trout, *Oncorhynchus mykiss* (1.6–1.9, Tekinay et al., 2009), and fermented fish offal in *Labeo bata* (1.2–1.7, Mondal et al., 2011).

The SGR values obtained in the present study is compared with reported values of fish fed diets containing various non-conventional animal protein sources and it was found that the SGR value of 4.21 obtained in diet T-3 (earthworm meal-based dry pelleted diet) is either comparable or better than the 0.84–3.07 reported in *P. gonionotus* and common carp Cyprinus carpio fed silkworm pupae-based diet (Mahata et al., 1994; Nandeesha et al., 1990), 0.59–0.99 in *L. rohita* fed offal meal based diets (Khan & Jafri, 1992), 3.2 in *Clarias gariepinus* fed mealworm-based diets (Ng et al., 2001), 1.73–2.48 in *L. rohita* fed slaughter house waste meal (Singh et al., 2005), 0.51–0.71 in C. gariepinus fed garden snail meal-based diets (Sogbesan et al., 2006), 0.71–1.45 in *Psetta maeotica* fed poultry by product meal diets (Yigit et al., 2006), 0.65–2.05 in *M. chrysops* × *M. saxatilis* fed turkey meal-based diets (Muzinic et al., 2006; Thompson et al., 2007), 0.62–0.98 in *Heterobranchus longifilis* fed earthworm meal-based diets (Sogbesan et al., 2006), 1.4–1.7 in *Cromileptes altivelis* fed poultry by-product meal (Shapawi et al., 2007), 2.7–3.1 in *Oreochromis niloticus* fed fisheries by-catch and processing meal waste-based diets (Goddard et al., 2008), 1.20–1.36 in *H. fossilis* fed fermented fish offal-based diets (Mondal et al., 2008), 0.6–0.7 in *O. mykiss* fed tuna by-product meal (Tekinay et al., 2009) and 1.05–1.34 in *L. bata* fed fermented fish offal meal (Mondal et al., 2011). The higher SGR obtained in the present study than many of the other researchers as mentioned here could be due to comparatively more weight gain of fish in a shorter rearing period of 35 days.

The PER of 1.26 obtained in D-3 is comparable to the PER reported by different authors using various non-conventional protein sources in the diet of fish (1.19–1.75, Singh et al., 2005 for *L. rohita* with slaughter house waste; 0.99–1.05, Thompson et al., 2007 for sunshine bass with turkey meal; 1.3–1.5, Tekinay et al., 2009 for rainbow trout with tuna by-product; 1.3–1.7, Shapawi et al., 2007 for *C. altivelis* with poultry by-product meal; 0.75–1.25, Mondal et al., 2011 for *Labeo bata* with fermented fish offal meal).

The protein retention of 23.0% found in fish fed diet D-3 in the present study is similar to the 14.7–23.3% reported by Mondal et al. (2011) for *L. bata* fed fermented fish offal-based diets and 18.31–31.80 by Shapawi et al. (2007) for *C. altivelis* with poultry by-product meal-based diets. The higher growth rate and whole body protein and also the proportionately lower dietary protein fed in relation to weight gain in this group of fish resulted significantly higher protein retention than D-1 and D-2 groups of fish. Similar to protein retention, the energy retention was also found to be significantly better in D-3 fed group than the D-1 and D-2 fed groups. This may be due to more energy gain in fish fed diet D-3 in relation to dietary energy fed through diet. The significant higher growth rate and comparatively more whole body energy in D-3 group of fish resulted more energy gain than other two groups.

Although the whole body moisture and energy contents did not vary significantly ($p > 0.05$) between the initial and final fish, there was a significant variation in whole body protein, ether extract and ash contents. The fish fed D-3 diet had significantly higher whole body protein content, but lower lipid and ash contents. The whole body protein and lipid are inversely related. The negative correlation between whole body protein and lipid contents was reported in many fish species (Hafedh, 1999; Mohanta, Mohanty, Jena, & Sahu, 2008). The higher whole body protein content in D-3 is reflected as more weight gain in this group of fish. The whole body moisture, protein, lipid, and ash contents in the present study are in the same ranges as reported by earlier researchers for other fish species fed with different non-conventional protein sources (Goddard et al., 2008; Mondal et al., 2011; Muzinic et al., 2006).

From the present study, it is concluded that the earthworm could be used as an ideal alternate protein source for formulating the diet of *L. rohita* advanced fry and the pelleted earthworm diet is better utilized by rohu for its growth and nutritional gains as compared to whole earthworm and earthworm custard diets.

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