International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2012; 2(5):117-123

Research Article

NUTRITIVE POTENTIAL OF EARTHWORM (*EISENIA FOETIDA*) MEAL IN THE DIET FOR NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLINGS

Mukti Pada Bag[1*], Subhash Chandra Mahapatra[2],Pavuluri Srinivasa Rao[3], Debajyoti Chakrabarty[4] and Himadri Pal[5]

1Rural Development Centre, Indian Institute of Technology, Kharagpur, West Bengal, India.
2 Rural Development Centre, Indian Institute of Technology, Kharagpur, West Bengal, India.
3 Agricultural and Food Engineering Department,Indian Institute of Technology, Kharagpur, West Bengal, India.
4 Associate Professor, Post Graduate Department of Zoology, Barasat Government College, Barasat. North 24Parganas West Bengal, India.
5 Singhania University, Pacheri bari, Jhujhunu, Rajastan.

(Received: 03 September, 2012; Accepted: 15 September, 2012; Published: 29 October, 2012)

Corresponding author's email: muktipbag@gmail.com

Abstract: Earthworm meal (ETM) was evaluated to investigate its effect on growth and fatty acid profile of juvenile Nile tilapia, *Oreochromis niloticus*. Triplicate groups of fish with average initial body weight (5.57 ± 0.15 g) were fed each with earthworm meal (ETM) and market available feed (MAF). After twelve weeks of feeding trials, fish fed with ETM revealed the significantly highest values for live weight gain, specific growth rate, better feed conversion ratio as well as protein efficiency ratio compared to the fish fed with MAF. Survival range was 80–100% in all the treatments. There were no significant differences (P>0.05) in the HSI and GSI of the fish. PUFA content and n3/n6 ratio were differed significantly in ETM fed fish. These results clearly indicate that earthworm had good effect on growth performance as well as fatty acid profile of fish.

Key words: Earthworm, fatty acid profile, specific growth rate, PUFA and n3/n6 ratio.

INTRODUCTION

Fish feed generally constitutes 60–70% of the operational cost in intensive and semi-intensive aquaculture system.[1] The fish feed used in aquaculture is quite expensive, irregular and short in supply in many third world countries. These feeds are sometimes adulterated, contaminated with pathogen as well as containing harmful chemicals for human health. Naturally there is a need for the preparation of healthy, hygienic fish feed which influences the production as well as determines the quality of cultured fish.

Considering the importance of nutritionally balanced and cost-effective alternative diets for fish, there is a need for research effort to evaluate the nutritive value of different non-conventional feed resources, including earthworm (*Eisenia foetida*). Earthworm is a bye product vermicompost farm contains substantial amount of protein and minerals.[2-4] Earthworms have been used as supplementary feeds in fish farming since the early times of freshwater fish culture [5-6] and still play an important role as fish feed in extensive culture systems. However, these materials are not evaluated for essential fatty acid content earlier. The beneficial fatty acid in fish body is synthesis from the feed materials they consumed.[7] The beneficial effects of fish lipids on human health have already been well established.[8] It is therefore of utmost importance to determine the influences of feed on growth as well as fat deposition in fish.[9] The aim of this study is to evaluate the growth of O. niloticus by using ETM and also to study the qualitative changes in fish flesh.

METERIALS AND METHODS

Experimental set up

Twenty fingerlings in triplicate groups used in two different treatment. Altogether one hundred and twenty (120) Nile tilapia (male and female ratio 1:1) fingerlings were used in this experiment.The fish fingerlings were treated with potassium permanganate solution (1 mg L^{-1}) to remove any external parasites and were acclimatized in a big tank for five days. Experiments were carried out at the tanks of aquacultural engineering section of IIT-Kharagpur, Paschim Medinipur, West Bengal, India. Each group of fingerlings also were initially weighed to record the initial biomass. They were stocked in six rectangular cemented cement tanks (1000 L). The water system was static in nature and the bottom of the tank was filled with local agricultural soil (pH 6.4 ± 0.05). The experiment was conducted for 60 days from June to July in the year 2010. Dechlorinated well water (temperature 26 ± 3 °C, pH 7.0 ± 0.05, free CO2 0.4 ± 0.01 mg L^{-1}, available nitrogen 0.5 ± 0.05 mg L^{-1} and dissolved

oxygen (DO) 6 ± 0.5 mg L^{-1}) was used in the experiment.

Feed formulation and preparation

The principal feed ingredient (earthworms) was collected from local vermicompost farm at very low cost. These substances were economically cheap but contained significant amount (36–40%) of crude protein (Sogbesan and Ugwumba, 2008). Biochemical composition of earthworm used in the feed for tilapia is shown in Table 1. Diets used for growth trial were prepared that feed formulations remain almost isonitrogenous (30 g 100 g^{-1}) and isoenergetic (4Kcal g^{-1}) in nature. The choice of these nutrient levels, particularly protein, was intended to reflect the practical diets used in India. Diet formulations are presented in Table 2. Mustard oil cake, wheat flour, rice bran, egg shell dust and vitamin premix were common ingredient in every feed tested. These ingredients were used to compensate lipid, protein and ash deficiency in formulated feed. Wheat flour was selected as binder. Each feed was fortified with egg shell dust which is available free of cost for calcium supplement. This was added keeping in mind that the developing fish needs huge quantity of calcium for its bone development. The different ingredients were thoroughly mixed using a food mixer (A200 Hobart Ltd). The proportion of different feed ingredients was determined by using Pearson's square method. The mixture was given the shape of pellets using a Pellet Mill (Model CL2) with a 12 mm die. The resulting pellets were dried in a hot air oven for 48 h at 50 ºC , packed in polythene bags and kept in dry and cool place.

Table 1 Biochemical composition of earthworm used for feed for tilapia (*O. niloticus*)

Ingredient (%)	Earthworm
Dry matter	92.54
Crude protein	40.43
Crude lipid	9.87
Carbohydrate	9.11
Ash	11.85
Nitrogen free extract	12.49
Crude fibre	8.79
Gross energy (Kcal g^{-1})	3.85

Feeding

The feed was given ad libitum in a feeding bag hung from an iron rod in four locations in each tank. Unconsumed feed was removed after 1hour from the beginning of feed administration and dried in a hot air oven at 50 °C. Feed consumption was estimated by subtracting the weight of the unconsumed feed from the weight of the feed offered. Fish, feed samples, and unconsumed feeds were weighed on pan electric balance to an accuracy of 0.1 mg.

Table 2 Detailed information of ETM diet

Ingredients	**% of CP in ingredient**	**% of ingredient in formulated feed**	**% of crude protein in feed**	**% of lipid in feed**	**% of carbohydrate in feed**	**Calorific value of feed (kcal/g)**
Earthworm	40.43	32.2	25.80	9.2	9.2	4.1
MOC	34.65	27.8				
Wheat flour	9.08	38.2				
Egg shell dust	1.8	1.8				

Growth calculation

Growth and nutrient utilization were determined in terms of feed intake (FI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and hepatosomatic index (HSI) as follows[10].

FI (g fish-1 day-1) = Total feed intake per fish/number of days

SGR (% day-1) = 100 × (ln[final body weight]-ln[initial body weight])/no. of Days

FCR = feed intake/live weight gain

PER = live weight gain/crude protein intake

HSI (%) = 100 × (liver weight/total body weight)

GSI (%) =100 × (weight of gonad /total body weight)

Analysis

Feeds and carcass samples were analyzed following standard procedures (AOAC, 199O)[11]: Dry dry matter (DM) after drying in a hot air oven (Gallenkamp, UK) at 105 °C for 24 h; crude protein (CP) by Kjeldahl method (N × 6.25) after acid hydrolysis, crude lipid (CL) after extraction with petroleum ether for 7-8 h by Soxhlet method (40–60 °C boiling range), total ash by igniting at 550 °C for 3 h in muffle furnace (Size 2, Gallenkamp, UK). Organic matter (OM) was calculated by subtracting total ash from DM [12] Crude fibre was determined using a moisture free defatted sample which was digested by a weak acid HCl (0.1N) followed by a weak base NaOH (0.1N) using the Fibertec System 2021 (FOSS, Denmark). Nitrogen-free extract was determined by subtracting the sum of crude protein, crude lipid, crude fibre and ash from DM [13]. Gross energy was determined using a Bomb Calorimeter Model-DFU 24 following the process as described below. The sample was combusted in a chamber pressurized with pure oxygen and resulting heat measured by increase in the temperature of the water surrounding the bomb.

Extraction of Lipids

The total lipids were extracted from all the samples, (fish flesh-2, feed-2) following the method of Bligh and Dyer (1959) using methanol-chloroform (2:1, v/v), methanol-chloroform-water (2:1:0.8, v/v/v), and then again with the first solvent system viz.,methanol-chloroform (2:1, v/v). Sample was ground with the solvent methanol-chloroform (2:1,v/v), filtered through Whatman no. 1 filter paper and residue was extracted with the next solvent system ,consisting of methanol-chloroform-water (2:1:0.8, v/v/v). The process was repeated once again with methanol-chloroform (2:1, v/v). Finally, the three extracts were pooled, diluted with three volumes of water (100-200 ml, depending on the volume of pooled extracts) and layer was allowed to separate in a separatory funnel made by Pyrex glass Co.. The chloroform layer at the bottom of the separatory funnel was withdrawn and dried over anhydrous sodium sulphate in glass stoppered conical flasks, by Pyrex. The chloroform solution of lipid was evaporated in a rotary vacuum evaporator by Rotavap under a pressure of 40-50 mm of Mercury, weighed on a micro-balance by Sartorius and redissolved in distilled n-hexane (10-20 ml) and kept at -20 °C for future use. BHT (butylated hydroxy toluene) was added at a level of 100 mg/L to the solvent as antioxidant.

Preparation of Methyl Ester of Fatty acids

Total lipid of various (fish flesh-2, feed-2) samples was dissolved in anhydrous methanol containing concentrated Sulfuric acid (1.0%, v/v) and the mixture was refluxed for 2 hours[14]. Methanol was evaporated to a small volume (1-3 ml) and cooled to 4 °C, in a freezer. Distilled water 10–15 ml was added to the cooled mixture (1-3 ml) in hard glass test tubes by Pyrex and the methyl esters of fatty acids were extracted 3 times with aliquots (5-10 ml) of diethyl ether, vortexed in a Vortex mixer. The ethereal extracts were taken out by Pasteur pipettes, pooled and dried over anhydrous sodium sulphate, (1-2 gm) in conical flasks (25-50 ml capacity) with glass stopper, filtered through Whatman no. 1 filter paper, vacuum dried, redissolved in n-hexane (1-2 ml volume) and kept in a freezer at 4 °C for future use.

Purification of Fatty Acid Methyl Ester (FAME) by Thin Layer Chromatography (TLC)

Fatty acid methyl esters were purified by TLC using a solvent system of nhexane- diethyl ether (90:10, v/v). [15-16] A standard methyl ester was also run on the same plate in a separate lane, for identification of the methyl ester bands in the samples. The location of methyl ester bands were indicated by placing the TLC plate in an iodine vapour chamber by Pyrex glass co.. The methyl ester bands corresponding to the standard were marked and then scrapped off the plate with a sharp rajor blade. Methyl esters were recovered by extracting the silica gel bands containing the methyl ester samples in a mini glass column (10 cm length x 0.8 cm internal diameter, by Pyrex) with chloroform (30-50 ml), the later was evaporated and the methyl esters were kept in n-hexane (1-2 ml) in a freezer at 4° C till analyzed by Gas Liquid Chromatography (GLC).

Gas Liquid Chromatography (GLC)

GLC of fatty acid methyl esters were done on a Chemito 1000 instrument, equipped with Flame Ionization Detector (FID). Quantifications were done by computer using specific Clarity Lite software.

Analysis of fatty acid methyl esters (FAME)

GLC of FAME was done on a BPX-70 megabore capillary column of 30 mt length and 0.53 mm internal diameter i.d. obtained from SGE, Australia. Oven temperature was programmed from 150 °C – 240 °C with a rate of 8 °C/min. Initial and final temperatures were kept isothermal for 1 minute and 20 minutes respectively. Injection port and detector temperatures were 250 °C and 300 °C respectively. Nitrogen gas was used as carrier gas and its flow rate was 6.18ml/min.

Statistical analysis

Data are presented as means ± SD. One-way ANOVA was used to find the significant effects of feed type and rearing period on the feed and growth rates and also to test the significance level of feed type on production of fingerlings[17.]

Table 3 Growth performance and nutrient utilization of *O. niloticus* fed with ETM and MAF

Particulars	ETM	MAF
Initial weight (g)	5.10 ±0.03[a]	5.10 ±0.02[a]
Final weight (g)	92.28 ±0.25[a]	80.26 ±0.24[b]
Initial length (cm)	4.50 ±0.01[a]	4.50 ±0.01[a]
Final length (cm)	15.40 ±0.11[a]	14.60 ±0.10[b]
Feed intake (g $fish^{-1}$ day^{-1})	2.08 ±0.05[a]	2.21 ±0.02[b]
Specific growth rate (% day^{-1})	0.96 ±0.04[a]	0.83 ±0.05[b]
Feed conversion ratio	2.15 ±0.05[a]	2.65 ±0.05[b]
Protein efficiency ratio	1.55 ±0.02[a]	1.25 ±0.04[b]
Hepatosomatic index	1.89 ±0.08[a]	1.87 ±0.07[a]
Gonado somatic index	1.65 ±0.07[a]	1.62 ±0.06[a]

RESULTS AND DISCUSSION

The highest weight gain (92.28 g) was observed in the ETM applied feed. The growth rate always faster in ETM fed fish than fish fed with MAF (Figure 1). This indicates that fish can consume the ETM feed well. This was possibly due to their higher palatability and preference of the fish to take it as their potential food.

But the amount of feed intake was highest (2.49 g) in MAF. The feed conversion ratio (FCR) was differed significantly and lowest value (2.15) was recorded from ETM fed fish indicating an encouraging effect on economic involvement in fish farming.

The specific growth rate (0.96) and protein efficiency ratio (1.55) were highest in ETM fed treatment. This indicates the better quality of protein in the feed produced from earthwarm (ETM). The hepatosomatic index (%) (HSI) was not differed significantly between two treatments (Table 3). The highest (1.64) value of gonadosomatic index (%) (GSI) was observed in ETM feed treatment. The higher value of GSI indicates that the ETM has better impact on the reproductive function. The Saturated Fatty Acid (SFA) content (%) was highest (53.9) in MAF fed treatment. The Mono Unsaturated Fatty Acid MUFA content (%) was highest (25.6) in MAF fed treatment. The Poly Unsaturated fatty acid (PUFA) and the n-3/n-6 ratio were highest in ETM fed fish (Table 4).

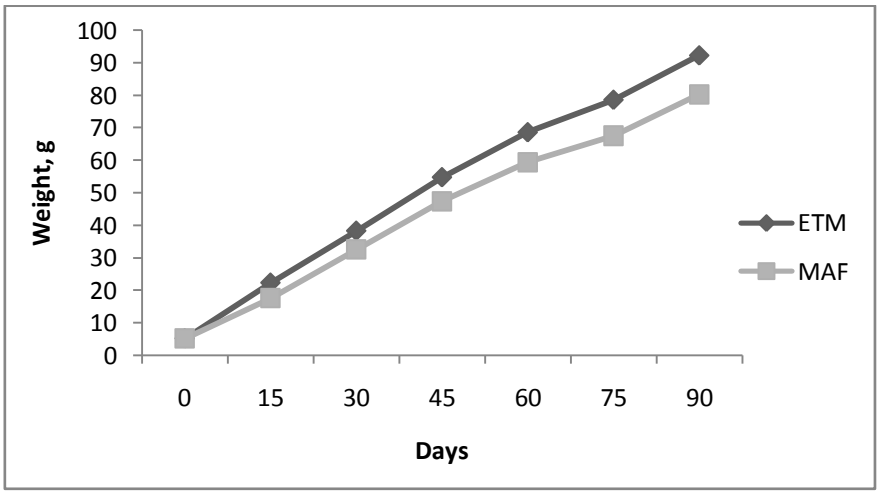

Figure 1: Growth rate of *O. niloticus* fed with ETM and MAF

Table 4 FA profiles of *O. niloticus* fed with ETM and MAF feeds (% w/w of each component in total fatty acids)

Components	ETM fed fish	MAF fed fish
Saturated		
14:0	5.0	4.5
15:0	1.1	1.8
16:0	29.2	28.0
17:0	0.6	2.4
18:0	6.6	8.5
20:0	0.8	0.6
22:0	5.2	6.7
24:0	1.0	1.4
Σ SFA	**49.5**	**53.9**
Monoene		
14:1	0.8	0.5
15:1	0.3	0.9
16:1	7.5	7.2
17:1	0.3	1.1
18:1ω9	11.9	12.5
20:1ω9	1.7	1.3
22:1ω11	1.0	0.6
24:1	1.5	1.5
Σ MUFA	**25.0**	**25.6**
Diene		
16:2	0.4	0.3
18:2ω6	6.0	5.9
20:2		0.1
Σ DUFA	**6.6**	**6.3**
Polyene		
18:3ω6	0.3	0.5
18:3ω3	4.0	3.1
20:3ω6	1.0	0.5
20:3ω3	0.15	0.00
20:4ω6	1.3	0.8
20:5ω3	2.0	1.1
21:5ω3	0.6	0.7
22:5ω6	0.2	0.3
22:5ω3	3.6	2.4
22:6ω3	5.9	5.4
Σ PUFA	**19.25**	**14.30**
Total -ω3	**16.25**	**12.70**
Total -ω6	**8.8**	**8.0**
n3/n6	**1.84**	**1.58**

Ackman [18] stated that only 14 fatty acids are really needed to describe the fatty acids of fish. However, Ackman et al. [19] listed 64 fatty acids from 5 fresh water fishes of West Bengal, India. The fish under discussion recorded 28 fatty acids of the total lipid (TL) and the result is more or less similar to those reported from other tropical and certain temperate zone fresh water fishes. According to Ackman et al. [19] dominant fatty acids in lipids of all the fishes were myristic (14:0), palmitic (16:0), stearic (18:0), palmitoleic (16:1ω7), oleic (18:1ω9), linoleic (18:2ω6), linolenic (18:3ω3), arachidonic (20:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids. The present results corroborate with the above findings. The total SFA of our experimental fish was nearly double than the amount reported from Ackman et al. [19].

Fatty acid deficiency in fish species is indicated by the presence of eicosatrienoic acid (20:3n-9) [20] .Thus, the absence of eicosatrienoic acid in these fish indicates that these fish are not suffering from any fatty acid deficiency. This observation corroborates that of Nematipour and Gatlin for hybrid striped bass in the USA.[21]

The n-3 PUFA is the chief group of components through which the beneficial effects of fish are mediated. The principal effects of n-3 PUFA are antithrombogenic and antiarrhythmic, whereas that of n-6 PUFA is antiatherogenic [22-23] stated that n3/n6 ratio should range 1–2 for fresh water fish. The n3/n6 ratio of our experimental fish was within the same range. The fish fed with ETM accumulates more n-3 fatty acids than n-6 fatty acids which increase n-3/n-6 ratio (1.84) in return.

CONCLUSION

The feed prepared from earthworm enhance growth and thereby yield of *Oreochromis niloticus*. It improves quality of fish by accumulating more n-3 PUFA in the flesh of the fish as well as increasing the n3/n6 ratio which is beneficial for human health. Moreover, the feed can be formulated at local level leading to employment generation in rural areas.

References

1. Singh PK, Gaur SR , Chari MS., Growth Performance of *Labeo rohita* (Ham.) Fed on Diet Containing Different Levels of Slaughter House Waste, *J. Fish. Aquat. Sci*. **2006**; 1 (1): 10–16.
2. Tacon AGJ, Stafford EA. and Edwards CA., A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. *Aquaculture*, **1983**; 35:187–199.
3. Kostecka J. and Pączka G., Possible use of earthworm *Eisenia fetida* (Sav.) biomass for breeding aquarium fish. *European Journal of Soil Biology*, **2006**; 42: S231-S233.
4. Sogbesan AO and Ugwumba AAA., Nutritional values of some non-conventional animal protein feedstuffs used as fishmeal supplement in aquaculture practices in Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences***, 2008**;8: 159-164.
5. Segun OA., Eudrilid Earthworms from Ile-Ife, Western Nigeria. *J. Natural History*, **1978**; 12: 15-31.
6. Omorinkoba WS., Bankole NO and Ita EO., A preliminary report on earthworm culture for sport fishing in Kainji Lake Research Institute (KLRI) reservoirs. KLRI Annual Report **1986**, pp. 62-65.
7. Horrobin DF, Manku MS., Clinical biochemistry of essential fatty acids. In D. F. Horrobin (Ed.), Omega-6 essential fatty acids. Pathophysiology and roles in clinical medicine New York: Wiley-Liss, 1985. pp. 21–53.
8. Mukhopadhyay PK., Fish as a nutritional source of long chain fatty acids. *Science and Culture*, **2009**;75, 53–60,
9. Cengiz EI, Unlu E and Bashan M., The effect of dietary fatty acids on the fatty acid composition in the phospholipids fraction of *Gambusia affinis. Turkish J. Biol*. **2003**; 27, 145–148.
10. Chakrabarty D, Das SK, Das MK and Bag MP., Assessment of Vermicompost as Direct Application Manure in Fish Farming Ponds. *Turkish J. Fisheries Aquat. Sci*. **2010**;10: 47-52.
11. AOAC., Official Methods of Analysis of the Association of Official Analytical Chemists. Helrich K. (Ed.) 15 edn, Arlington, VA, USA.**1990**
12. Giri SS, Sahoo SK, Sahu AK and Mukhopadhyay PK., Nutrient digestibility and intestinal enzyme activity of *Clarias batrachus* (Linn.) juveniles fed on dried fissh and chicken viscera incorporated diets. *Bioresource Technology*. **2000**; 71, 97–101.
13. Maynard L, Loosil J, Hintz H, Warner R., In: C.R. Zappa (Ed.),Animal Nutrition, [7th] edition, McGraw-Hill, New York: **1979**; 13-14.
14. Christie WW., In Lipid Analysis 2nd Edn. Pergamon Press, Oxford (England). **1982.**
15. Mangold HK., In Thin Layer Chromatography (E. Stahl Ed.), Springer, New York, **1969**;155
16. Misra S, Ghosh A, Dutta J., Production and composition of microbial fat from *Rhodotorula glutinis*. *J. Sci. Food. Agric.* **1984**; 35, 59–65.
17. Zar JH., Biostatistical analysis. Prentice-Hall Inc, Englewood Cliffs, NJ. **1974**.;pp. 101-162.
18. Ackman RG., Fatty acids in fish and shell fish. In: Chow, C.K. (Ed.), Fatty Acids in Foods and their Healh Implication. M. Dekker, Inc, N.Y. and Basel, **2000**; pp. 153–172.
19. Ackman RG, Mcleod C, Rakshit S and Misra KK., Lipids and fatty acids of five freshwater food fishes of *India. J. Food Lipids* **2002**; 9:127–145.
20. Watanabe T., Lipid Nutrition in Fish. *Comparative Biochemistry and Physiology;* **1982**; 73B:. 3-15.
21. Nematipour GR and Gatlin III DM., Requirementof hybrid striped bass for dietary (n-3) highly unsaturated fatty acids. *J. Nutr*. **1993**;123, 744–753.
22. Christensen JH, Skou HA, Fog L and Hansen V., Marine n-3 fatty acids, wine intake, and heart rate variability in patients

referred for coronary angiography. *Circulation*, **2001**;103: 651–657.
23. Dewailly EE, Blanchet C, Gingras S, Lemieux S and Sauve L., Relations between n-3 fatty acid status and cardiovascular disease risk factors among Quebecers. *Am. J. Clin. Nutr*. **2001**;74: 603–611.