

Influence of replacement of fish meal with the earthworm *Eisenia fetida* on growth performance, feed utilization and blood parameters of Nile tilapia (*Oreochromis niloticus*)

Abstract

A total of 450 of *Oreochromis niloticus* fingerlings with initial average weight of 4.10 ± 0.02 g were obtained by the Fish Hatchery of the General Authority for Fisheries Development, Aswan governorate, to evaluate the effect of replacement of fish meal with earthworm meal (EWM), on growth performance, survival and blood parameters of Nile tilapia. The stocking density was 30 fingerlings per tank and each diet was tested in triplicate. Fish were manually fed with experimental diets at the rate of 5%, twice/day for 60 days. Once every ten days, the total number of survivors in each tank was counted and fish biomass determined. Crude protein content of Nile tilapia juveniles increased significantly ($P < 0.01$) for juveniles maintained at the control, 75, 100% EWM. While average lipid content less for Juveniles fed at 0% EWM. The Juveniles maintained at 75, 50, and 100% EWM were have significantly ($P < 0.01$) higher SGR, PPV, PER, ER and the best food conversion ratio FCR (weight/gain) than the other Juveniles. There were no significant differences ($P < 0.01$) in mean values of creatinine, triglyceride, lipase, glucose, albumin and globulin among all the diets, while cholesterol and amylase varied significantly.

Keywords: blood parameters, *Eisenia fetida*, creatinine, triglyceride, food conversion ratio, tilapia

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Ragaa A Ahmed,¹ Hemdan Sayed Eissa,²
Manal Esam Shafi,³ Mohamed YM Aly,⁴
Omayma M Abd Al-Kareem⁵

¹Department of Aquaculture, Aswan University, Egypt

²Mariculture research centre, Egypt

³Zoology department of biological sciences, King Abdulaziz University, Saudi Arabia

⁴Pollution Laboratory, National Institute of Oceanography and Fisheries (NIOF), Egypt

⁵Department of Fish Health and Diseases, Aswan University, Egypt

Correspondence: Mohamed YM Aly, Pollution Laboratory, National Institute of Oceanography and Fisheries (NIOF), Egypt, Email myahya120@yahoo.com

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Introduction

In the last three decades, global fish production has doubled, with an average annual growth rate of 8%. Freshwater fish account for the largest share of global fish production, exceeding 56.4%, which means 33.7 million tonnes of total fish production.¹ The global population is doubling rapidly, especially in recent decades, and 76% of this increase is concentrated in Africa and Asia, mostly in developing countries, where, the population increase is expected to reach 12 billion people in the year 2100, Population increase means increased consumption and thus the depletion of environmental resources available for agricultural development.² It is predictable that the large population increase is the main reason for this increase in the fish production sector, in addition to the growing interest in fish production in fresh water, where it is characterized by ease and cheapness compared to fish production of marine water. Goddard³ believes that fish meal, which is the main protein source in fish feed composition, is the best source of protein due to its growth rate, palatable taste, fish attractiveness, amino acid content balance, vitamins. However, according to Sogbesan⁴ higher fish production costs and higher prices of high-quality fishmeal with increasing demand have led fish production specialists to seek alternatives to fishmeal to ensure continuity and expansion of global fish production. Also, high-quality fishmeal will not be available to supply endless global fish production as it is a constrained source of fisheries.⁵

In previous research, fishmeal was replaced by earthworm meal in a semi-intensive aquaculture system.⁶ The fingerlings of *Heteroclaris* spp. were fed after replacing fishmeal in feed with

earthworm *Eudrilus eugeniae*⁷ of domesticated earthworms, *Eisenia fetida* is known to be the most active species in the production of vermicompost.⁸ Vermicompost is a natural environmentally friendly technology that can convert organic waste into a good and beneficial organic product, a fertilizer containing many humus and antibacterial enzymes, known as vermicompost.⁹

E.fetidaisa promising product, and a good basis for animal nutrition, containing 60 to 70% of raw protein on the basis of dry matter and many of the ingredients that feed raw materials lack,¹⁰ for example, essential amino acids such as alanine, threonine, lysine and arginine. In addition, it is rich in fatty acids, such as linolenic acid (C18:3), linoleic acid (C18:2) and octadecanoic acid (C18:0).¹¹ Also Gunya et al.¹² have found large amounts of other nutrients such as zinc, manganese, copper and phosphorus iron.

The aim of the current study is to evaluate the effect of replacement of fish meal with *E. fetida* on growth performance, survival and blood parameters of Nile tilapia (*Oreochromis niloticus*).

Materials and methods

Experimental fish

A total of 450 of *O. niloticus* fingerlings with initial average weight of 4.1 ± 0.02 g were obtained by the Fish Hatchery of the General Authority for Fisheries Development, the Lake Development Authority, Aswan governorate. The fish were acclimated for a period of one week in the laboratory. The stocking density was 30 fingerlings per tank and each diet was tested in triplicate. Fish were manually fed

with experimental diets at the rate of 5%, twice per day for a period of 60 days.

Water temperature was recorded by mercury thermometer, dissolved oxygen by metteler Toledo, model 128.s/No1242. pH was measured every three days by pH meter (Extech pH/temp pen model pH 60). Total alkalinity, Ammonia and free carbon dioxide were determined according to Standard Methods.¹³

Once every ten days, the total number of survivors in each tank was counted and fish biomass determined. The proximate composition of *E. fetida* and formulated diets was evaluated. 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.

Collection of earthworms

Five plastic buckets (Plates) were used as culturing containers for earthworms. Cow manure was used as sources of nutrients for earthworms with dried rice straws and tree leaves as bedding materials.

Three kg of soil from earthworms' natural environment was collected and mixed with 20% cow manure. The culture was set with bedding materials at the bottom of each Plastic buckets, followed with equal number of adult earthworms in the substrates. Thereafter bedding materials placed on the top of mixtures to improve their housing as advised by Sherman S.¹⁴ The worm cultures were placed under shade and covered with the mosquito nets. Each culture substrate was moisturized by one half liter of water once a week. Earthworms was washed thoroughly with water to ensure any impurities stuck to them were eliminated. Then it was grinded and dried at 30°C for 24h using an oven and sieved to obtain earthworm meal in homogenized form.

Experimental diets

Five iso-nitrogenous (25% crude protein) experimental diets were formulated and prepared in the fish nutrition lab in faculty of fish and fisheries technology– Aswan University. Experimental diets were prepared to incorporate earthworm meal at four graded levels namely 0.25% (T₂₅), 50% (T₅₀), 75% (T₇₅) and 100% (T₁₀₀). Diet without earthworm served as control (T₀). The composition chemical analyses of the experimental diets (on dry matter (DM) basis) are presented in Table 1.

Table 1 The composition and chemical analysis (% on dry matter basis) of the experimental diets

Ingredients	Experimental diets composition/kg				
	T ₀	T ₂₅	T ₅₀	T ₇₅	T ₁₀₀
Fish meal	98.0	73.5	49.0	24.5	0.0
Earthworm meal	0.00	24.50	49.00	73.50	98.00
Soybean meal	140.0	140.0	140.0	140.0	140.0
Yellow corn	170.0	170.0	170.0	170.0	170.0
Gluten	200.0	200.0	200.0	200.0	200.0
Rice bran	180.0	180.0	180.0	180.0	180.0
Wheat flour	110.0	110.0	110.0	110.0	110.0
Premix ¹	8	8	8	8	8
Fish oil	30	30	30	30	30
Sunflower oil	30	30	30	30	30
Ascorbic acid	4	4	4	4	4
Total	1000	1000	1000	1000	1000
Chemical composition %					
Dry mater	89.65	89.4	89.61	89.52	89.86
Moisture	10.35	10.6	10.39	10.48	10.14
Crude protein (N×6.25)	30.08	29.96	29.84	29.71	29.59
Crude fat	14.24	14.02	13.09	12.21	11.52
Crude fiber	1.56	1.51	1.2	1.35	1.24
Ash	6.12	6.72	7.13	7.64	8.28
Carbohydrate (NFE) ²	37.65	37.19	38.35	38.61	39.23
Gross energy kcal/100g ³	459.779	456.9	456.2	452.4	451

¹Vit./min. Premix (mgkg⁻¹); ¹Premix Composition: Each 1kg contains Vit A (400000 i.u.), Vit D3 (100000 i.u.), Vit E (230mg) Vit K3 (165mg) Vit B1 (300mg), Vit B2 (80mg), Vit B6 (200mg), Vit B12 (1mg), Vit C (650mg), Niacin (1000mg), Methionine(3000 mg), Choline chloride (10000mg), Folic acid (100mg), Biotin (2mg), Pantothenic acid (220mg), Magnesium sulphate (1000mg), Copper sulphate (1000mg), Iron sulphate (330mg), Zinc sulphate (600 mg), Cobalt sulphate (100mg), Calcium carbonate up to (1000mg).

²NFE = 100- [% Ash + % lipid + % protein + % Fiber]

³Gross energy (GE) was calculated as 5.64, 9.44 and 4.11 kcal/100g for protein, lipid and NFE, respectively (NRC, 1993).

The feed were calculated using Pearson's square method, with these as the major ingredients;

- a. Fish meal-crude protein (65%)
- b. Earthworm meal-crude protein (60%)
- c. Soybean meal-crude protein (45%)=0.68kg
- d. Yellow corn-crude protein (9%)
- e. Gluten-crude protein (60%)
- f. Rice bran-crude protein (13%)
- g. Wheat flour-crude protein (14.4%)

Fish oil was added at 6% of the total formulated feeds weight for protein-sparing effect on the fish and to improve the feed palatability and binding of the ingredients together. Feed ingredients were fully mixed, homogenized, moistened by the addition of 35% water and pelleted in a mincing machine. The pellets were dried at 50°C for 8h.

Growth performance and feed utilization efficiency

At the end of the experiment, fish were harvested, counted, and weighed. The growth performance and feed utilization parameters were calculated as follows:

A) Specific growth rate (SGR) = $[(\ln \text{FBW} - \ln \text{IBW}) / t] \times 100$

where, FBW and IBW = final mean weight and the initial mean weight, respectively; T=feeding period in days.

B) Survival rate % = $[(\text{NF} / \text{Ni}) \times 100]$

where, NF=final total number of fish;

Ni=initial total number of fish.

C) Feed conversion ratio (FCR) = Total feed intake (g) / Total weight gain (g)

D) Protein efficiency ratio (PER) = Total weight gain (g) / Protein intake

E) Body weight gain (BWG) = $W_t - W_i$

F) Protein efficiency ratio (PER) = wet weight gain (g) / total protein intake (g)

Blood sampling and biochemical analyses

At the end of experiment which was conducted over 8 weeks, five fish were removed from the tank and used as replicates. Blood samples were taken from the caudal vein of each fish as described by Congleton and La Voie.¹⁵ Each sample was centrifuged at 3000rpm for 10 minutes to obtain serum for biochemical studies. The serum was kept at -20°C until analysis of creatinine, cholesterol, triglycerides, amylase, lipase, glucose, albumin and globulin. colorimetric determinations of biochemical parameters were performed using a spectrophotometer. We measured the following biochemical assays; quantitative estimation of creatinine (mg/dl) was estimated according to Henry.¹⁶ Blood serum glucose was measured by Glucose Oxidase method. Serum cholesterol (mg/dl) was estimated by Enzymatic Colorimetric test. Serum triglycerides by colorimetric method. All these parameters follow procedures according to Chawla.¹⁷

Statistical analysis

Data collected on the investigated traits (growth performance, feed utilization, biochemical and blood serum analyses) were analyzed with one-way analysis of variance (ANOVA) using the SPSS version 16 statistical package (SPSS Company Inc., Chicago, IL, USA) to evaluate the differences between the tested treatments. The differences within each experimental treatment were assessed using Duncan's multiple range test at the $P < 0.01$ level.

Results and discussion

Water quality parameters

Water temperature were recorded by mercury thermometer, and dissolved oxygen by metteler Toledo, model 128.s/No1242. Where the average range of dissolved oxygen was above 6mg/l. pH was measured every three days by pH meter (Extech pH / temp pen model pH 60). Total alkalinity, Ammonia and free carbon dioxide were determined according to Standard Methods. The mean values of these variables are shown in Table 2.

Table 2 Mean values ± Se of water quality parameters recorded in different feeding during experimental period

Treatment	Temperature °C	pH	Dissolved Oxygen mg/l	Ammonia mg/l
C	23.4±0.6	8.1±0.14	6.8±0.11	0.014±0.012
T25	24.0±0.26	8.5±0.05	7.3±0.16	0.016±0.002
T50	26.06±0.22	7.7±0.13	6.5±0.30	0.014±0.003
T75	27.6±0.06	7.8±0.10	7.5±0.19	0.063±0.008
T100	27.2±0.3	7.6 ±0.11	7.3±0.13	0.042±0.002

Growth performance and feed utilization

The effects of replacement of fish meal with *E. fetida* on growth performance and feed utilization of *O. niloticus* are presented in Table 3. IW, FW and WG at five levels of EWM replacement (T_0 , T_{25} , T_{50} , T_{75} and T_{100}) were significantly increased ($P < 0.01$) with increasing level of EWM from T_{25} to T_{75} replacement after 8 weeks. The survival of the Juveniles maintained at T_{100} EWM was significantly ($P < 0.01$)

less than Juveniles maintained at the other levels. There were no significant differences for Juveniles maintained at T_0 , T_{25} , T_{50} , and T_{75} .

SGRs of the Juveniles maintained at T_{75} , T_{50} , and T_{100} were significantly ($P < 0.01$) higher than Juveniles maintained at other experimental diets. The least significant SGRs were for Juveniles that maintained at T_0 and T_{25} .

Table 3 Mean values±Se of Growth performance and feed utilization of Nile tilapia (*O. niloticus*) during fed experimental diet for 8 weeks

Parameters	C	T ₂₅	T ₅₀	T ₇₅	T ₁₀₀
Initial BW	4.1	4.16	4.07	4.13	4.13
Survival	93.33+1.33 ^a	93.33+1.33 ^a	93.33+2.67 ^a	94.67+1.33 ^a	92+2.31 ^b
Final wt	27.27+0.12 ^c	28.43+0.09 ^{bc}	29.4+0.11 ^{ab}	30.50+0.15 ^a	29.36+0.14 ^{ab}
Gain	23.17+0.18 ^c	24.27+0.13 ^{bc}	25.33+0.09 ^{ab}	26.37+0.18 ^a	25.23+0.12 ^{ab}
SGR	3.38+0.03 ^c	3.43+0.04 ^{bc}	3.53+0.01 ^a	3.57+0.04 ^a	3.50+0.01 ^{ab}

The best FCR was for those Juveniles fed on diet T¹⁰⁰. Juveniles maintained at T₂₅, T₅₀, and T₇₅ showed not significant differences in FCR, while the Juveniles maintained at T₀ showed the highest FCR.

ER, PPV and PER were affected by different level of FM replacement with EWM. The highest PPV and PER were observed with the larvae fed at T₁₀₀, T₇₅, and T₅₀, respectively but no significant differences. For the control PPV and PER were significantly lower than the other.

It was also observed that ER was significantly higher in T₅₀, T₇₅, and T₁₀₀ than T₂₅ and T₀, showing that earthworms contain a high rate of protein,¹⁸ which has been confirmed to be rich in amino acids necessary for optimal fish growth. Several studies have shown that the earthworm powder content ranges from 48 and 71% protein. For instance, Serna¹⁹ showed that *E. fetida* contained 58 to 71% protein.

In this study, replacing FM with EWM has led to significant differences between experimental diets on both growth performance and food utilization, as the use of T₇₅ resulted in the highest WG and SGR followed by T₅₀ and then by T₁₀₀. The fish fed with the control diet achieved a significantly lower WG and SGR. This last result is consistent with Omeru and Solomon.²⁰

Whole replacement of FM with EWM in the diet of catfish *Clarias gariepinus* promote growth performance and survival. It may be sense that this improvement was attributed to containing EWM on eight essential amino acids.²¹

Protein quality generally depends on two important factors, the biological value and the efficiency of use with *E. fetida* contain protein of 84% biological value and 79% efficiency in rat growth test and These values are also found in fish and chicken tissues.²²

From Table 4, it can be concluded that there is a significant increase in the food utilization by increasing the EWM in diets of tilapia juveniles compared to juveniles that were fed a diet 0% that did not contain EWM. The fish maintained at T₅₀, T₇₅, and T₁₀₀ got the highest values in each of the PPV, PER, ER. While the values were the least significant for the juveniles that maintained at T₀.

It seems that the earthworms will soon become a source of nutritional protein in animal feed, in addition to the possibility of using it for humans as well. Earthworm meal protein is easily soluble in digestive enzymes, which is a clear indication of the ease of digestion by fish.

Table 4 Mean values±SE of feed utilization of Nile tilapia (*O. niloticus*) during fed experimental diet for 8 weeks.

Parameters	C	T ₂₅	T ₅₀	T ₇₅	T ₁₀₀
Offered feed	35.29+0.04 ^b	35.95+0.16 ^b	37.01+0.26 ^{ab}	38.20+0.16 ^a	36.22+0.23 ^b
FCR	1.52+0.01 ^a	1.48+0.01 ^{ab}	1.46+0.01 ^b	1.45+0.01 ^b	1.43+0.01 ^b
PPV	32.11+0.65 ^b	33.18+0.06 ^{ab}	33.85+0.35 ^a	33.89+0.13 ^a	34.49+0.22 ^a
PER	2.19+0.02 ^b	2.25+0.02 ^{ab}	2.28+0.01 ^a	2.30+0.01 ^a	2.32+0.01 ^a
ER	15.68+0.54 ^c	16.04+0.07 ^{bc}	17.03+0.21 ^{ab}	17.53+0.07 ^a	17.30+0.08 ^a

Biochemical parameters of blood

The results obtained of biochemical parameters assessments of fish blood at termination of the experiment are presented in Table 5. There were no significant differences (P<0.01) in mean values of creatinine triglyceride, lipase, glucose, albumin and globulin among all the diets, while cholesterol and amylase varied significantly. For the juveniles maintained at T₀ and T₂₅ were significantly higher in cholesterol than the other juveniles. But juveniles maintained at T₇₅

were having significantly (P<0.01) higher amylase than the other group. Study the blood file in fish is one of the most important indicators of the physiological state of fish. Some studies showed that the most important fish fatigue due to exposure to toxins leads to high blood glucose levels as a result of glycolysis.²³ As the low content of albumin and globulin in the blood leads to proteinaemia, as it is a sign of impaired liver function.²⁴ or lack of protein synthesis in the liver²⁵ and accumulative creatinine may be due to kidney disease and muscle

breakdown.²⁶ cholesterol is involved in building the cell structure and is therefore an important blood component, as metabolic disturbances of both fats and lipoproteins lead to high levels of cholesterol in the serum as well as an indication of liver disease.²⁷ Also, other studies have shown the main enzymes responsible for digestion in fish are amylase, protease and lipase. These enzymes control the fish's efficiency in digesting food and its protein, carbohydrate and fat components mentioned by Lemieux et al.²⁸

In the present study, the results showed that the use of different levels of EWM did not lead to significant differences in lipase, while there was a significant increase in amylase in fish that were fed by T₅₀ and T₇₅ than the other. Also, the blood analysis of Nile tilapia did not show significant differences in cholesterol, triglyceride, lipase, glucose, albumin and globulin according to the different experimental diets and a decrease in the serum content of cholesterol by increasing the content of EWM. Therefore, it is possible to predict that EWM works to facilitate digestion and improve liver function.

Table 5 Mean values±Se of Biochemical blood parameters of Nile tilapia (*O. niloticus*) at the end of the experimental diet

Parameters	C	T ₂₅	T ₅₀	T ₇₅	T ₁₀₀
Creatinine	0.43±0.02 ^a	0.43±0.01 ^a	0.42±0.02 ^a	0.44±0.02 ^a	0.43±0.01 ^a
Cholesterol	113±2.08 ^a	112±2.52 ^a	105±3.79 ^b	103±1.15 ^b	101±1.15 ^b
Triglyceride	115.66±2.08 ^a	114.71±3.06 ^a	114.86±2.08 ^a	114.72±1.53 ^a	114.01 ±1.53 ^a
Amylase	208.6±3.46 ^b	208.3±5.13 ^a	208±3.21 ^b	209.42±2.89 ^a	208.82±2.89 ^b
Lipase	3.02±0.12 ^a	3.10±0.15 ^a	3.02±0.06 ^a	3.01±0.10 ^a	3.10±0.10 ^a
Glucose	103.11±3.21 ^a	104.21±2.08 ^a	103.30±1.73 ^a	103.31±0.58 ^a	103.01±0.58 ^a
Albumin	4.31±3.21 ^a	4.22±3.21 ^a	4.52±3.21 ^a	4.55±3.21 ^a	4.71±3.21 ^a
Globulin	1.63±3.21 ^a	1.33±3.21 ^a	1.59±3.21 ^a	1.71±3.21 ^a	1.40±3.21 ^a

Biochemical composition of the experimental diets

The results obtained of means of biochemical composition of Nile tilapia juveniles are shown in Table 6. Crude protein content increased significantly (P<0.01) when fed at T₀, T₇₅, T₁₀₀. Differences in body moisture content at different replacement levels were not significant. While average lipid content less significantly (P<0.01) when fed the T₀ diet.

It was noted at the end of this study that Biochemical composition of Nile tilapia body showed significant differences according to the differences in experimental diets. Fish maintained at T₅₀ and T₇₅ showed a significant increase in the total body protein than the other fish (Table 6). Body EE for fish using EWM exceeded than control diet, while there were no significant differences in the total body content of moisture.

Table 6 Mean values±SE of Biochemical composition of Nile tilapia (*O. niloticus*) at the end of the feeding trial (dry matter weight basis).

Parameters	T ₀	T ₂₅	T ₅₀	T ₇₅	T ₁₀₀
Moisture	76.29±0.64 ^a	76.19±0.52 ^a	76.08±1.09 ^a	76.22±0.66 ^a	76.05±0.36 ^a
CP	65.68±0.18 ^a	64.76±0.37 ^b	64.52±0.32 ^b	66.63±0.05 ^a	65.35±0.11 ^a
EE	17.74±0.08 ^b	20.10±0.45 ^a	21.41±0.26 ^a	19.49±0.34 ^{ab}	19.34±0.18 ^{ab}
Ash	16.54±0.36 ^a	15.13±0.35 ^{ab}	14.03±0.58 ^b	13.86±0.33 ^b	15.26±0.55 ^a

Conclusion

From the results of this study, the assimilation of earth worm in the diet of *Oreochromis niloticus* has supported good growth performance. Based on the results obtained from the study, it can be concluded that 75% replacement of fishmeal with earthworm meal in the diet may be used as an ideal alternative protein source for better growth performance of this fish species.

Conflicts of interest

The author declares that there is no conflicts of interest.

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