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±0.02g 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 (wait/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

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 endlessly global fish production as it is a constrained source of fisheries.3

In previous research, fishmeal was replaced by earthworm meal in a semi-intensive aquaculture system.5 The fingerlings of Heteroclarias spp. were fed after replacing fishmeal in feed with earthworm Eudrilus eugeniae of domesticated earthworms, Eisenia fetida is known to be the most active species in the production of vermicompost.6 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.7 E.fetida is a 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,8 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).9 Also Gunya et al.,10 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±0.02g 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
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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 graded 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% (T₀), 50% (T₂₅), 75% (T₅₀) 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          | T₀  | T₂₅ | T₅₀ | T₇₅ | T₁₀₀ |
|----------------------|-----|-----|-----|-----|------|
| Fish meal            | 98.0| 73.5| 49.0| 24.5| 0.0  |
| Earthworm meal       | 0.00| 24.5| 49.0| 73.5| 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|
| Premix²              | 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 matter           | 89.65                                |
| Moisture             | 10.35                                |
| Crude protein        | 30.08                                |
| Crude fat            | 14.24                                |
| Crude fiber          | 1.56                                 |
| Ash                  | 6.12                                 |
| Carbohydrate (NFE)³  | 37.65                                |
| Gross energy kcal/100g² | 459.797                            |

¹Vit./min. Premix (mg/kg²); ²Premix 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(2000 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).
³NFE =100- [% Ash + % lipid + % protein + % Fiber]
²Gross energy (GE) was calculated as 5.64, 9.44 and 4.11 kcal/100g for protein, lipid and NFE, respectively (NRC, 1993).

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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 FBW-ln IBW)/t]=100
where, FBW and IBW = final mean weight and the initial mean weight, respectively; T=feeding period in days.

B) Survival rate %=[(NF/Ni)×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)=Wt–Wi

**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.11 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 mettler 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      | 7.9±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 (T100, T75, T50, T25 and T10) were significantly increased (P<0.01) with increasing level of EWM from T25 to T100 replacement after 8 weeks. The survival of the Juveniles maintained at T100 EWM was significantly (P<0.01) less than Juveniles maintained at the other levels. There were no significant differences for Juveniles maintained at T75, T50, T25 and T10.

SGRs of the Juveniles maintained at T100, T75, and T50 were significantly (P<0.01) higher than Juveniles maintained at other experimental diets. The least significant SGRs were for Juveniles that maintained at T25 and T10.

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The best FCR was for those Juveniles fed on diet T\textsuperscript{100}. Juveniles maintained at T\textsubscript{25}, T\textsubscript{50}, and T\textsubscript{75} showed not significant differences in FCR, while the Juveniles maintained at T\textsubscript{75} 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 T\textsubscript{100}, T\textsubscript{50}, and T\textsubscript{75}, 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 T\textsubscript{50}, T\textsubscript{75}, and T\textsubscript{100} than T\textsubscript{0} and T\textsubscript{25}, showing that earthworms contain a high rate of protein, 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, Serna\textsuperscript{19} 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 T\textsubscript{25} resulted in the highest WG and SGR followed by T\textsubscript{50} and then by T\textsubscript{75}. The fish fed with the control diet achieved a significantly lower WG and SGR. This last result is consistent with Omeru and Solomon.\textsuperscript{20}

### 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\textsubscript{25} | T\textsubscript{50} | T\textsubscript{75} | T\textsubscript{100} |
|------------|------------|---------------------|---------------------|---------------------|---------------------|
| Initial BW | 4.1        | 4.16                | 4.07                | 4.13                | 4.13                |
| Survival   | 93.33±1.33 | 93.33±1.33          | 93.33±2.67          | 94.67±1.33          | 92±2.31             |
| Final wt   | 27.27±0.12 | 28.43±0.09          | 29.4±0.11           | 30.5±0.15           | 29.36±0.14          |
| Gain       | 23.17±0.18 | 24.27±0.13          | 25.33±0.09          | 26.37±0.18          | 25.23±0.12          |
| SGR        | 3.38±0.03  | 3.43±0.04           | 3.53±0.01           | 3.57±0.04           | 3.50±0.01           |

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.\textsuperscript{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.\textsuperscript{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 diets 0% that did not contain EWM. The fish maintained at T\textsubscript{25}, T\textsubscript{50}, and T\textsubscript{75} got the highest values in each of the PPV, PER, ER. While the values were the least significant for the juveniles that maintained at T\textsubscript{0}.

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 mail 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\textsubscript{25} | T\textsubscript{50} | T\textsubscript{75} | T\textsubscript{100} |
|------------|------------|---------------------|---------------------|---------------------|---------------------|
| Offered feed | 35.29±0.04  | 35.95±0.16         | 37.01±0.26           | 38.20±0.16           | 36.22±0.23          |
| FCR        | 1.52±0.01   | 1.48±0.01           | 1.46±0.01           | 1.45±0.01           | 1.43±0.01           |
| PPV        | 32.11±0.65  | 33.18±0.06         | 33.85±0.35           | 33.89±0.13           | 34.49±0.22          |
| PER        | 2.19±0.02   | 2.25±0.02           | 2.28±0.01           | 2.30±0.01           | 2.32±0.01           |
| ER         | 15.68±0.54  | 16.04±0.07         | 17.03±0.21           | 17.53±0.07           | 17.30±0.08          |

Biochemical parameters of blood

The results obtained of biochemical parameters assessments of fish blood at termination of the experiment are presented in Table5. 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 T\textsubscript{0} and T\textsubscript{25} were significantly higher in cholesterol than the other juveniles. But juveniles maintained at T\textsubscript{75} 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.\textsuperscript{23} As the low content of albumin and globulin in the blood leads to proteinemia, as it is a sign of impaired liver function.\textsuperscript{24} or lack of protein synthesis in the liver\textsuperscript{25} and accumulative creatinine may be due to kidney disease and muscle

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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 at $T_{50}$ and $T_{75}$ 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 in 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   | 112±2.08$^a$ | 112±2.52$^a$ | 105±3.79$^a$ | 103±1.15$^a$ | 101±1.15$^a$ |
| 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$^a$ | 208.3±5.13$^a$ | 208±3.21$^a$ | 209.42±2.89$^a$ | 208.8±2.89$^a$ |
| Lipase        | 3.02±0.12$^a$ | 3.10±0.15$^a$ | 3.02±0.06$^a$ | 3.01±0.10$^a$ | 310±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 $T_{50}$, $T_{75}$, $T_{100}$. Differences in body moisture content at different replacement levels were not significant. While average lipid content less significantly (P<0.01) when fed the $T_{50}$ 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 $T_{50}$ and $T_{75}$ 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$^a$ | 64.52±0.32$^a$ | 66.63±0.05$^a$ | 65.35±0.11$^a$ |
| EE         | 17.74±0.08$^a$ | 20.10±0.45$^a$ | 21.41±0.26$^a$ | 19.49±0.34$^a$ | 19.34±0.18$^a$ |
| Ash        | 16.54±0.36$^a$ | 15.13±0.35$^a$ | 14.03±0.58$^a$ | 13.86±0.33$^a$ | 15.26±0.55$^a$ |
Conclusion

From the results of this study, the assimilation of earthworm 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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