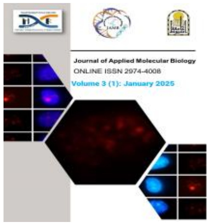
Journal of Applied Molecular Biology (JAMB)
Molecular biology Research & Studies institute , Assuit University, Egypt
ISSN 2974 – 4008
Volume 3 (1): January 2025

https://jamb.journals.ekb.eg



Potential Use of Earthworm (*Eisenia foetida*) as Dietary Replacement of Fishmeal for Tilapia (*Oreochromis niloticus*) in Aquaponics System: Growth Performance, Blood Chemistry, Histochemical invistigation, and Growth Hormone Gene Expression

Hamdy A. M. Soliman[1,2,*], Maha Abbass[1,2], Ahmed E. A. Badry [3], Mostafa A.M Soliman[4], Alaa Osman[3]
1 Department of Zoology, Faculty of Science, Sohag University, Sohag 82524, Egypt
2 Aquaponics unit, Upper Egypt Center of Development, Sohag, Egypt
3 Department of Zoology, Faculty of Science, Al-Azhar University, Assiut 71524, Egypt.
4 Department of Animal System Production Research, Animal Production Research Institute(APRI), & Agriculture Research Center, Ministry of Agriculture, Dokky-Giza-Egypt.
*Corresponding author: E-mail: hamdy_soliman@science.sohag.edu.eg

ARTICLE INFO

Article History:
Received:16-11-2024
Accepted: 17-12-2024
Online: 27-01-2025

Keywords:
Aquaponics,
Growth hormone gene,
Liver,
Weight-Gain,
Nile tilapia.

ABSTRACT

For the sake of sustainability and ecosystem preservation, it is crucial to look for alternate protein sources for aquatic organisms fed in captivity. In the current study, many biomarkers were used to evaluate the potential applications for earthworms (*Eisenia foetida*) in aquaponic systems as alternative fishmeal for feeding tilapia (*Oreochromis niloticus*). A four-month feeding experiment using Nile tilapia (*O. niloticus)* was conducted to determine the impact of substituting 25% of the fish meal in the feed formula with earthworms (*Eisenia foetida*). Growth performance, food consumption, haematological parameters, biochemical parameters, and histochemical investigation as well as growth hormone gene expression values have been evaluated in *O. niloticus*. Our data indicated that final weight, weight gain, feed conversion ratio (FCR) and feed efficiency ratio (FER), in addition to haematological parameters and biochemical parameters, showed non-significant differences between fish fed a normal diet and fish consumed an earthworm diet under aquaponic systems. In contrast, fish get a regular diet; the gut and liver of tilapia fed an earthworm diet showed a normal histological structure, no indication of any particular pathology, and a normal amount of protein and carbohydrates. Growth hormone level expression in muscle of fish fed earthworm was similar to fish fed a normal diet. To summarise, the partial incorporation of earthworm meals in the diets of *O. niloticus* has a substantial and favourable influence on Tilapia's health status, implying that earthworm meal is appropriate as an animal protein source in the diet of Tilapia in an aquaponics system.

1. INTRODUCTION

Recently the River Nile become pollutant naturally or due human activaties [1, 2, 3, 4] and search for other fish sources is very important. Aquaponics may be seen as a viable agricultural cultivation system since it does not utilize any nonrenewable resources necessary for agribusiness in order to continue using agricultural techniques [5]. In these systems, hydroponically grown plants receive nutrients generated by bacteria's breakdown of organic matter or exhaled directly by fish [6]. The demand for feed materials is increasing due to the change in the availability of fish around the world, particularly for excellent quality lipid and protein feed ingredients, such as fishmeal [7, 8]. Due to the enormous demand for fishmeal over the past ten years, the price has significantly risen [9]. Smaller aquaculture enterprises operating in remote places are unable to boost fish output by employing higher quality feed inputs as a result. Consequently, the quest for replacement, highly nutrient-rich feed components for aquafeeds is sparked [9]. Although the earthworm (Eisenia fetida) is considered a waste product or byproduct of a vermiculture item, it can serve as a new animal protein- source for fish diet [10]. They are highly useful for producing worm meal and are advised for processing dry and pulverised form for feed preparation due to the high rate of reproduction and biomass production of earthworm species. In general, additional study is required to expand the usage of earthworm meals while addressing its application's drawbacks, such as scaling up production to a more intensive scale [11].

Earthworms were reported as excellent source of protein and beneficial amino acids [12 - 17]. Earthworms' lipid component was thoroughly analysed by Hansen and Czochanska [18], who discovered that it contains a significant amount of polyunsaturated fatty acids (linolenic; -3 fatty acids), which are crucial for producing fish diets for several species. In addirtion, the protein and fat levels of earthworms range from 50 to 70% and 5 to 10%, respectively [10, 14, 16, 18-20]. According to Sogbesan and Ugwumba [64], the earthworm's supply of salt, calcium, and potassium is adequate to meet the needs of catfish and other tropical fish. Earthworms can be added either alone or in conjunction with other feed additives to fish meal compositions to produce a high-nutrient meal [20-25].

Fish physiological research might focus on hemato-biochemistry because of its importance in clinical disease. Hemato-biochemical assays are crucial diagnostics for assessing the health of many kinds of farmed fish [26]. Particularly, blood markers have been revealed to be the most important physiological markers for determining how fish respond to stress [27], inadequate nutrition [28, 29], unsuitable quantity of stock [29], farm practises [31], handling and shipping [30, 32]. The availability of food in the wild and feeding regime quality and amount of dietary protein [31] are two dietary factors that have a significant impact on the energy metabolism of fish and cause significant variations in their different biochemistry serum parameters. Fish reared under intensive cicumstances are subjected to more stress than fish farmed in the wild, which could impact their wellbeing and growth [34]. Understanding the hemato-biochemical changes in fish raised under stressful conditions can lead to improvements in aquaculture technology, aquatic animal welfare, and productivity [26, 31].

Fish tissues have been examined histologically and histochemically as a biomarker for evaluating inside (nutrition) and outside (aqueous environment) parameters [35 ,36]. Fish are typically examined for digestive capability and morphological changes that may be related to the digestive system (liver and gastrointestinal tract) when nutritional changes take place [37]. Animals' somatic development and growth depend on the pleiotropic endocrine hormone known as growth hormone (GH) [38, 39]. Growth is a crucial quality since it is correlated with aquaculture production's productivity and profitability [40]. Although under genetic control, phenotypic manifestation also depends on several environmental factors, primarily nutrition [41].

The purpose of this study was to evaluate if earthworm meal could be utilized as a replacement to fish meal in an aquaponic system in terms ofdevelopment performance, feed consumption, body composition, plasma biochemical elements, intestinal and liver histology, and growth hormone gene expression.

2.MATERIALS and METHODS

2.1. Construction of an aquaponics system

In Egypt's Sohag Governorate, the ASRT owns the Upper Egypt Center of Development, where Floating Raft cultivation techniques have been created in its greenhouse [42]. The fish tank has a two cubic metre volume. A mechanical filter separates sludg from water that is fed by gravity.

The water first passes through the mechanical filter before passing through the net filter. Water from the biofilter is then poured into the building's vinyl-coated grow bed. Following that, a submersible pump fills the fish tank with water (Figure 1). To make sure there is adequate dissolved oxygen in the fish tank and the hydropnic unit, an air pump aerates the entire system.

Figure 1. Aquaponics system , the Upper Egypt Center of Development owned by the ASRT in Sohag Governorate, Egypt (1- Fish tank, 2- Mechanical filter, 3- Net filter, 4- Biological filter, 5- Cultivate plants unit).

2.2.Experimental Design

A total of six hundred Nile tilapia fingerlings (*O. niloticus*) were purchased from the Alahaywh government hatchery (General Authority for Fish Resources Development), Sohag Governorate, Egypt, and housed in an aquaponics system unit at the Upper Egypt Centre of Development, Sohag Governorate, having an average starting body weight of 19±1.4 g (mean ±SD). Before being put into the tank, the fish have been checked and acclimated in the aquaponics unit. The fish were devided into two groups, each with 300 fish. The second group was fed a 25% earthworm meal once daily at a rate of 3 % of their body weight, whereas the control group received a commercial extruded floating fish meal (which included 25% crude protein). Both at the beginning and final stages of the experiment (4 months later), the fingerlings were weighed. The water content of dissolved oxygen, pH, and temperature were 5.23±1.5 mg/L, 8.1 ± 0.34 and 21 °C ± 2 °C, respectively. Following feeding experiment, 12 randomly chosen fish from each group were randomly selected and anesthetized by ice to reduce stress during processing. 1.5 ml of blood were drawn from the caudal vein, with part used for haematological indices and the other centrifuged under refrigeration for biochemical analysis. Tissues from the liver and intestine were used for histological and histochemical research. In addition, 2 mL sterile eppendorf tubes containing tissue samples from each group's white muscle were shocked in liquid nitrogen, and then had their RNA extracted. The Sohag University Faculty of Science in Egypt's Research and Ethical Committee (No.CSRE-41-24) gave its approval for the experimental design, procedures, and fish handling.

2.3.Feed stuff preparation:

The earth worm (*E. fetida*) employed in this study is cultivated locally at Sohag Government's vermicompost unit. In order to ensure that any impurities stuck to the earthworms were removed, they were thoroughly cleaned in water. Then, after drying in an oven set at 90° for 24 hours, the earthworm meal was made by powdering it.. Using a kitchen mixer, the necessary amounts of dried earthworm meal, fish meal, soybean meal, corn gluten, yellow corn, wheat bran, rice bran and Premix1 were well combined (**Table 1**). An electric heater was used to heat the requisite volume of water in a 1 L beaker to 80°C, and the necessary quantity of gelatin was added while stirring slowly. The gelatin was added to the feed mixture once it had properly dissolved. The feed mixture was combined appropriately with the necessary amount of tepid water to create a dough. To create feed pellets with a 2 mm diameter, The wet mixture was passed through a hand pelletizer (**Figure. 2**). In a refrigerator, the pellets were kept at 4°C while being dried at 60°C.

2.4. Chemical Analyses of Feed and Fillets

Following the accepted procedures, fish samples (five fingerliners/tank) and feed samples were analyzed for their approximate composition [43]. Using the hot air oven, samples were dried at 105 ± 2 °C until a stable weight was attained, at which point the moisture content was calculated. Bligh and Dyer [44] used chloroform/methanol (1/1, v/v) to measure total lipid. By converting the nitrogen content obtained using Kjeldahl's technique (N x 6.25), crude protein content was calculated. After 20 hours of 550 °C combustion, the amount of ash was measured.

Table 1. Composition (% dry matter) of the experimental diets

Ingredients composition (g)	The normal diet	The earthworm diet
Fish meal (65%)	70	52.5
meal Earthworm	0	17.5
Soy bean meal	250	250
Corn gluten	80	80
Yellow corn	100	100
Wheat bran	150	150
Rice bran	300	300
Fish oil	20	20
Premix1	30	30
Total	1000	1000

Figure 2. Earth worm *(E. fetida*) meal preparation steps.

2.5. Growth performance

The mean weight of individual fish (g) was recorded at the begining and finish of the trial. the following parameters were computed as (mean±SD):

Weight gain (g) = final weight – initial weight.

Growth rate (g/day) = final weight – initial weight/experiment period.

Feed conversion ratio (FCR) = food intake/weight gain.

Feed efficiency ratio (FER) = weight gain/feed fed × 100.

Survival rate (%) = [Number of fish harvested/Number of fish stocked] x 100

2.6. Hematological parameters

According to Bain et al. [45], an automated technical analyzer (BC-2800 from Mindray) was used to determine a number of hematological indices, including the count of red blood cells (RBCs) and white blood cells (WBCs); differential WBCs; blood platelets; hematocrit level (Hct); hemoglobin level (Hb); and erythrocyte indices, such as mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC).

2.7. Biochemical parameters

Based on the technique proposed by Bricknell et al. [46], blood will be drawn, shaken for 10 minutes at 4000 rpm, and blood serum will then be isolated for biochemical analysis. A spectrophotometer T80+ UV/VIS will be used to evaluate the biochemical characteristics of the collected serum. Glucose (GLU), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, globulin, albumin, total cholesterol, creatinine, and uric acid will all be examined throughout the trials.

2.8. Histological and histochemical studies

Following collection, liver and intestinal samples were preserved in 10% neutral buffered formalin. Fixed samples were typically prepared by paraffin embedding, sectioned at a thickness of 5 µm and stained with Harris's hematoxylin and eosin (H&E), as well as Periodic acid-Schiff (PAS), and bromophenol blue, which can be used to stain polysaccharides and proteins, respectively. Sections were examined using an Olympus microscope model BX50F4 (Olympus Optical Co., Ltd., Tokyo, Japan).

2.9. Growth hormone gene expression:

The extraction of RNA, reverse-transcription polymerase chain reaction, cDNA synthesis, and quantitative real-time PCR were carried out following Pfaffl [47] and Elbialy et al. [48]. Primer sequences and accession codes are displayed in Table 2.

Table2. Primers sequence used in the current study

Gene	Primer sequence (5'-3')	NCBI gene bank accession number	Reference
β-actin (internal control)	F: CCACACAGTGCCCATCTACGA R: CCACGCTCTGTCAGGATCTTA	EU887951.1	[44]
Growth hormone gene	F:GTTGTGTGTTTGGGTCTCC R: CAGGTGCGTGACTCTGTTGA	HM565014.1	[45]

2.10. Statistical analysais

The data is presented using means and standard errors. To estimate the differences between treatments, a T-test was performed by SPSS software.

3 . RESULTS

3.1.Proximate analysis of diet and fish

There was no alteration in the proximate analyses of the usual diet and the earthworm diet in Table (3). The initial and final fish carcass proximate compositions varied ($p < .05$), as indicated in Table (4). The harvested fish had considerably ($p < 0.05$) greater protein, fat, and ash contents than the initial carcass. On the other hand, before the experiment, the moisture levels of fish carcasses were considerably greater ($p < 0.05$). When compared to fish consumed the normal diet, the carcasses of fish fed an earthworm diet had analogous moisture, protein, fat, and ash levels ($p > 0.05$).

Table 3. Proximate analysis of the normal diet and earthworm diet.

Diet Constituents	The normal diet	The earthworm diet
Dry matter (%)	87.23±0.72	89.33±0.81
Crude protein (%)	24.93±1.34	25.03±0.45
Crude fibre (%)	3.3±1.12	3.73±0.17
Crude fat (%)	5.13±0.22	5.0±0.08
Ash (%)	8.3±1.25	8.26±1.20

*Significant at (0.05)

Table 4. Proximate analysis of the flesh of Nile tilapia (*Oreochromis niloticus*) fed the normal diet and earthworm diet under aquaponics systems.

Items		Normal diet	Earthworm diet
Initial	Moisture (%)	74.1±0.9*	
	Protein (%)	16.2±0.4*	
	Lipid (%)	6.1±0.2*	
	Ash (%)	3.2±0.2*	
At the end	Moisture (%)	70.3±0.8	70.9±1.1
	Protein (%)	18.5±0.3	17.9±0.2
	Lipid (%)	7.9±0.05	7.9±0.1
	Ash (%)	2.9±0.1	3.1±0.4

*Significant at (0.05)

3.2. Growth proficiency and feed utilization

Table (5) compares the impact of different feeds on Tilapia development and feed consumption. The FBW (final body weight), WGR (weight gain rate), SGR (specific growth rate), and FCR (feed conversion ratio) of fish given an earthworm meal did not vary substantially from those consumed the standard feed ($P > 0.05$). However, these characteristics didn't significantly decrease in fish consumed an earthworm diet ($P > 0.05$). The survival rate (SR) did not alter in a statistically meaningful manner.

3.3. Hematological indicators

Table (6) displays the results of haematological parameters. Between fish given the conventional meal and fish fed the earthworm diet, no haematological parameters changed substantially ($P > 0.05$), with the exception of lymphocytes, which were lesser in the fish fed the earthworm diet.

3.4. Biochemical indicators

The serum biochemical indicators data are illustrated in Table (7). No notable changes were seen in any of the serum biochemical indicators activities ($P > 0.05$) among fish received the standard formula and those fed the earthworm meal. The total protein, ALP, glucose, and globulin contents of fish consumed earthworm food were insignificantly greater than those of fingerlings fed standard diet. In contrast, the values of ALT and Uric acid in fish fed a conventional diet did not have significantly higher levels than fish fed an earthworm diet. Furthermore, the levels of Cholesterol were the same in both groups.

Table 5. Growth performance parameters and feed utilization of Nile tilapia (*Oreochromis niloticus*) fed the normal diet and earthworm diet under aquaponic systems.

Parameters	Normal diet	Earthworm diet
Initial weight (g)	20.3 ± 1.03	18.3± 0.94
Final weight (g)	130.3 ± 4.8	118.6 ± 1.8
WG(g)	110.3 ± 8.8	100.3 ± 10.8
GR (g per day)	1.07 ± 0.02	0.91 ±0.09
FCR	1.25 ± 0.0	1.47± 0.17
FER (%)	79.72 ± 1.9	67.79 ± 7.3
Survival (%)	96	94

*Significant at (0.05)

Table 6. Hematological parameters of Nile tilapia (*Oreochromis niloticus*) fed the normal diet and earthworm diet under aquaponic systems.

Hematological parameters	Normal diet	Earthworm diet
(RBCs) (million/mm^3)	2.1 ± 0.3	2.1 ± 0.3
Hemoglobin (Hb) (g/dl)	9.5 ± 0.8	8.9 ± 1.5
Ht (PCV) (%)	35.3 ± 4.5	33.8 ± 4.6
MCV (µm³)	166 ± 16	165± 13
MCH (Pg)	42.5 ± 1.6	42.9 ± 1.7
MCHC (%)	25.7 ± 2	26.4 ± 2.5
Thrombocytes (Thou./mm3)	120 ± 20.3	105 ± 22
(WBC's) (10^9/l)	41.8 ± 6	40.6 ± 5.7
Lymphocytes (%)	92.9 ± 1.4	90 ± 2.9*
Monocytes (%)	5.7 ± 0.9	5.6± 1.0

3.5. Expression of growth hormone gene

Figure 3 depicts values of growth hormone expression of genes in muscles. The levels of GH mRNA expressed did not change significantly between fish given an earthworm diet and those fed a conventional diet.

Table 7. Biochemical parameters of Nile tilapia (*Oreochromis niloticus*) fed the normal diet and earthworm diet under aquaponic systems.

Biochemical parameters	Normal diet	Earthworm diet
AST activity (U/l)	52.1±1.1	53.1±1.1
ALT activity (U/l)	41.1±12	34.1±6.6
ALP activity (U/l)	71±7.6	76.4±0.4
Glucose (mg/dl)	62.7±17	65±2.4
Cholesterol(mg/dl)	152.1±16	152±36
Total protein (mg/dl)	6.3±2	7.1±3.2
Globulin	3.3±1.2	3.7±1.7
Albumin	2.9±1	3.2±1.6
Creatinine (mg/dl)	0.4±0.1	0.56±0.1
Uric acid (mg/dl)	3.7±0.6	3.1±0.9

*Significant at (0.05)

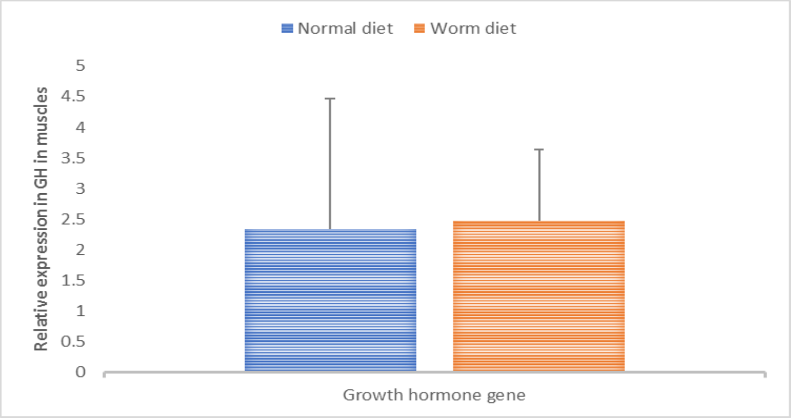


Figure 3. The relative expression of growth hormone gene (GH) in tissue of Nile tilapia (Oreochromis niloticus) fed the normal diet and earthworm diet under aquaponic systems.

3.6. Histological and histochemical analysis of the intestine and liver

Fish fed on a typical diet exhibited normal architecture in their livers, with most cells around the central vein displaying unstained cytoplasm and polygonal hepatocytes with vesicular nuclei inserted between blood sinusoids (Figure 4a). Apart from minimal fat buildup, the liver of Tilapia fed an earthworm diet showed a normal histological structure and no signs of particular pathology (Figure 4b). The livers of fish given an earthworm diet and fish on a regular diet were stained with periodic acid-schiff, which revealed normal glycogen levels (granules appeared magenta in the cytoplasm of the hepatocytes) (Figure 4c and d). The livers of fish fed an earthworm diet and fish consumed a conventional formula were stained with bromophenol blue, which revealed the presence of proteins (blue colour) in the cytoplasm of the hepatocytes (Figure 4 e and f).

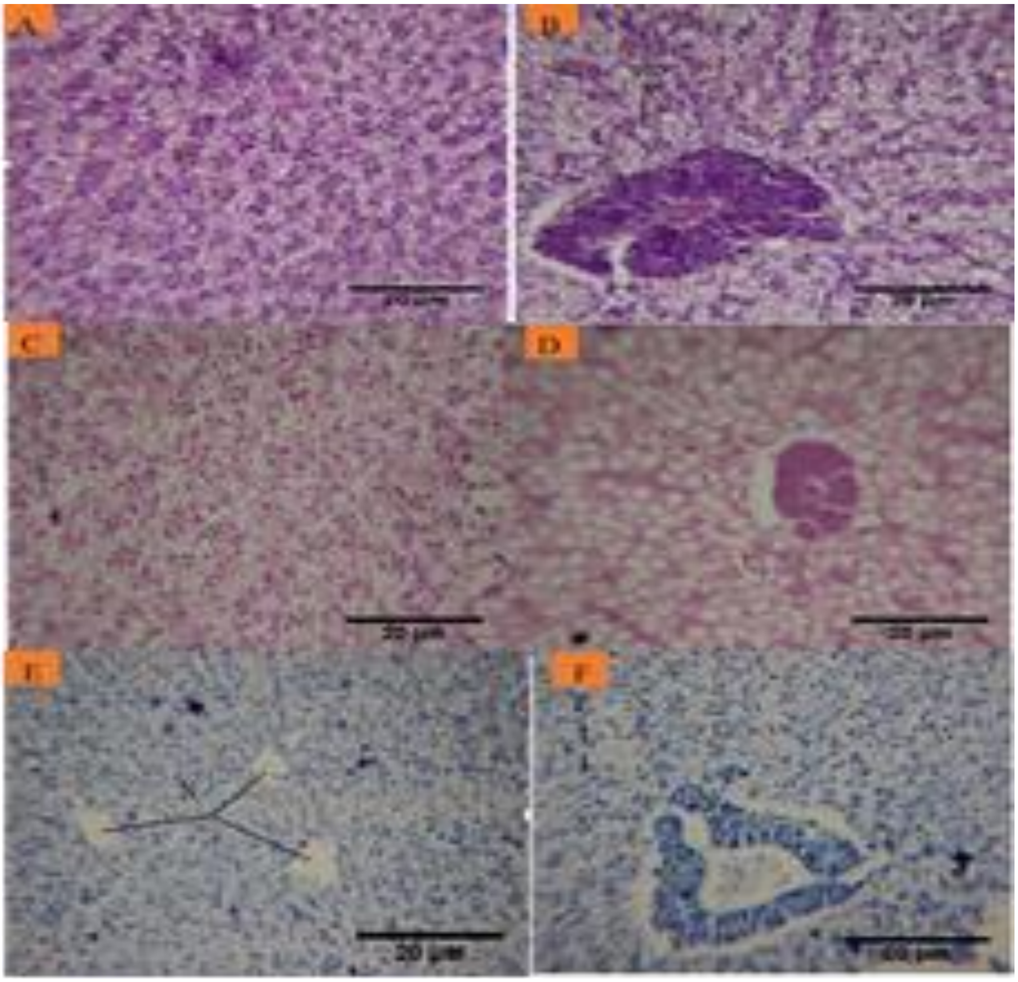


Figure 4. Photomicrographs of the liver of Nile tilapia (Oreochromis niloticus) stained with H&E; A. fish fed the normal diet and B. earthworm diet, stained with PAS reagent; C. fed the normal diet and D. earthworm diet, and stained with bromophenol blue; E. fed the normal diet and F. earthworm diet. H: hepatocytes, BS: blood sinusoids, CV: central vein (bar=20µ).

The mucosa, submucosa, lamina propria, inner muscle layer, external m uscle layer, and serosa of the tilapia intestine all had normal-appearing morphology. The mucosa was composed mostly of columnar, darkly pigmented, ovoid basal nuclei and goblet cells

(Figure 5a). Tilapia intestines given an earthworm diet had normal histological structure and no pathological signs (Figure 5b). The intestines of fish fed an earthworm diet and fish fed a normal diet stained with periodic acid-schiff revealed normal glycogen levels (granules appeared magenta in the cytoplasm of epithelial cells) (Figure 5c and d). The intestines of fish fed an earthworm feed and fish fed a conventional diet stained with bromophenol blue revealed the presence of proteins (blue colour) in the cytoplasm of epithelial cells (Figure 5 e and f).

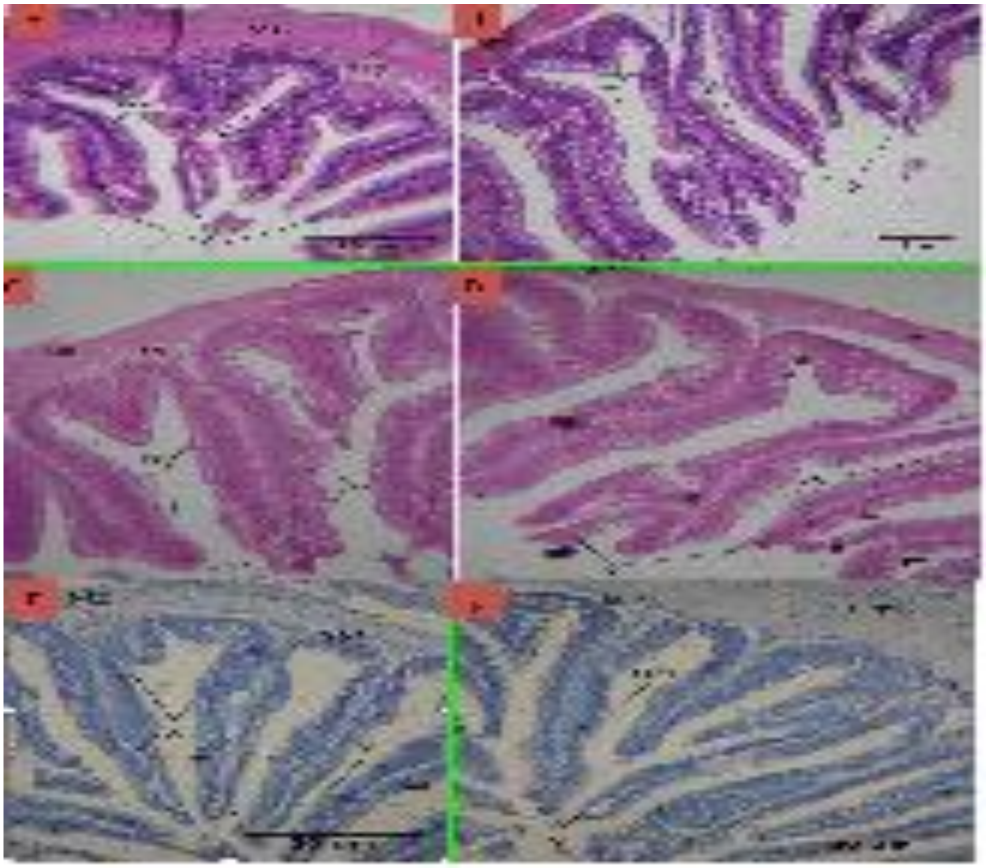


Figure 5. Photomicrographs of the intestine of Nile tilapia (Oreochromis niloticus) stained withH&E; A. fed the normal diet and B. earthworm diet, stained with PAS reagent; C. fed the normal diet and D. earthworm diet, and stained with bromophenol blue; E. fed the normal diet and F. earthworm diet. ME: mascularis externa, SM :submucosa, LP: lamina propria, GC: goblet cells and V: Villi. (Bar=20µ).

4 . DISCUSSION

Global fish supplies are increasingly coming from integrated aquaculture industries. However, numerous of elements, such as the costly and erratic fish meals, seriously jeopardize its viability. The three main factors that affect how long fish feeds in aquaculture may be used sustainably are price, quality, and quantity. In addition to being nutritionally full, affordable, tasty, and simple to digest, along with improving disease resistance and reducing mortality, sustainability feed for fish should also be able to maintain water quality. These qualities encourage a quicker rate of fish development, robust health, and high levels of reproductive efficiency, which translate to a higher output and hence maximum profitability. Therefore, obtaining high-quality, plentiful fish feeds at a fair price is every farmer's goal in order to achieve economic success.

Our research revealed that there was no distinction between the usual diet and the earthworm diet in the proximate analysis. According to Mi et al. [25], the earthworm (*E. foetida*) deit contains a substantial protein quantity and an amino acid composition similar to a fish meal and a chicken egg. The earthworm's crude protein level was inferior to previously reported information, but total body fat content was found to be equivalent [12, 14, 16, 20]. The increased body protein level may have resulted in a lower body lipid amount since protein and lipid levels are commonly inversely related in animal tissue. In addition, each species of earthworm has a different protein and lipid composition. Once more, the nutritional composition of earthworms is significantly affected by the culture environment (nutrient/medium). Since we are uninformed of the cultural conditions illustrated by the prior researchers, it is impossible to contrast the nutritional content of the earthworm discovered in the present study to the previous findings.

In current investigation, the harvested fish had much greater protein, fat, and ash contents than that of the initial carcass. In contrast, fish carcasses before the experiment had far greater moisture levels. When comparing between fish raised on a conventional meal, the carcasses of fish received an earthworm diet contained similar amounts of moisture, protein, lipids, and ash. Protein in full body, ether extract, and ash levels also varied significantly. Though the total body moisture and energy contents of both the initial and final fish did not significantly differ from one another [22]. According to Mi et al. [25], fish fed a diet of (15.1% EM and 1.8% WC) had a noticeably increased whole body protein values, but decreased lipid and ash concentrations. The greater weight increase in fish fed on this treatment diet is due to the higher overall body protein content (15.1% EM and 1.8% WC). Furthermore, the current findings for the total body moisture, protein, lipid, and ash values are similar to previous findings for other species of fish that receive nourishment with non-traditional protein sources [51, 52, 53].

According to our findings, there were no appreciable differences between the fish given an earthworm diet and the fish fed on a the conventional diet depends on the final body weight, weight gain, specific growth rate, or feed conversion ratio. Salmonids' growth performance was improved when earthworm meal (*Dendrodrilus subrubicundus, Allolophora foetida*) was substituted for fishmeal at low levels [54, 55]. In order to swap fish meal entirely or in part in the rearing of common carp with dried worm meal (*Eudrilus eugeniae*), Nandeesha et al. [56] found that the diet that contained 5% sardine oil and only the best outcomes came from partially replacing fish meal with worm meal. According to Keshavappa et al. [57], the weight development of carp (*Catla catla*) fry-fed with 30% earthworm meal and 30% fish meal was no variation. Additionally, the former had a greater survival rate (75.75%) than the latter (66.66%). Whenever applied to supplant 25 to 50% of the fish meal component in diets for rainbow trout, worm meal generated from E. foetida led to greater growth rates in fish given these diets than control diets with no worm meal [58]. Carp (*Cirrhinus mrigala*) given a diet that included meal made from *E. foetida* worms showed superior development and nutrient utilisation than fish meal-based diet, according to Ganesh et al. [54]. In India, fresh earthworms (*Eisenia foetida*) were fed to catfish (*Clarias batrachus*) observed that the fish gained more weight than the control fish given a standard feed without earth worm mealEWM . Aquarium fish (*Poecilia reticulate*) consumed earthworm biomass (*E. foetida*) produced twice as many offspring and had a much higher brood number than fish fed a diet devoid of earthworms. EWM (*Eisenia foetida*) was evaluated by Vodounnou et al. [23] on *Parachanna obscura* for six weeks as a substitute for fishmeal. The fish feed with 50% EWM produced greater growth rates and FCR. Using earthworms (whole, custard, and pellet from *Eisenia foetida*), Mohanta et al. [22] investigated gaining weight, rate of development, and FCR in rohu (*Labeo rohita*) and discovered that the pellet produced the best results for all measurements tested. According to Karabulut et al. [60], rainbow trout (*Oncorhynchus mykiss*) could grow more effectively if earthworm meal (EM) were substituted for 30% of the fish meal.

However, according to Tacon et al. [13], providing rainbow trout with 100% worm powder (*E. foetida*) protein in the form of frozen slices had no encouraging development or nutritive results. For monogastric animals and fish, worm meal can replace fish meal, providing 25–50% of the diet's protein needs, according to Edwards and Niederer [60]. Common carp lack free accessibility to rich natural sources of nourishment, the substitution of dried earthworm meal for fishmeal caused decreased development [55]. According to Pereira and Gomes [10], the development rate and feed utilization efficiency of rainbow trout were negatively impacted by feeding them diets enriched in frozen worms. According to Shiau and Yu [62], hybrid tilapia (*Oreochromis niloticus*× *O. aureaus*) consumed diets including as low as 2% chitin showed impaired growth and feed efficiency. According to Pereira and Gomes [10], a absence of particular amino acids such as lysine, methionine, and cystine might also contribute to poor development and nutritional absorption in fish fed solely or at significant amounts of earthworm in their diets. According to Ng et al. [63], the abundance of chitin in meal worms resulted in growth drooping, inadequate nutrition and protein utilization in catfish fed large amounts or entirely on feed worms. Marble goby (*Oyxerlotris marmorata*) and pangasius catfish (*Pangasius hypophthalmus*) growth was inhibited by feeds that gradually replaced fishmeal with frozen *Perionyx excavates,* however, aquatic organsims in an experiment demonstrated considerably greater development rates when given fresh earthworms as opposed to frozen ones. White prawns that received meals with fermented soybean meal and earthworms had a comparable reduction in growth [21]. Similar outcomes were observed when Nile tilapia (*O. niloticus*) in a semi-intensive culture type, earthworms, Eisenia, and bed feed were used as protein sources [24]. Mi et al. [25] found no changes between the Control, 15.4% EM, and 15.1% EM and 1.8% WC groups regarding of WGR, SGR, and FCR; however, these variables significantly decreased in the 14.8% EM and 3.7% WC, and 14.5% EM and 5.8% WC groups. These findings demonstrated that the high WC did not promote the growth of common carp.

Other haematological indicators in our results showed little variations between fish fed an earthworm diet and fish given a regular diet, with the exception of lymphocytes, which were lower in the latter group.. According to Rawling et al. [64], earthworm meal (*Perionyx escavatus*) fed fish mirror carp (*Cyprinus carpio*) had a significantly higher haemoglobin level than control and soybean meal (SBM) provide food for fish. As a result, fish that were fed earthworms (EW) demonstrated a significant rise in both the average corpuscular hemoglobin concentration and mean hemoglobin. On the other hand, total leukocyte levels were markedly lowered in earthworms (EW) treatment in contrast to soybean meal and the control (SBM) fed carp. As reported by Razzaghi et al. [60], the greatest concentrations of haemoglobin, hematocrit, and white blood cells (WBCs) were found in treatments that included 25% earthworm meal plus garlic powder, 25% earthworm meal with and without garlic powder, and 75% earthworm meal without garlic powder, respectively. The smallest concentrations of red blood cells (RBCs) were found in treatments containing 50% earthworm meal without garlic powder. The treatment incorporates 2.5% garlic powder and 0.0% earthworm meal had MCV, MCH, and MCHC values that were significantly greater than those of the another treatments, with the exception of the control treatment. The diets formula with zero earthworm meal and zero to two percent garlic powder showed the greatest blood protein levels. The lowest levels of triglycerides and blood sugar were seen in the treatment containing 2.5% garlic powder and 25% earthworm meal. The treatments comprising 75% earthworm meal with 0 and 2.5% garlic powder had the lowest cholesterol values. When earthworm meal (*Perionyx excavatus*) was added to the diets of young butter catfish (*Ompok pabda*), the haematological parameters did not exhibit any abnormalities [66].

The respiratory burst activity, hematocrit, phagocytic activity (PA), phagocytic index (PI) super oxide dismutase (SOD), and leucocyte differentiation were all considerably raised when fermented earthworms (FE) supplemented at 2.5%, but natural agglutination was unaffected.Collectively, the findings imply that using FE as a feed addition is a useful and practical method for enhancing catfish non-specific immunity. According to Pimentel et al. [67]. There have been no noticeable variations in performance responses or haematological profiles between factors affecting hematocrit andMCHC In contrast, the treatment containing 20% earthworm meal was equivalent to the treatments involving 5 and 10% earthworm meal, but significantly different from the control treatment in terms of haemoglobin levels, demonstrating its capability as a fish feed additive . As reported by Mi et al. [25], after feeding common carp (*Cyprinus carpio L.*) earthworm meal (EM) and earthworm cast (WC), serum AKP, AST, and ALT activities did not significantly change. All substitution groups significantly reduced their TC contents as contrasted to the Control group, the 14.5% EM and 5.8% WC members had considerably lower TG and LDL-C levels. In contrast, the 14.5% EM and 5.8% WC group had considerably higher levels of HDL-C. Additionally, all substitution groups had considerably lower MDA levels than the Control group. SOD activity was markedly increased in the14.5% EM and 5.8% WC groups. The group with the highest LYZ activity was 15.4% EM group

According to our findings, there was no discernible difference between fish given an earthworm diet and fish on normal diet in terms of the mRNA expression values of GH. Growth hormone (GH) gene expression was shown by Zhou et al. [68] to increase when dietary carbohydrate levels rose to 38% and then fall in Wuchang Bream (*Megalobrama amblycephala*). The control group showed the highest levels of insulin-like growth factor-1 (IGF-1) gene expression in the brain, liver, and tissue. These levels were statistically similar to those for the detoxified *Jatropha curcas* kernel meal group had considerably greater rates than other of the groups. According to Hassaan et al. [69], when compared to other groups, the group given cotton seed meal (CSM) and lacking protease enzyme had the highest growth hormone (GH) gene expression in the tilapia brain and liver. Even though there was no discernible change in the levels of GH gene expression among the groups, research suggests that adding up to 0.5% of cysteic acid to the diet can help fish grow [70]. This research suggests that Japanese flounder can make taurine from cysteic acid. In Nile tilapia fish fed soybean and maize cultivars, Al-Hawary et al. [71] discovered that the expression of liver and muscle insulin-like growth factor 1 (IGF-1) did not change. When compared to soybean meal-based diets with black soldier fly prepupae (BSFP), the expression levels of the GH gene in the liver were noticeably higher in largemouth bass (Micropterus salmoides). Contrary to the liver, the expression levels of GH in the gut remained constant throughout several feeding regimes [72].

According to our findings, the liver and intestine of tilapia received an earthworm diet displayed a normal histological structure, no indication of any particular pathology, and normal protein and carbohydrate contents. The findings of a study on hybrid snakeheads (*Channa argus*× *Channa maculata*) also suggested that supplementing with 7.5% earthworm meal could encourage the formation of intestinal villi. According to Zhao et al. [73], providing solely earthworms in feeds can improve the villus height and width of common carp. This compares to consuming simply earthworms and duckweed, or duckweed alone. According to Mi et al. [25], both the (15.1% EM and 1.8% WC) and (14.8% EM and 3.7% WC) groups had higher intestinal villus heights. According to Song et al. [74], one possible explanation is that the gastrin contained in earthworms encourages diversity and development of intestinal epithelial cells, hence creating favorable circumstances for the creation of intestinal villi.

CONCLUSION

The present findings demonstrated that the health status of tilapia (*O. niloticus*) was significantly and favourably impacted by the partial inclusion of earthworm meals in their diets. As a result, we were able to confirm that EM might be a viable and sustainable source of protein for tilapia (*O. niloticus*).

ACKNOWLEDGEMENT

The study was conducted in a recently created Floting Raft Culture system (FTC), a prototype created at the Upper Egypt Centre of Development, which is owned by the ASRT and located in the Egyptian governorate of Sohag. The ASRT provided financial support for the project. The support has been greatly appreciated by the authors. The ASRT has funded Maha Gamal with a grant from the Next Generation Scholars Programme (seventh session 2020).

REFERENCES

1. Naira Ali Mohamed; Ameer ELfarash; Mohamed A. Abdel-Sater; Elhagag Ahmed Hassan, 2024. Fungal diversity and composition in River Nile water polluted with chlorpyrifos insecticide. Journal of Applied Molecular Biology, Vol. 2(1): 31-51
2. Alaa El-Din Hamid Sayed; Sary Kh. Abdel-Ghaffar; Asmaa A. A. Hussein, 2024. Environmental Assessment and Experimental Trial on the Effects of BTX Exposure on Adults and Embryos of Bufo regularis. Journal of Applied Molecular Biology, Vol. 2(1).
3. Reem Ahmed Mohamed; Ameer Effat M. Elfarash; Elhagag Hassan Ali, 2023. Evaluation the fungal biodiversity and structure in Nile River water treated with the emerging pollutant ibuprofen. Journal of Applied Molecular Biology, Vol. 1(1): 32-50

4. Fatma Elzahraa Abdelhamed; Elhagag Hassan; Mohamed Abdel-sater; Ameer Effat M. Elfarash, 2023. Analysis of fungal diversity and structure in Nile River water polluted with crude oil and naphthalene using microcosm experiments. Journal of Applied Molecular Biology, Vol. 1(1): 51-72
5. Lehman, H., Clark, E. A.,Weise, S. F., 1993. Clarifying the definition of sustainable agriculture. Journal of Agricultural and Environmental Ethics, 6, 127-143.
6. Adler, P. R., Summerfelt, S. T., Glenn, D. M.,Takeda, F., 2003. Mechanistic approach to phytoremediation of water. Ecological Engineering, 20(3), 251-264.
7. Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliott, M., Farrell, A. P., Forster, I., Gatlin, D. M., Goldburg, R. J.,Hua, K., 2009. Feeding aquaculture in an era of finite resources. Proceedings of the National Academy of Sciences, 106(36), 15103-15110.
8. Tacon, A. G.,Metian, M., 2009. Fishing for aquaculture: non-food use of small pelagic forage fish—a global perspective. Reviews in Fisheries Science, 17(3), 305-317.
9. Hardy, R. W., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture research, 41(5), 770-776.
10. Pereira, J. ó.,Gomes, E. F., 1995. Growth of rainbow trout fed a diet supplemented with earthworms, after chemical treatment. Aquaculture International, 3, 36-42.
11. Bhuvaneshwaran, T., Sanjay, G., Jayakumar, N., Ahilan, B., Felix, N.,Prabu, E., 2019. Potentiality of earthworm as a replacement for fish meal and its role in aquaculture. Journal of Aquaculture in the Tropics, 34(1/2), 115-127.
12. Guerrero, R., 1981. The culture and use of Peronya excavatus as a protein resource in the Phillippines, Dorwin Centenary Symp. On Earthworm ecology, Inst. Terrestrial Ecology UK Aug, 30.
13. Tacon, A., Stafford, E.,Edwards, C., 1983. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture, 35, 187-199.
14. Reinecke, A.,Alberts, J., 1987. The chemical and ammo acid composition of the compost worm Eisenia fetida as potential protein source in animal feeds. Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie, 6(4), 144-149.
15. Lieberman, S., 2002. Worms, beautiful worms. International worm digest, 4, 11-18.
16. Medina, A., Cova, J., Vielma, R., Pujic, P., Carlos, M.,Torres, J., 2003. Immunological and chemical analysis of proteins from *Eisenia foetida* earthworm. Food and Agricultural Immunology, 15(3-4), 255-263.
17. Istiqomah, L., Sofyan, A., Damayanti, E.,Julendra, H., 2009. Amino acid profile of earthworm and earthworm meal (Lumbricus rubellus) for animal feedstuff. Journal of the Indonesian Tropical Animal Agriculture, 34(4), 253-257.
18. Hansen, R. P.,Czochanska, Z., 1975. The fatty acid composition of the lipids of earthworms. Journal of the Science of Food and Agriculture, 26(7), 961-971.
19. Dynes, R. A. (2003). *Earthworms: technology information to enable the development of earthworm production*. Canberra, Rural Industries Research and Development Corporation.
20. Sogbesan, A.,Madu, C., 2008. Evaluation of earthworm (Hyperiodillus euryaulos, clausen, 1914; oligocheata: eudrilidae) meal as protein feedstuffs in diet for

Heterobranchus longifilis valenciennes, 1840 (teleostei, clariidae) fingerlings under laboratory condition. Research Journal of Environmental Sciences, 2(1), 23-31.

21. Chiu, S. T., Wong, S. L., Shiu, Y. L., Chiu, C. H., Guei, W. C.,Liu, C. H., 2016. Using a fermented mixture of soybean meal and earthworm meal to replace fish meal in the diet of white shrimp, Penaeus vannamei (Boone). Aquaculture Research, 47(11), 3489-3500.
22. Mohanta, K. N., Subramanian, S.,Korikanthimath, V. S., 2016. Potential of earthworm (*Eisenia foetida*) as dietary protein source for rohu (Labeo rohita) advanced fry. Cogent Food & Agriculture, 2(1), 1138594.
23. Vodounnou, D., Kpogue, D., Tossavi, C., Mennsah, G.,Fiogbe, E., 2016. Effect of animal waste and vegetable compost on production and growth of earthworm (Eisenia fetida) during vermiculture. International Journal of Recycling of Organic Waste in Agriculture, 5, 87-92.
24. Musyoka, S. N., Liti, D., Ogello, E. O., Meulenbroek, P.,Waidbacher, H., 2020. Earthworm, Eisenia fetida, bedding meal as potential cheap fishmeal replacement ingredient for semi-intensive farming of Nile Tilapia, Oreochromis niloticus. Aquaculture Research, 51(6), 2359-2368.
25. Mi, J., Lu, R., Yan, X., Song, D., Yang, L., Qin, C., Yang, G., Zhang, H., Lin, M.,Nie, G., 2022. Evaluating the mixture of earthworm meal and wormcast as a protein source for common carp (Cyprinus carpio L.) based on growth performance, antioxidant, immune capacity, lipid metabolism and intestinal health. Aquaculture Reports, 24, 101118.
26. Fazio, F., Marafioti, S., Torre, A., Sanfilippo, M., Panzera, M.,Faggio, C., 2013. Haematological and serum protein profiles of Mugil cephalus: effect of two different habitats. Ichthyological research, 60, 36-42.
27. Seibel, H., Baßmann, B.,Rebl, A., 2021. Blood will tell: what hematological analyses can reveal about fish welfare. Frontiers in Veterinary Science, 8, 616955.
28. Groff, J. M.,Zinkl, J. G., 1999. Hematology and clinical chemistry of cyprinid fish: common carp and goldfish. Veterinary Clinics of North America: Exotic Animal Practice, 2(3), 741-776.
29. Khan, Y. M., Khan, M. A., 2021. Optimization of dietary pyridoxine improved growth performance, hematological indices, antioxidant capacity, intestinal enzyme activity, non-specific immune response, and liver pyridoxine concentration of fingerling major carp Catla catla (Hamilton). Aquaculture, 541, 736815.
30. Refaey, M. M.,Li, D., 2018. Transport stress changes blood biochemistry, antioxidant defense system, and hepatic HSPs mRNA expressions of channel catfish Ictalurus punctatus. Frontiers in Physiology, 9, 1628.
31. Uiuiu, P., Lațiu, C., Păpuc, T., Craioveanu, C., Ihuț, A., Sava, A., Răducu, C., Șonea, C., Constantinescu, R.,Cocan, D., 2021. Multi-approach assessment for stress evaluation in rainbow trout females, Oncorhynchus mykiss (Walbaum, 1792) from three different farms during the summer season. Animals, 11(6), 1810.
32. Ghirmai, S., Eriksson, L., Wu, H., Axelsson, M.,Undeland, I., 2020. Improving the stability of red blood cells in rainbow trout (Oncorhynchus mykiss) and herring (Clupea harengus): Potential solutions for post-mortem fish handling to minimize lipid oxidation. Food and Bioprocess Technology, 13, 1344-1355.

33. Ahmed, I.,Ahmad, I., 2020. Effect of dietary protein levels on growth performance, hematological profile and biochemical composition of fingerlings rainbow trout, Oncorhynchus mykiss reared in Indian himalayan region. Aquaculture Reports, 16, 100268.
34. Magnoni, L. J., Novais, S. C., Eding, E., Leguen, I., Lemos, M. F., Ozório, R. O., Geurden, I., Prunet, P.,Schrama, J. W., 2019. Acute stress and an electrolyte-imbalanced diet, but not chronic hypoxia, increase oxidative stress and hamper innate immune status in a rainbow trout (Oncorhynchus mykiss) isogenic line. Frontiers in Physiology, 10, 453.
35. Matulić, D., Barišić, J., Aničić, I., Tomljanović, T., Safner, R., Treer, T., Gao, J., Glojnarić, I.,Čož-Rakovac, R., 2020. Growth, health aspects and histopathology of brown bullhead (Ameiurus nebulosus L.): replacing fishmeal with soybean meal and brewer's yeast. Scientific Reports, 10(1), 1-16.
36. Walaa M. Shaalan; Axel Mosig; Aya Elshaer, 2024. Histopathological and molecular responses of goldfish (Carassius auratus) exposed to sodium and manganese sulfates. Journal of Applied Molecular Biology, Volume 2 (2): 203-223
37. Elia, A. C., Capucchio, M. T., Caldaroni, B., Magara, G., Dörr, A. J. M., Biasato, I., Biasibetti, E., Righetti, M., Pastorino, P.,Prearo, M., 2018. Influence of Hermetia illucens meal dietary inclusion on the histological traits, gut mucin composition and the oxidative stress biomarkers in rainbow trout (Oncorhynchus mykiss). Aquaculture, 496, 50-57.
38. Chen, W. Y., Chen, N., Yun, J., Wagner, T. E.,Kopchick, J. J., 1994. In vitro and in vivo studies of antagonistic effects of human growth hormone analogs. Journal of Biological Chemistry, 269(22), 15892-15897.
39. Forsyth, I. A.,Wallis, M., 2002. Growth hormone and prolactin-molecular and functional evolution. Journal of mammary gland biology and neoplasia, 7(3), 291.
40. De-Santis, C.,Jerry, D. R., 2007. Candidate growth genes in finfish—Where should we be looking? Aquaculture, 272(1-4), 22-38.
41. Moriyama, S., Ayson, F. G.,Kawauchi, H., 2000. Growth regulation by insulin-like growth factor-I in fish. Bioscience, biotechnology, and biochemistry, 64(8), 1553-1562.
42. Soliman, H.A.,_Badrey, A. E., & Osman, A. G. (2022). Growth performance and hematological, biochemical, and histological characters of the Nile tilapia (*Oreochromis niloticus, L.*) cultivated in an aquaponic system with green onion: The first study about the aquaponic system in Sohag, Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 26(2), 119-131.
43. Association of Official Analytical Chemists (AOAC) (1990): Official Methods of Analysis of the 15th ed. Atomic Absorption Method for fish. Washington, D. C.
44. Bligh, E. G.,Dyer, W. J., 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 37(8), 911-917.
45. Bain, B. J., Bates, I.,Laffan, M. A. (2016). *Dacie and lewis practical haematology e-book*. Elsevier Health Sciences.
46. Bricknell, I., Bowden, T., Bruno, D., MacLachlan, P., Johnstone, R.,Ellis, A., 1999. Susceptibility of Atlantic halibut, Hippoglossus hippoglossus (L.) to infection with typical and atypical Aeromonas salmonicida. Aquaculture, 175(1-2), 1-13.

47. Pfaffl, M. W., 2001. A new mathematical model for relative quantification in real-time RT–PCR. Nucleic acids research, 29(9), e45-e45.
48. Elbialy, Z. I., Gamal, S., Al-Hawary, I. I., Shukry, M., Salah, A. S., Aboshosha, A. A.,Assar, D. H., 2022. Exploring the impacts of different fasting and refeeding regimes on Nile tilapia (Oreochromis niloticus L.): Growth performance, histopathological study, and expression levels of some muscle growth-related genes. Fish Physiology and Biochemistry, 48(4), 973-989.
49. Qiang, J., He, J., Yang, H., Wang, H., Kpundeh, M., Xu, P.,Zhu, Z., 2014. Temperature modulates hepatic carbohydrate metabolic enzyme activity and gene expression in juvenile GIFT tilapia (Oreochromis niloticus) fed a carbohydrate-enriched diet. Journal of thermal biology, 40, 25-31.
50. Abo-Raya, M. H., Alshehri, K. M., Abdelhameed, R. F., Elbialy, Z. I., Elhady, S. S.,Mohamed, R. A., 2021. Assessment of growth-related parameters and immune-biochemical profile of nile tilapia (oreochromis niloticus) fed dietary ulva fasciata extract. Aquaculture Research, 52(7), 3233-3246.
51. Muzinic, L., Thompson, K., Metts, L., Dasgupta, S.,Webster, C., 2006. Use of turkey meal as partial and total replacement of fish meal in practical diets for sunshine bass (Morone chrysops× Morone saxatilis) grown in tanks. Aquaculture Nutrition, 12(1), 71-81.
52. Goddard, S., Al-Shagaa, G.,Ali, A., 2008. Fisheries by-catch and processing waste meals as ingredients in diets for Nile tilapia, Oreochromis niloticus. Aquaculture Research, 39(5), 518-525.
53. Mondal, K., Kaviraj, A.,Mukhopadhyay, P., 2011. Partial replacement of fishmeal by fermented fish-offal meal in the formulation of diet for Indian minor carp Labeo bata. Journal of Applied Aquaculture, 23(1), 41-50.
54. Akiyama, T., Murai, T., Hirasawa, Y.,Nose, T., 1984. Supplementation of various meals to fish meal diet for chum salmon fry. Aquaculture, 37(3), 217-222.
55. Stafford, E.,Tacon, A., 1984. Nutritive value of the earthworm, Dendrodrilus subrubicundus, grown on domestic sewage, in trout diets. Agricultural Wastes, 9(4), 249-266.
56. Nandeesha, M., Srikanth, G., Basavaraja, N., Keshavanath, P., Varghese, T., Bano, K., Ray, A.,Kale, R. D., 1988. Influence of earthworm meal on the growth and flesh quality of common carp. Biological wastes, 26(3), 189-198.
57. Keshavappa, G., Devaraj, K.,Seenappa, D. (1989). Evaluation of three animal based feeds for rearing of catla fry. Proceedings of the National Symposium on Freshwater Aquaculture.
58. Velasquez, L., Ibanez, I., Herrera, C.,Oyarzun, M., 1991. A note on the nutritional evaluation of worm meal (Eisenia fetida) in diets for rainbow trout. Animal Science, 53(1), 119-122.
59. Ganesh, P., Mohan, M., Subha, R.,Vijayalakshmi, G., 2003. Earthworm meal for fish feed formulation and its influence on growth of mrigal fingerlings. Journal of Ecobiology, 15(3), 181-184.
60. Karabulut, H. A., Kurtoglu, I. Z., Yuksek, T.,Bayraktar, Z., 2020. The investigation of the possibility of using red California earthworm (Eisenia Fetida) meal as an alternative protein source in Rainbow Trout (Oncorhynchus Mykiss) diet. Fresenius Environmental Bulletin, 29(4 A), 2951-2959.

61. Edwards, C.,Niederer, A., 1988. Production and processing of earthworm protein. Earthworms in waste and environmental management/edited by Clive A. Edwards and Edward F. Neuhauser.
62. Shiau, S.-Y.,Yu, Y.-P., 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, Oreochromis niloticus× O. aureus. Aquaculture, 179(1-4), 439-446.
63. Ng, W. K., Liew, F. L., Ang, L. P.,Wong, K. W., 2001. Potential of mealworm (Tenebrio molitor) as an alternative protein source in practical diets for African catfish, Clarias gariepinus. Aquaculture Research, 32, 273-280.
64. Rawling, M., Merrifield, D., Snellgrove, D., Kühlwein, H., Adams, A.,Davies, S., 2012. Haemato-immunological and growth response of mirror carp (Cyprinus carpio) fed a tropical earthworm meal in experimental diets. Fish & Shellfish Immunology, 32(6), 1002-1007.
65. Razzaghi, B., Yeganeh, S., Keramat Amirkolaie, A.,Janikhalili, K., 2018. Effects of dietary earthworm (Eisenia fetida) meal and garlic (Allium sativum) powder on hematological parameters of Rainbow trout (Oncorhynchus mykiss).
66. Chakraborty, P., Islam, M. R., Hossain, M. A., Fatema, U. K., Shaha, D. C., Sarker, M. S. A.,Akter, T., 2021. Earthworm meal (Perionyx excavatus) as an alternative protein source to fish meal in feed for juvenile butter catfish (Ompok pabda). Aquaculture International, 29, 2119-2129.
67. Pimentel, S., Cardoso, I. L., Ferri, G. H., Silva, L. C., de Queiroz, J. F., Ivo, M. A.,Ishikawa, M. M., 2022. Use of earthworm (Eisenia fetida) meal as a supplement for feeding tilapia juveniles (Oreochromis niloticus).
68. Zhou, C., Ge, X., Liu, B., Xie, J., Xu, P., Zhou, Q., Pan, L.,Chen, R., 2013. Effect of dietary carbohydrate level on growth performance, blood chemistry, hepatic enzyme activity, and growth hormone gene expression in Wuchang bream (Megalobrama amblycephala). The Israeli Journal of Aquaculture-Bamidgeh, IJA_65, 8.
69. Hassaan, M., El-Sayed, A., Soltan, M., Iraqi, M., Goda, A., Davies, S., El-Haroun, E.,Ramadan, H., 2019. Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth, feed performance, hematological indices and associated gene expression markers (GH, IGF-I) for Nile tilapia, Oreochromis niloticus. Aquaculture, 503, 282-292.
70. Nakamura, K., Gonzales-Plasus, M. M., Ushigusa-Ito, T., Masuda, R., Kabeya, N., Kondo, H., Hirono, I., Satoh, S.,Haga, Y., 2021. Taurine synthesis via the cysteic acid pathway: effect of dietary cysteic acid on growth, body taurine content, and gene expression of taurine-synthesizing enzymes, growth hormone, and insulin-like growth factor 1 in Japanese flounder Paralichthys olivaceus. Fisheries science, 87, 353-363.
71. Al-Hawary, I. I., Elbialy, Z. I., Barsem, D. B., Abdel-Mawgood, A., Sallah, A. S., Rabie, T. S., Assar, D. H.,Dawood, M. A., Evaluating local strains of soybean and corn cultivars in the diets of Nile tilapia (): growth and insulin-like growth factor 1, intestinal health, and inflammation features. Annals of Animal Science, 22(4), 1301-1309.
72. Fischer, H., Romano, N., Renukdas, N., Kumar, V.,Sinha, A. K., 2022. Comparing black soldier fly (Hermetia illucens) larvae versus prepupae in the diets of

largemouth bass, Micropterus salmoides: Effects on their growth, biochemical composition, histopathology, and gene expression. Aquaculture, 546, 737323.

73. Zhao, L., Luo, J., Liu, Q., Du, J., Yang, H., Li, B., Zhou, Y.,Yang, S., 2020. Different diets can affect the digestion and immunity of common carp (Cyprinus carpio) according to enzyme activity assay and transcriptome sequencing. Aquaculture, 523, 735176.
74. Song, S., Wang, Y., Ji, K., Liang, H.,Ji, A., 2016. Effect of earthworm active protein on fibroblast proliferation and its mechanism. Pharmaceutical biology, 54(4), 732-739.