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Use of earthworm (*Eisenia fetida*) meal as a supplement for feeding tilapia juveniles (*Oreochromis niloticus*)

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Abstract

Earthworm meal comprises a class of food additives with potential to replace or supplement conventional proteins used in fish feed. Considering the potential of earthworm meal, as a protein source for fish feed, this study aimed to investigate the productive performance and the hematological profile of Nile tilapia juveniles fed with a commercial diet enriched with earthworm meal at levels (0.0; 5.0, 10 and 20%). The assay was conducted for 32 days and the productive performance and hematology profile of the animals were evaluated. Tilapia juveniles (n=144; 25.30±3.27g) were distributed in 12 aquariums (300 L) in a static system with partial water changes. The experimental design was completely randomized with four treatments and three replications. There were no significant differences ($p>0.05$) for performance responses and hematological profile related to variables on hematocrit and CHCM. However, for hemoglobin values the treatment with 20% earthworm meal was similar to the treatments with 5 and 10% earthworm meal, and significantly differed from the control treatment, suggesting its potential as a fish feed supplement. Thus, earthworm meal can be used at 5, 10, and 20% as a supplement for Nile tilapia juveniles without compromising their performance and hematological profile.

Keywords: Fish farm; Food supplement; Hematological response; Performance response

1 Introduction

Fishmeal has been used in large scale as a protein source for animal feed [1]. The capture of marine fish, which are the main sources used for fishmeal production, is causing a series of drastic variations on natural stocks. The intensification of such extractive activity and the negative impacts caused by climatic phenomena can compromise the supply of fishmeal on the market [2], [3] Complementary or alternative sources must be evaluated to supply the increase demand of protein for fish feed. Thus, earthworm meal can be used as a viable option to replace fish protein in commercial feeds used by the aquaculture industry, Several studies with different fish species were conducted successfully to evaluate its effects on zootechnical performance [4],[5],[6].

Earthworm meal has several beneficial characteristics that justify its use for the formulation of feed for fish and other animals. Earthworm meal has high levels of protein, energy, minerals, vitamins, and it can be also included in

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commercial feeds for poultry, swine and fish. [7], [8]. In addition, earthworm meal is an easy-to-produce product, as it uses organic waste for its growth favoring the low cost of its production [9]. *Eisenia fetida* is one of the most interesting earthworm species for meal production, as it stands out for its ability to use a wide variety of organic residues and for its high reproductive rate [10].

Few studies have been conducted using earthworm meal as supplement for Nile tilapia (*Oreochromis niloticus*). Tilapia is the common name used to refer to several species of fish belonging to the Cichlidae family [11]. Native to Africa, Israel and Jordan, tilapia are now cultivated in more than 135 countries under different climates, production systems and salinities [12], [13]. Tilapia is also adaptable to adverse environmental situations, has different feeding habits, being omnivorous, phytoplanktophagous or herbivorous [14]. Tilapia also accepts easily other types of food what makes this species of great interest for fish farming [15].

Global production of tilapia exceeds one million tons per year, and it is in continuous expansion [16]. According to [17], Nile tilapia (*Oreochromis niloticus*) is probably the major species produced by aquaculture worldwide. Tilapia fillet yield is around 35 to 40%, and it is absent from myocepts (Y-shaped spines), what makes it suitable for industrialization [18]. In addition, the quality of its meat is appreciated worldwide, what is one of the factors that have highlighted tilapia in the world market [19].

Nowadays, the great challenge for the aquaculture industry is to search for alternative sources of protein to use in fish feed. At same time, it must be considered economic and environmental issues in order to improve fish productivity and animal health, which are essential to assure environmental quality.

Therefore, the main objective of the present study was to evaluate the effects of earthworm (*Eisenia fetida*) meal supplementation at different levels on zootechnical performance and hematological profile parameters of Nile tilapia juveniles during a period of 32 days.

2 Material and methods

The experiment was conducted at the Laboratory of Aquatic Ecosystems at Embrapa Environment, Jaguariúna-SP. The experiment was approved by the Animal Ethic Committee AEC (Embrapa Environment) Protocol n º 009/2018.

2.1 Experimental diets

The experimental diets were prepared with a commercial feed (Nutripiscis TR 32® specific for tilapia: (4 mm; 12 % moisture; 32 % protein; 6 % fat) supplemented with earthworm meal at 0 %, 5 %, 10 % and 20 %. All the ingredients – commercial feed and earthworm meal - were processed with a laboratory mill to obtain 0.5 mm average size, further mixed, moistened with 20% water at 50 ºC and processed in a meat mill (2.5 mm). Finally, all the experimental diets were dried in a forced ventilation oven (55 ºC) for 24 hours and then stored in pots.

2.2 Performance test

Nile tilapia juveniles (25.30±3.27g) were randomly distributed in 12 aquariums with useful volume of 300 L provided with a closed water recirculation system consisted by a physical and biological filter. Twelve fish per aquarium were stocked, and a 1.0 hp blower was used for supplementary aeration. Temperature was kept constant by electrical heaters and thermostats.

The experimental design was completely randomized with four treatments (0; 5; 10 and 20%) and three replications for each treatment. The experiment was conducted during 32 days, and the experimental diets were supplied to all aquariums three times a day (8 h; 12 h; and 16 h) in small amounts until the fish were apparently satiated.

The following variables of fish performance were evaluated - weight gain (WG (g) = final weight (g) – initial weight (g)), feed consumption (FC (g) = total food consumed (g) / experimental period), apparent feed conversion rate (AFCR = feed provided (g) / weight gain (g)).

2.3 Water quality monitoring

Water quality was monitored daily in the morning before feeding the fish. Measurements of physical and chemical variables were done with a multiparameter probe (U-50, Horiba, Minami-ku, Kyoto, Japan). The following variables were measured, temperature ºC, pH units of pH, dissolved oxygen mg/L-1, electrical conductivity (µs/cm).

2.4 Hematological analysis

At the end of the experimental period five fish from each aquarium were subjected to anesthetic induction with 100 mg L-1 benzocaine (immersion bath), and blood collection was performed by caudal puncture with syringes containing EDTA (3%).

Hematocrit (Htc%) was determined by the microhematocrit method and centrifuged in a model NI 1807 centrifuge (Nova Instruments, Piracicaba, SP, Brazil) for 5 min and 10,000 rpm. Hemoglobin (Hb; g.dL-1) analyzes were performed using the hemoglobin cyanide (HCN) method with a Labtest Diagnóstica Kit (Labtest Diagnóstica, MG, Brazil). After blood collection, the animals received an overdose of benzocaine at 200 mg L-1 (immersion bath) and then euthanized. Muscle, liver, intestine and viscera were collected from 3 animals per treatment for later histological analysis.

2.5 Statistical analysis

Normality test was applied and the means were submitted to analysis of variance (ANOVA), and when significant, applied to the Tukey test at 5% of significance. The analyzes were performed with the statistical program Rstudio.

3 Results

Water quality of all aquariums was measured daily during the whole experimental period (Table 1).

Table 1 Water quality parameters of aquariums supplemented with different levels of earthworm meal

Parameter	Earthworm Meal (%)			
	0	5	10	20
Temperature ºC	25.90±0.23[a]	25.98±0.21[a]	25.82±0.10[a]	25.90±0.25[a]
Conductivity	0.199±0.01[a]	0.197±0.01[a]	0.189±0.01[a]	0.194±0.02[a]
pH	6.79±0.02[a]	6.79±0.11[a]	6.74±0.10[a]	6.79±0.16[a]
Dissolve Oxygen	5.67±0.09[a]	5.56±0.06[a]	5.59±0.03[a]	5.76±0.21[a]

Means followed by different letters on the same line differ by Tukey's test (P < 0.05).

There were no significant differences (p>0.05) for initial weight, final weight, weight gain, feed consumption and feed conversion between treatments (Table 2). Although, the commercial feed was crushed and pelletized again in order to make the experimental diets composed by different levels of earthworm meal, the similar results of fish productive performance between treatments showed that none of the levels of earthworm meal supplementation altered the performance variables evaluated.

Table 2 Mean values and standard deviation performance of Nile tilapia juveniles fed with diets supplemented with different levels of earthworm meal

Parameter	Earthworm Meal (%)			
	0	5	10	20
IW (g)	25.52±0.17[a]	25.63±0.15[a]	25.23±0.11[a]	25.29±0.13[a]
FW (g)	64.80±1.83[a]	62.38±1.07[a]	65.55±0.66[a]	68.22±1.64[a]
WG (g)	39.28±1.73[a]	36.52±0.97[a]	40.47±0.50[a]	42.93±1.76[a]
FC	46.03±2.86[a]	45.76±4.35[a]	46.82±0.82[a]	46.57±1.39[a]
FCR	1.30±0.06[a]	1.34 ±0.08[a]	1.31±0.07[a]	1.17±0.10[a]

Initial weight (IW), Final weight (FW), weight gain (WG), feed consumption (FC), feed conversion rate (FCR). Means followed by different letters on the same line differ by Tukey's test (P < 0.05).

Hematological variables of hematocrit and CHCM did not differ significantly (p>0.05) between treatments (Table 3).

Table 3 Blood variables of juvenile tilapia fed with diets supplemented with different levels of earthworm meal

Parameters	Earthworm Meal (%)			
	0	5	10	20
Hemoglobin (gdL^{-1})	11.68±4.00^{b}	12.38±2.30^{ab}	12.91±1.90^{ab}	13.88±2.87^{a}
Hematocrit (%)	27.3±8.67^{a}	33.06±3.1^{a}	31.0±5.5^{a}	32.13±5.8^{a}
CHCM (%)	27.5±2.1^{a}	24.8±2.4^{a}	26±3.4^{a}	27.7±5.3^{a}
PPT	4.8±0.44^{a}	4.3±2.4^{a}	4.6±0.35^{a}	4.8±0.32^{a}

Means followed by different letters on the same line differ by Tukey's test ($P < 0.05$).

4 Discussion

No differences for physical and chemical parameters of water quality were observed between the treatments composed with different sources of earthworm protein and the control. These results are within the acceptable values for tilapia culture [20].

Considering the need to find alternative sources of protein to replace fish and soybean meal in commercial fish feeds, the results of this study indicated that earthworm meal has the potential to be an alternative source for these conventional proteins. The supplementation of up to 20% of earthworm meal in the experimental diets did not negatively affect the development of the fish during the study period of 32 days.

Similar results were obtained in an experiment carried out during 63 days with jundiá juveniles (*Rhamdia voulezi*). Fish were fed with a replacement level of up to 3.75% of earthworm meal and did not present negative effects on productive performance. The same authors recommend the use of earthworm meal as an alternative protein source for different growing phases of this species [21].

In another study, carried out with Nile tilapia (*Oreochromis niloticus*) post-larvae, the results showed that replacing fish meal with earthworm meal up to the level of 20% can be a suitable substitute for fish meal without harm the development of fish. It was also observed by the authors that it did not promote any negative effects on the animals during an experimental period 41 days [7].

[22], also observed positive results of earthworm meal on productive performance when evaluating the partial replacement of earthworm meal to fishmeal and soybean meal in Nile tilapia, concluding that the inclusion level of 1.25% of this ingredient improved weight gain. According to [23] in their experiment with African catfish, the replacement of viscera meal by the mixture of earthworm meal plus larvae meal resulted in a significant increase in the size of the animals supplemented with these diets.

For the hemoglobin values the treatment with 20% earthworm meal was similar to the treatments with 5 and 10% and differed significantly from the control treatment. This increase may be due to the availability in greater quantity of some nutrients in the earthworm meal. This fact suggests the potential of earthworm meal as a food supplement for tilapia.

Hematological parameters are important in understanding the normal and pathological conditions of fish, helping to identify adverse conditions [24]. In addition, hematology can be used as a tool for interpreting the effects of diets regarding aspects of fish immune function and health [25],[26].

The results of this study indicate that the earthworm meal did not compromise fish health during the experimental period of 32 days. This fact can be explained in part by the nutritional enrichment promoted by the earthworm meal in the 20% treatment compared to the control. However, the exact influence of earthworm meal on hemoglobin concentration still needs to be elucidated.

It was also observed that the increasing of the nutritional properties of the commercial feed used, such as, protein, energy and existing minerals, caused by the addition of earthworm meal did not affect the zootechnical performance and health parameters of Nile tilapia. Although, the results presented are preliminary they can be used for future studies using earthworm meal in partial and total replacement of fish meal and soybean meal as a sustainable and easily accessible alternative.

5 Conclusion

The inclusion of earthworm meal in a concentration of up to 20% in a commercial fish feed demonstrated its potential as a protein supplement for feeding Nile tilapia juveniles. In addition, the use of earthworm meal as a food supplement for juvenile tilapia did not compromise fish performance and hematological profile parameters.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest whatsoever among the authors in the cause of executing this research work.

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