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Culture of earthworm (*Eisenia fetida*), production, nutritive value and utilization of its meal in diet for *Parachanna obscura* fingerlings reared in captivity

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Abstract

The impact of the substitution of fish meal (FM) with earthworm meal (EM) in *Parachanna obscura* fingerlings diets was investigated for 42 days. Growth parameters, nutrient utilization and economical incidence of the fish were measured. Earthworms were cultured using animal wastes for 90 days and harvested, dried and stored. Five triplicate groups of fingerlings with initial average body weight of 1.13 ± 0.05 g were respectively fed with earthworm meal containing 0, 25, 50, 75 and 100% isonitrogenous diet (45% crude protein). The fish were reared in 15 circular tanks which volume of 90 l each, at a density of 20 fingerlings per tank. Better growth rate and feed conversion efficiency were obtained with the fish's diet containing 50% of earthworm meal. Feed efficiency, protein efficiency rate and specific growth rate between the groups of fish fed with 25% and 50% EM content diets were statistically similar ($p > 0.05$).

Keywords: Earthworm meal, culture, growth performance, feed utilization and *Parachanna obscura*

1. Introduction

Aquaculture is an important economy generating activity worldwide. It generates employment in rural areas and also provides food security (Ogunremi and Obasa 2009) [31]. More than half of the fish consumed in the world is provided by aquaculture (Naylor *et al.* 2009) [29]. Among African Snakeheads, *Parachanna obscura* is the most common (Bonou and Teugels 1985) [2]. Its rapid growth potential (2 g/day), tasty flesh, few bones, tolerance of high stocking density, utilization of atmospheric oxygen for respiration in water with low dissolved oxygen and high ammonia levels, make it a desirable species for culturing (Gras 1961;) [12]. Dietary protein requirement, feeding rate requirement, optimum stocking density of *P. obscura* have been previously studied (Kpogue, 2013) [21]. Because of the worldwide application of FAO capture fishery down regulation measure (FAO 2012) [11], the permanent availability of fish meal the main protein source for fish feeding, is problematic. In contrast, the development of global fish supply is increasing the demand for feed resources, especially high quality protein and lipid feed such as fish meal and fish oil (Naylor *et al.*, 2009, Tacon and Metian 2009) [29, 39]. As a result, fish meal prices are constantly rising, adversely affecting the profitability of aquaculture enterprises and increasingly forcing the aquaculture industry to explore new raw material like insects, worms and vegetable oils that can be used to feed fish. Earthworm meal was used in replacement of fish meal in plant based feeds for common carp in semi-intensive aquaculture (Pucher *et al.*, 2014) [34]. Such previous studies include the replacement of fish meal with Kikuyu grass and Moringa's Leaves (Hlophe and Moyo 2014) [17] and the substitution of fish meal with the earthworm meal (*Eudrilus eugeniae*) to feed the fingerlings of Heteroclaris (Monebi *et al.*, 2012) [26].

Among the many earthworm species which could be used for fish meal substitution. *Eisenia fetida* is the one which supports tropical climatic conditions (Tomlin 1981; Lee 1985; Curry 1998) [41, 22, 7]. According these authors, these species can reproduce normally at a temperature ranging between 20 and 29 °C. This epigeic earthworm lives on soil surface, and mainly feeds on plant litter and other organic debris. Consequently, these earthworms are the most suitable for converting organic wastes into useful organic manures (Chattopadhyay 2012; Vodounnou 2016) [4, 43]. This species is rich in amino acids and fatty acid such as Omega 3 (Dynes 2003) [10].

Some of the difficulties of aquaculture are the unavailability, the poor nutritive quality and the expensive cost of fish meal. Yet, because of its nutritional quality and palatability properties, this feedstuff is still the main dietary protein source (Hardy and Tacon 2002) [14]. The aim of the current study is to evaluate the effect of fish meal substitution with earthworm meal in *Parachanna obscura* fingerlings diet.

2. Materials and methods

2.1 Culture and meal production of earthworms.

Earthworms were cultivated in a mixture of dungs from (cow, sheep and rabbit) in 24 concrete cylindrical tanks (118 cm x 40 cm x 55 cm) for 90 days. The animal wastes were collected from farms located in Abomey-Calavi Benin. Because pre-composting is very essential to avoid the death of the worm (Gunadi and Edwards 2003) [13], dung was used 14 days after collection. Adults of *E. fetida* (clitella accented) weighting 0.7 to 1.2 g and measuring 7 to 8 cm in length were collected from garbages at the National Institute of Agricultural Research of Benin (INRAB). The experiment lasted for 90 days. Substrate mixture was inoculated to 20.62 ± 1.12 g of adult earthworms at a depth of 10 cm. During the study period substrate moisture was maintained by sprinkling water on the dung daily. The earthworms were weighed and rinsed with pure water and put into in bowl which was placed in a freezer. After drying them by using a lyophilisator, the dry matter was ground with a mill and then, stored at 0 -20 °C.

2.2 Use of earthworm meal in *P. obscura* fingerlings diet

A total of 300 fingerlings of *Parachanna obscura* (mean weight: 1.13± 0.05g) were provided by the Laboratory of Wetland Research of Abomey-Calavi University in Benin. The study was carried out for 42 days in 15 circular concreted tanks containing 90 l of water each under atmospheric conditions. Five isocaloric experimental diets were formulated to meet the nutrient requirements of *P. obscura* fingerlings (Kpogue 2013) [21]. Fish meal in the control diet was replaced with earthworm meal at the inclusion level of 25, 50, 75 and 100 (Table 1). Each experimental diet was assigned three tanks at a density of 20 fingerlings per tank. The feed was distributed at a rate of 5.01(fish biomass)^{-0.23} (Kpogue, 2013) [21]. The fingerlings were fed three times a day (Kpogue, 2013) [21] and the fish were examined weekly (7days).

Table 1: Formulation of experimental diets

Ingredients	Experimental diets				
	A0	A 25	A50	A75	A100
Cotton bran meal	20	20	20	20	20
Maize meal	12	12	12	12	12
Fish meal	45	33.75	22.5	11.25	0
Earthworm meal	0	11.25	22.5	33.75	45
Soy bean meal	17	17	17	17	17
God fish liver oil	2	2	2	2	2
Premix(min – vit)*	2.5	2.5	2.5	2.5	2.5
Ferrous sulphate	0.5	0.5	0.5	0.5	0.5
Carboxymethylcellulose	1	1	1	1	1

* premix (vitamin – mineral) contains (%):Vitamin A 4 000 000 U.I; Vitamin D 800 000 U.I; Vitamin E 40 000U.I; Vitamin K3 1600 mg; Vitamin B1 4 000 mg; Vitamin B2 3 000 mg; Vitamin B6 3 800 mg; Vitamin B12 3 mg; Vitamin C 60 000 mg; Biotin 100 mg; Inositol 10 000 mg Pantothenic acid 8 000 mg; Nicotinic acid 18 000 mg; Folic acid 800 mg; Choline chloride 120 000 mg; Cobalt carbonate 150 mg; Ferrous sulphate 8 000 mg; Potassium iodide 400 mg; Manganese oxide 6 000 mg; Copper 800 mg; Sodium selenite 40 mcg; Lysine 10 000 mg; Methionine 10 000 mg; Zinc sulphate 8 000 mg

2.3 Biochemical analysis

Temperature, pH and dissolved oxygen were monitored daily with a thermometer, a pH meter and an oxygen meter respectively.

Using the Association of Analytical Chemist methods (AOAC), (2000) [1], the proximate composition of the diets was analyzed for crude protein, crude lipid and gross energy. Fish carcasses were analyzed for crude protein before and after experiment. The amino-acids and crude protein of the earthworms were determined in laboratory of aquatic animal nutrition of the Faculty of Fishery of Kagoshima University in Japan.

2.4 Data and statistical analysis

At the end of the experiment, different parameters were calculated:

Specific Growth Rate (SGR; %/d) = 100 × [Ln (Final Body Weight (g)) - Ln (Initial Body Weight (g))] / Duration (days) of the experiment

Feed Efficiency (FE) = (FB+DB-IB) / FD, Survival Rate (SR, %) = 100 x FN/IN;

Condition factor (K %) = 100 x Final Body Weight /L³, Protein Efficiency Ratio (PER) = (FB - IB) / (FD x Dietary Protein), Protein Productive Value (PPV) = 100 x (Final Protein in fish – Initial Protein in Fish) / (Total Feed Intake per Fish x Dietary Protein).

With:

IB: Initial Biomasses (g), FB: Final Biomasses (g), DB: Dead fish Biomass (g), FD: Feed Distributed (g), IN: Initial Number, FN = Final Number, L: Fish Length (cm).

The data were analyzed using a one-way analysis of variance (ANOVA) with the facilities of STATVIEW version 5.01 software, after the verification of variance homogeneity, using Hartley’s test. Significant differences among means were determined using Fisher’s test p= 0.05 significance level.

3. Results

During the study, the water temperature, the dissolved oxygen and the pH averaged 27.60 ± 0.36 °C, 6.26 ± 0.23 mg/l⁻¹ and 6.67 ± 0.13 respectively.

Crude protein and essentials amino acids profile of earthworm meal are presented in table 2.

Table 2: Amino acids and crude protein composition in earthworm meal

Aminoacids	Values (%)
Threonine	1.76
Valine	1.32
Methionine	0.76
Isoleucine	1.16
Leucine	3.12
Phenylalanine	1.84
Histidine	1.36
Tryptophane	0.12
Lysine	2.68
Arginine	2.84
Crude Protein	56.90%

The chemical composition and the gross energy (kj/g⁻¹) of each experimental diet are presented in Table 3. The different diets used were isonitrogenous and isolipidic. The crude protein content of the diets ranged from 44.40 (A100) to 44.85(A0) and lipid between 7.36 (A100) and 7.63(A0). The gross energy varied from 17.18 (A0) to 18.13 (A100).

Table 3: Proximate composition of experimental diet

Parameters	Experimental diets				
	A0	A25	A50	A75	A100
Crude Protein (%)	44.85	44.84	44.62	44.51	44.40
Crude Lipid (%)	7.63	7.57	7.50	7.43	7.36
Gross Energy (kj/g)	17.78	17.81	17.92	18.05	18.13

Before the experiment, the initial levels of proximate proteins in the carcasses were measured (50.27±0.71 %). After the experiment, the different proteins in carcasses in each diet

were also determined (Table 4). Significant differences were observed among proximate proteins in carcasses $p < 0.05$. The effect of different diets on growth performance parameters was determined. The lowest Final Biomass (FB), Specific Growth Rate (SGR) were obtained with A100 diet whereas the highest values of these parameters were obtained with fingerlings fed with A25 and A50 (Table 4). The Protein Efficiency Rate ranged from 0.53±0.02 (A100) to 1.29±0.08 (A50). The Protein Productive Value exhibited significant differences ($P < 0.05$). The highest Protein Productive Value (10.08±0.85) was observed with A50 diet (Table 4).

Table 4: Growth performances, feed utilization and body composition of *P. obscura* fingerlings fed with experimental diet

Parameters	A0	A25	A50	A75	A100
IB (g)	21 ± 0.00 ^a	22.33 ± 1.15 ^a	22.33 ± 1.15 ^a	23.00 ± 1.00 ^a	24.00 ± 1.00 ^a
FB (g)	39.33 ± 2.52 ^a	54.33 ± 4.5 ^b	54.33 ± 7.02 ^b	41.66 ± 0.58 ^a	31.66 ± 3.06 ^c
SGR (%/d)	1.50 ± 0.15 ^a	2.11 ± 0.11 ^b	2.11 ± 0.14 ^b	1.45 ± 0.14 ^a	0.74 ± 0.06 ^c
FE	0.39 ± 0.04 ^a	0.57 ± 0.02 ^b	0.58 ± 0.03 ^b	0.36 ± 0.02 ^a	0.24 ± 0.02 ^c
PI	50.27 ± 0.71 ^a	50.27 ± 0.71 ^a	50.27 ± 0.71 ^a	50.27 ± 0.71 ^a	50.27 ± 0.71 ^a
PF	51.01 ± 0.10 ^a	52.15 ± 1.17 ^b	53.09 ± 0.08 ^b	50.93 ± 2.03 ^a	50.87 ± 0.04 ^a
PER	0.88 ± 0.03 ^a	1.27 ± 0.06 ^b	1.29 ± 0.08 ^b	0.81 ± 0.02 ^a	0.53 ± 0.02 ^c
PPV	3.57 ± 0.13 ^a	7.55 ± 0.76 ^b	10.08 ± 0.85 ^c	2.77 ± 0.13 ^a	3.72 ± 0.13 ^a
K(%)	0.81 ± 0.09 ^a	0.82 ± 0.01 ^a	0.80 ± 0.03 ^a	0.81 ± 0.07 ^a	0.79 ± 0.03 ^a
SR (%)	100 ± 0.00 ^a	100 ± 0.00 ^a	100 ± 0.00 ^a	98.33 ± 1.67 ^a	96.66 ± 3.33 ^a

Each value is mean ± SE of triplicates. Means on the same line followed by different superscripts are significantly different ($p < 0.05$).

Variation of Feed Efficiency (FE) among the experimental diets is shown in Figure 1. The FE of the experiment diet were significantly different ($p < 0.05$). The lowest FE was obtained with A100 whereas the highest values were recorded with A25 and A50. The body composition showed that the experimental

diets have significant impact of the crude proteins in the carcass of *P. obscura* fingerlings ($p < 0.05$). The highest crude Protein in Final carcass (PF) was obtained with diets A25 and A50. No significant difference was found between PF for fish fed diets A0, A75 and A100 ($p > 0.05$).

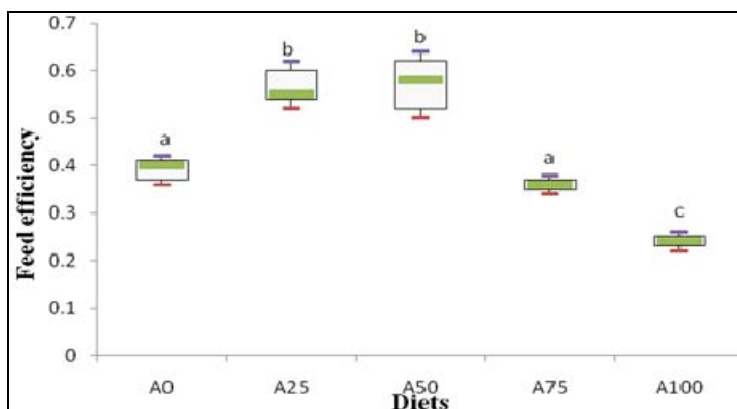


Fig 1: Effect of earthworm meal inclusion level on feed efficiency

4. Discussion

This study showed the importance of earthworm meal as an animal protein capable of supplementing the diet of *P. obscura* fingerlings. The physico-chemical parameters in our study were monitored within the suitable range for tropical fish, indicating that environmental conditions of the fish during the experimental period were adequate (Sogbessan *et al.* 2007) [36]. The different parameters like temperature (27.60 ± 0.36 °C), the dissolved oxygen (6.26 ± 0.23 mg/l⁻¹) and the pH (6.67 ± 0.13) have been recorded in our study. These results are accepted by *P. obscura* in natural environment (Bonou and Teugels 1985; Riehl and Baensch 1991) [2, 35]. The fish average survival rate (98.99 ± 0.22) can be explained by the higher physico-chemical quality in the rearing environment and the good quality of different diets. This result is in agreement with the study of Kpogué (2013) [21] on the *P.*

obscura fingerlings reared in captivity. The high specific growth rate (2.11 ± 0.11) obtained with A25 and A50 diets are similar to that reported (2.55 ± 0.04) by Kpogué (2013) [21] in *P. obscura* fingerlings. This value also agreed with the results that Monebi and Ogwumba (2012) [26] obtained while studying the utilization of the earthworm *Eudrilus eugeniae* in the diet of *Heteroclaris* fingerlings where the optimum inclusion level of the earthworm meal in diet was 25%. Similar results were also reported in a study by Dedeke *et al.*, (2013) [8]. According to Sogbessan *et al.* (2007) [36], the optimum inclusion level of the earthworm (*Hyperiodrilus euryaulos*) meal in diet of *Heterobranchus longifilis* fingerlings have ranged from 7.5% to 25%. Our data is different from their results where the optimum inclusion level of earthworm meal in diet of *P. obscura* fingerlings ranged between 25% and 50% because of the carnivorous

characters of African snakehead (*P. obscura*) is contrary to the omnivorous characters of *Heterobranchus longifilis*.

The high feed efficiency (0.58 ± 0.03) is obtained with A50 diet (50% inclusion level). The poor feed efficiency (0.24 ± 0.02) was obtained with A100 diet. This result can be explained by hemolytic factors in the coelom fluid of *Eisenia fetida* (Medina *et al.* 2003; Kostecka and Paczka 2006) [25, 20]. Indeed, Coelomic fluid was shown to play an important role in the immune reactions of earthworms and also can affect the immune reactions in other animals treated with coelomic fluid. The coelomic fluid of *Eisenia fetida* has toxic effects on fish (Kobayashi *et al.*, 2001) [19]. Lysenin, a component of the coelomic fluid, produce toxic effects (Kobayashi *et al.*, 2001, Kobayashi *et al.*, 2004) [19, 18].

The diet have a significant impact on the protein efficiency rate ($p < 0.05$). The high value (1.29 ± 0.08) obtained in our study with 50% inclusion level is close to the 1,52 obtained by Sogbessan *et al.*, (2007) [36] on earthworms as animal protein, where 25% of fish meal was supplement e by earthworm meal in the diet of *Heterobranchus longifilis* fingerlings. Our study agreed with the one on *Heteroclaris* fingerlings fed with earthworm meal in hatchery tanks where the best protein efficiency rate (0.6) was observed when 50% of fish meal was replaced by earthworm meal (Olele 2011) [32].

Other studies showed that fish meal can be replaced in diets for carnivorous fish species (Chou *et al.* 2004; Lim *et al.* 2004; Hernandez *et al.* 2006; Pham *et al.* 2007; Lim and Lee 2008) [6, 24, 16, 33, 23]. Particularly, fish meal can be substituted by animal proteins or vegetable proteins (Hlophe and Moyo 2014, Pucher *et al.* 2014) [17, 34]. Earthworm is an alternative protein source in fish diet and can replace fish meal (Hasanuzzaman *et al.* 2010) [15]. The protein quality of earthworm meal is comparable to that of fish meal with high levels of essential amino acids for fish (Tacon *et al.* 1983; Tacon and Metian 2009; Dong *et al.* 2010; Tuan 2010; NRC 2011) [40, 39, 9, 42, 30]. This efficiency of earthworm meal was also proved in a study where it was used to feed tilapia post larvae (Chaves *et al.* 2015) [5]. However, this efficiency of earthworm meal can depend on the earthworm species and the feed substrate (Tacon *et al.* 1983; Stafford and Tacon, 1984; Sun *et al.* 1997; Changguo *et al.* 2006; Sogbesan *et al.* 2007; Dong *et al.* 2010; Tuan 2010) [40, 37, 38, 3, 9, 42].

5. Conclusion

The inclusion of earthworm meals in the diets of *P. obscura* have significant and positive impact ($p < 0.05$) on the growth performance of snakeheads; therefore suggesting that earthworm meal is suitable as a protein source in the diet of *P. obscura* fingerlings.

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