



# International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129  
P-ISSN: 2394-0506  
(ICV-Poland) Impact Value: 5.62  
(GIF) Impact Factor: 0.549  
IJFAS 2017; 5(1): 268-273  
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www.fisheriesjournal.com  
Received: 10-11-2016  
Accepted: 11-12-2016

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## Earthworm amino acids and effect of 3 different forms of earthworm (meal, frozen and living) on growth, feed utilization and survival rate of African Snakehead fingerlings (*Parachanna obscura*) reared in captivity

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### Abstract

The earthworm amino acids and the effect of earthworm ingredient form were investigated on African snakehead fingerlings (*Parachanna obscura*) reared in captivity for 42 days. Feed utilization, growth parameters, whole body composition and survival rate were measured. A total of 180 fingerlings of *Parachanna obscura* (mean weight:  $2.77 \pm 0.15$ g) were provided by the Laboratory of Wetland Research. The fingerlings were fed with diet containing earthworm meal, frozen and living form. The fish were reared in 15 circular tanks whose volume were 90 l each, at a density of 20 fingerlings per tank and feed was distributed at a rate of  $5.01$  (fish biomass)<sup>-0.23</sup>. The different earthworm ingredient forms affect the growth performance, feed utilization and whole body composition ( $P < 0.05$ ) of *P. obscura*. The living and meal form of the earthworm gave the best results in terms of growth parameters and whole body composition compared to the frozen form.

**Keywords:** Earthworms, effect, form, *P. obscura*

### 1. Introduction

More than the half of the world fish consumption is provided by aquaculture. Aquaculture is an important activity to be promoted to ensure food security. It remains an important employment and income generating activity for the countries (Naylor *et al.*, 2009, Ogunremi and Obasa 2009) [26, 29]. Fish meal is one of the main ingredients in fish feeds because it offers a balanced source of essential amino acids, essential fatty acids, vitamins, minerals and generally enhances palatability (Samocha *et al.*, 2004, Kristofersson and Anderson 2006, Muzinic *et al.*, 2009) [36, 22, 25]. Nowadays, aquaculture is influenced by the unavailability and the high price of fish meal. The solution to this problem is to evaluate others protein sources for partial or total fish meal substitution (Gao *et al.*, 2013, Yuan *et al.*, 2013, Jung *et al.*, 2016) [12, 43, 15]. Fish meal used as feedstuffs in fish feeding in aquaculture has become unprofitable. Consequently, several authors have evaluated protein sources of plant origin and animal origin in fish diets to replace fish meal (Bairagi *et al.*, 2004, Ramachandran *et al.*, 2005, Ramachandran and Ray 2008) [2, 33, 34]. Earthworm meal can be used to replace fish meal in fish diet (Vodounnou *et al.*, 2016) [42].

Earthworms are terrestrial invertebrates grouped into three categories according to their behavior in the natural environment (Vodounnou *et al.*, 2015) [41]. It meal is good such as fish meal in terms of quality and has been found to be an efficient substitute for fish meal in domestic animal diets (Bhorgin and Uma 2013) [4]. Earthworms have been shown to contain 60-70% crude protein and very little crude fibre (<5%), contain amino acids, fat acids Omega3 and also several minerals that are nutritionally important (Sabine 1983, Dynes 2003, Paoletti *et al.*, 2011) [35, 11, 31]. The content of lysine in earthworm meal is significant, representing the daily requirement of children between the age of 2 and 5 years (Segovia 1996). The earthworm meal has been used to substitute the fish meal in diet of African Snakeheads, *Parachanna obscura* (Vodounnou *et al.*, 2016) [42].

*P. obscura* is the commonest African Snakeheads. *P. obscura* has a paramount economic value for African aquaculture because its flesh is highly appreciated, its rapid growth potential (2g/day),

tolerant of high stocking density, use of atmospheric oxygen for respiration in water with low dissolved oxygen and high ammonia levels (Bonou and Teugels 1985, Ng and Lim 1990, Victor and Akpocha 1992, O' Bryen and Lee 2007, Bolaji *et al.*, 2011) [6, 27, 40, 28, 5]. Dietary protein requirements, feeding rate requirement, optimum stocking density of *P. obscura* have been previously studied (Kpogue and Fiogbe 2012, Kpogue *et al.*, 2013) [21, 20]. It was demonstrated that meal form of earthworm can be used to substitute fish meal in diet of African Snakeheads *P. obscura* (Vodounnou *et al.*, 2016) [42]. But the frozen and living forms were not hitherto tested in diet of *P. obscura* fingerlings. The aim of the current study is to evaluate the earthworm amino acids and the effect of its 3 different ingredient forms (meal, frozen and living) on growth, feed utilization, and whole body composition and survival rate of *P. obscura* reared in captivity.

## 2 Materials and Methods

### 2.1 Earthworms culture and meal production

Earthworms were cultivated in cow manure in 9 concreted tanks (3 triplicates) 80 cm x 80 cm x 60 cm for 90 days. The cow manure was collected from farms located in Abomey-Calavi district in Benin. To prevent worm death the manure was pre-composted for 14 days after collection before its utilization (Gunadi and Edwards 2003) [13], manure was used. The experiment lasted 90 days. During the study period substrate moisture was maintained by sprinkling water on the manure, on daily basis.

### 2.2 Different earthworm ingredient form utilization in *P. obscura* fingerlings diet

The meal, frozen and living ingredient form of the earthworm were tested in *P. obscura* fingerlings diet. To obtain the worm meal ingredient, the harvested concreted tanks were weighed, rinsed with pure water and put in freezer. After drying them by using a lyophilisator, the dry matter was ground with a mill

and then, stored at 0 -20 °C. The worm from the second triplicate concreted tanks was harvested and conserved by freezing. The living worm ingredient was maintained in the last triplicate concreted tanks. The fish meal was replaced by earthworm ingredient in *P. obscura* diet at the inclusion level of 50% (Vodounnou *et al.* 2016) [42].

### 2.3 Experiment procedure

One hundred eighty fingerlings of *P. obscura* (mean weight: 2.77± 0.15g) were provided from experimental site of Laboratory of Wetland Research of Abomey-Calavi University in Benin. Prior to receiving the experimental diet, the fish were acclimated with a mixture of different experimental diets for one week. The study was carried out for 42 days in 9 circular concreted tanks containing 90 l of water each under atmospheric conditions. The water was renewed continuously in all the tanks at the rate of 1l per min. 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)<sup>-0.23</sup> (Kpogue 2013) [20]. Temperature, pH and dissolved oxygen were monitored daily with a thermometer, a pH meter and an oxygen meter respectively. The fingerlings were fed three times a day (Kpogue 2013) [20] and the fish were examined weekly.

### 2.4 Experimental diet

Three experimental diets were formulated to meet the nutrient requirements of *P. obscura* fingerlings (Table 1). The various ingredients were ground with a hammer mill, weighed with scale and blended with a mixer. The feed was prepared by mixing the dry ingredients with oil and water until a desirable paste-like consistency was reached. The resulting paste was transformed into pellets of 1.5 mm using the feed blender. The nutritional compositions of the different diet were determined.

**Table 1:** Formulation of experimental diets and nutrition composition

Ingredients (%)	Experimental diets		
	T1	T2	T3
Cotton bran meal	20	20	20
Maize meal	12	12	12
Fish meal	22.5	22.5	22.5
Earthworm meal	22.5	0	0
Earthworm frozen	0	X	0
Earthworm living	0	0	Y
Soy bean meal	17	17	17
Godfish liver oil	2	2	2
Premix (min – vit)*	2.5	2.5	2.5
Ferrous sulphate	0.5	0.5	0.5
Carboxymethyl cellulose	1	1	1
Total	100	77.5 + X	77.5 + Y
Nutrition composition			
Moisture	9.80	9.19	9.22
Crude protein	45.72	32.27	32.28
Ash	14.14	4.08	4.11

\*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; Pantothenicacid 8 000 mg; Nicotinicacid 18 000 mg; Folicacid 800 mg; Cholinchloride 120 000 mg; Colbat carbonate 150 mg; Ferroussulphate 8 000 mg; Potassium

iodide 400 mg; Manganeseoxide 6 000 mg; Copper 800 mg; Sodium selenite 40 mcg; Lysine 10 000 mg ; Methionin 10 000 mg; Zinc sulphate 8 000 mg

With:

T1: the diet in which earthworm meal form was used to replace fish meal at the inclusion level of 50%.

T2: the diet in which earthworm frozen form was used to replace fish meal at the inclusion level of 50%.

T3: the diet in which the living form of earthworm was used to replace fish meal at the inclusion level of 50%.

X= the quantity of the frozen form of earthworm, calculated based on the dry matter, inclusion level and used as complement in diet.

Y= the quantity of the living form of earthworm, calculated based on the dry matter, inclusion level, and used as complement in diet.

**2.5 Biochemical analysis and calculations**

Fish carcasses were analyzed for crude protein analysis before and after experiment using the Association of Analytical Chemist methods (AOAC, 2000). The proximate proteins in diets were also analyzed. 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.

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

Specific Growth Rate (SGR; %/d) =  $100 \times [\text{Ln}(\text{Final Body Weight (g)}) - \text{Ln}(\text{Initial Body Weight (g)})] / \text{Duration (days)}$  of the experiment

Final Body Weight (FBW) = FB/FN; Initial Body Weight (IBW) = IB/IN; Feed Efficiency (FE) =  $(\text{FB} + \text{DB} - \text{IB}) / \text{FD}$ ; Feed Conversion Rate =  $1/\text{FE}$ ; Survival Rate (SR %) =  $100 \times \text{FN}/\text{IN}$ ; Protein Efficiency Ratio (PER) =  $(\text{FB} - \text{IB}) / (\text{FD} \times \text{Dietary Protein})$ ; Protein Productive Value (PPV) =  $100 \times (\text{Final Protein in fish} - \text{Initial Protein in Fish}) / (\text{Total Feed Intake per Fish} \times \text{Dietary Protein})$ .

With:

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

**2.6 Statistical analysis**

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**

Essentials amino acids, crude protein and ash of the earthworm used in diet are presented in table 2. Earthworm is rich in Leucine, Lysine and Arginine, and poor in Tryptophane and Methionine (table 2).

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

Amino acids	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%
Ash	17.52 %

Temperature, pH and dissolved oxygen monitored daily are presented in table 3. In this study, physico-chemical parameters (temperature, dissolved oxygen and pH) were monitored and maintained within the suitable range for tropical fish.

**Table 3:** Physico-chemical parameters monitored

Parameters	T1	T2	T3
pH	6.66 ± 0.13 <sup>a</sup>	6.65 ± 0.08 <sup>a</sup>	6.69 ± 0.11 <sup>a</sup>
Temperature (°C)	27.64 ± 0.26 <sup>a</sup>	27.68 ± 0.31 <sup>a</sup>	27.59 ± 0.16 <sup>a</sup>
Dissolved Oxygen ( mg/l-1)	6.16 ± 0.25 <sup>a</sup>	6.22 ± 0.23 <sup>a</sup>	6.19 ± 0.18 <sup>a</sup>

Each value is mean ± SE of triplicates. Means in the same line followed by same superscripts are not significantly different ( $P>0.05$ ).

The result of whole-body composition analysis is presented in Table 4. Moisture, Crude protein and ash were significantly affected by the different forms of earthworm included in the diet ( $P < 0.05$ ). The crude proteins observed in the whole-body of the fish fed with experimental diets are different from

that observed in the initial whole body. There was a significance difference between the living, the frozen and the meal form of earthworm, fed ( $P<0.05$ ). Moisture in fish with initial condition and the fish fed with experimental diet was similar. Ash ranged between 11.32±0.31 (initial body) and 12.56±0.34 (T3: living form). The ash content of the fish initial body was less than that of body of fish fed the experimental diets.

**Table 4:** Effect of diets with different forms of earthworm on *P. obscura* whole body composition

Parameters	Initial body	Experimental diets		
		T1	T2	T3
Moisture	69.70±0.05 <sup>a</sup>	69.94±0.12 <sup>a</sup>	69.84±0.09 <sup>a</sup>	70.13±0.14 <sup>a</sup>
Crude protein	56.77±0.23 <sup>a</sup>	57.88±0.28 <sup>b</sup>	56.69±0.14 <sup>a</sup>	58.17±0.19 <sup>b</sup>
Ash	11.32±0.31 <sup>a</sup>	11.58±0.22 <sup>a</sup>	11.40±0.37 <sup>a</sup>	12.56±0.34 <sup>b</sup>

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

The effect of different diets on growth performance parameters and feed utilization were shown in table 5. The highest Final Biomass (FB), Specific Growth Rate (SGR) and Feed Efficiency (FE), were obtained with the earthworm living form diet (T3). The lowest values of these parameters

were obtained with the frozen earthworm diet (T2). Survival Rate (SR) were similar among all the experimental fish ( $P>0.05$ ) (Table 5). The highest Protein Productive Value ( $2.57 \pm 0.06$ ) was observed with diet T3 with a significant difference among treatment groups ( $P<0.05$ ). The Protein Efficiency Rate ranged from  $0.96 \pm 0.10$  (T2) to  $1.58 \pm 0.08$  (T3) with also a significant difference among treatment groups ( $P<0.05$ ).

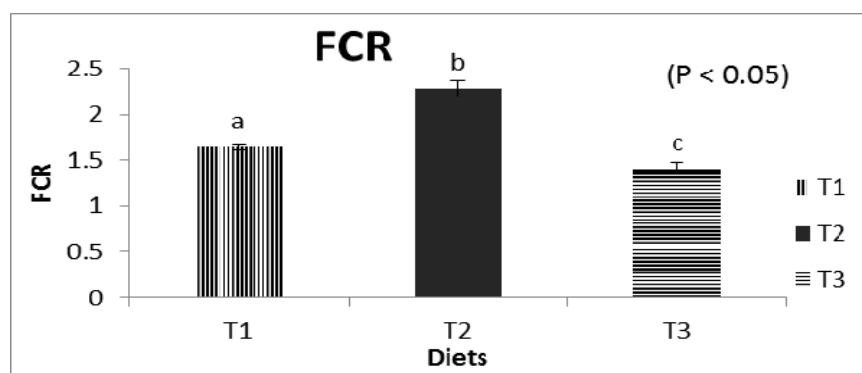
**Table 5:** Growth performances and feed utilization of *P. obscura* fingerlings fed with earthworm different forms

Parameters	T1	T2	T3
IB(g)	55.30±0.05 <sup>a</sup>	55.43±0.06 <sup>a</sup>	55.30±0.10 <sup>a</sup>
FB(g)	124.00±0.57 <sup>a</sup>	103.66±1.76 <sup>b</sup>	140.00±3.60 <sup>c</sup>
IBW (g)	2.76±0.00 <sup>a</sup>	2.77±0.00 <sup>a</sup>	2.76±0.00 <sup>a</sup>
FBW (g)	6.20±0.03 <sup>a</sup>	5.46±0.08 <sup>b</sup>	7.00±0.18 <sup>a</sup>
FE	0.61±0.04 <sup>a</sup>	0.44±0.05 <sup>b</sup>	0.71±0.02 <sup>a</sup>
FCR	1.64±0.03 <sup>a</sup>	2.28±0.09 <sup>b</sup>	1.41±0.06 <sup>a</sup>
SGR (%/d)	2.23±0.03 <sup>a</sup>	1.90±0.06 <sup>b</sup>	2.57±0.07 <sup>c</sup>
PER	1.33±0.06 <sup>a</sup>	0.96±0.10 <sup>b</sup>	1.58±0.08 <sup>a</sup>
PPV (%)	2.15±0.07 <sup>a</sup>	0.42±0.03 <sup>b</sup>	2.57±0.06 <sup>a</sup>
SR (%)	100.00±0.00 <sup>a</sup>	95.00±0.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>

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

The Feed Conversion Rate is shown in figure 1. Feed Conversion Rate was significantly affected by the different

form of earthworm ingredient included in the diet ( $P < 0.05$ ). It was ranged between 1.41±0.06 (T3) and 2.28±0.09 (T2). The best Feed Conversion Rate was observed in T3 diet (figure 1). But no significant difference was observed between T3 and T1.



**Fig 1:** Effect of different forms of earthworm diet on *P. obscura* Feed Conversion ratio

#### 4. Discussion

The unavailability and the high price of fish meal have forced the aquaculture industry to explore new raw material like vegetable proteins, vegetable oils, insects and worms that can replace fish meal (Stickney *et al.*, 1996, Kikuchi 1999, Pham *et al.*, 2007, Lim and Lee 2008) [39, 16, 32, 23]. It is proved that earthworm meal is rich in proteins, amino acids and fat acids Omega3 (Dynes 2003) [11] and can be used to feed a carnivorous fish (Hasanuzzaman *et al.*, 2010) [14]. Crude protein (56.90%) of earthworm in this study is better than crude protein in some fish such as *Channa striatus*, *P. obscura* and *Sarotherodon melanotheron* (Baliu *et al.* 2007, Zuraini *et al.* 2006, Ama-Abassi 2013) [3, 44, 1]. It is also rich in crude protein better than crude protein in some vegetable feedstuff using in aquaculture such as *Azolla filiculoides*, *Moringa oleifera* and *Dialium guineense* (Djissou *et al.*, 2016) [9]. The essential amino acids in earthworm are better than some animal and vegetable feedstuff in aquaculture. For example methionine in earthworm in this study 0.76% is superior that of *Azolla filiculoides* (0.00%) and commercial fish meal 0.4% (Djissou *et al.*, 2016) [9].

Fish average survival rate (98.33± 0.14) can be explained by the higher physico-chemical quality of the rearing water and the good quality of the different experimental diets. In this study, physico-chemical parameters (temperature, dissolved oxygen and pH) were monitored and maintained within the suitable range for tropical fish, indicating that rearing water conditions of the fish during the experimental period were adequate (Sogbessan *et al.*, 2007) [38]. This result agreed with

the study of Kpogue (2013) [20] and Vodounnou *et al.*, (2016) [42] on the *P. obscura* fingerlings reared in captivity.

Previous study concerning, the culture of earthworm (*Eisenia fetida*), its production, nutritive value and the utilization of its meal in diet of *P. obscura* fingerlings or reared in captivity has shown that earthworm meal can be used at 50% level to replace fish meal in African snakehead fingerlings (*P. obscura*) reared in captivity (Vodounnou *et al.*, 2016) [20]. This study provides substantial information on the different form of earthworm that can be used in *P. obscura* diet on the basis of 50% fishmeal replacement rate of. It is showed that earthworm efficiency in fish diet depends on the earthworm species and the feed substrate (Changguo *et al.*, 2006; Dong *et al.*, 2010) [7, 10]. This study showed that ingredient form of earthworm affects significantly ( $p < 0.05$ ) the growth performance, nutrient utilization the whole body and the survival rate of *P. obscura*.

The highest Specific Growth Rate (2.57±0.07) and final body weight (7.00±0.18) are obtained with living form of earthworm content diet (T3). For these parameters, the values recorded with fish fed with T2 are lower. This result can be explained in one hand by the carnivorous character of *P. obscura* that like capturing its prey and on the other hand by the higher nutritional quality of the living earthworm which hasn't undergone any transformation. The non-consumption of the frozen earthworms is confirmative of this carnivorous aspect of the fish. Consequently, the Specific Growth Rate and the final body weight parameters were better than the result obtained in frozen earthworm content diet (T2), but no

significance difference was so far observed between T1 and T3 ( $P>0.05$ ). This result agreed with the study of Vodounnou *et al.* (2016)<sup>[20]</sup> on fish meal substitution by earthworm meal in *P. obscura* diet where the optimum substitution rate was 50%. It agreed also with a study on partial replacement of fish meal by earthworm meal (*Libyodrilus violaceus*) in diets of African catfish, *Clarias gariepinus* (Dedeke *et al.*, 2013)<sup>[8]</sup>. The low Specific Growth Rate ( $1.90\pm 0.06$ ) and the low final body weight ( $5.46\pm 0.08$ ) were obtained with T2 diet (frozen earthworm content diet)

The different earthworm ingredients forms tested in fish diet have had a significant effect on Feed Efficiency, Protein Efficiency Rate and Protein Productive Value ( $P<0.05$ ). The highest Feed Efficiency ( $0.71 \pm 0.02$ ) is obtained with the living form of earthworm and the lowest value ( $0.44 \pm 0.05$ ) with frozen earthworm. The highest value ( $1.58 \pm 0.08$ ) of Protein Efficiency Rate is obtained with earthworm living form content diet, with no significant difference with that of the earthworm meal form content diet ( $1.33 \pm 0.06$ ) ( $P>0.05$ ). This value is lightly better than that obtained by Vodounnou *et al.* (2016)<sup>[42]</sup> in substituting fish meal by earthworm meal, with 50% inclusion level, but close to the 1.52 obtained by Sogbessan *et al.* (2007)<sup>[38]</sup> on the use of earthworms as animal protein in fish diet, where 25% of fish meal were supplemented by earthworm meal in the diet of *Heterobranchus longifilis* fingerlings. The low value ( $0.96\pm 0.10$ ) is obtained with frozen earthworm. The Protein Productive Value result is also higher ( $2.57 \pm 0.06\%$ ) in earthworm living form content diet and lower ( $0.42\pm 0.03\%$ ) in frozen worm content diet. But this result is lower compared to the one obtained by Vodounnou *et al.* (2016)<sup>[42]</sup> on the substitution of fish meal by earthworm meal with 50% inclusion level ( $10.08\pm 0.85\%$ ).

The different forms of earthworm ingredient used in this study have not undergone a heat treatment, yet a heat treatment is recommended before using earthworm in animal diet (Medina *et al.*, 2003, Kostecka and Paczka 2006)<sup>[24,19]</sup>. Indeed, the coelom fluid of earthworm has a hemolytic effect that can be reduced by heat. It is shown that coelomic fluid plays an important role in the immune reactions of earthworms and can affect the immune reactions of fish fed with earthworm (Kobayashi *et al.*, 2001, Ohta *et al.*, 2003)<sup>[18,30]</sup>. This effect on immune reactions is due to Lysenin in coelomic fluid which is a toxic effect producing (Kobayashi *et al.*, 2001, Kobayashi *et al.*, 2004)<sup>[18,17]</sup>.

## 5. Conclusion

The different earthworm forms (meal, frozen and living) affected the growth performance, feed utilization and whole body composition ( $P<0.05$ ) of *P. obscura* reared in captivity. At the end of this experiment, the living form and meal form of earthworm have given the best results in terms of growth parameters and whole body composition compared to the frozen form.

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