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Full Length Research Paper

The Role of *Eudrilus eugeniae* Earthworms in Feeding Heteroclarias Fingerlings

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The earthworm, *Eudrilus eugeniae* was cultured for 12 weeks and its meal utilized as a replacement for fish meal in the diet of fingerlings of the mud catfish hybrid (♂*Heterobranchus longifilis* X ♀*Clarias gariepinus*) called Heteroclarias. Two hundred and seventy adult *E. eugeniae* were cultured in three substrates: Soil, cellulose, or a mixture of cellulose and soil. The worms were harvested at the end of the culture period, processed by oven-drying and grounded into powdery form as earthworm meal. Cellulose substrate was the best for *E. eugeniae* culture with the highest weight gain and relative growth rate. The earthworm meal produced was used to replace commercial fish meal in five compounded diets at levels of 0 (control), 25, 50, 75 or 100%. The diets were fed to Heteroclarias fingerlings to evaluate the effect of earthworm meal in comparison with fish meal on the growth and nutrient utilization of the fingerlings. The feeding experiment was carried out in 40 L plastic tanks using 5 tanks per treatment and 15 fingerlings per tank, and the fingerlings were fed 5% of their body weight on a daily basis for 10 weeks. Highest mean weight gain was in fish fed 75% earthworm meal, while relative and specific growth rates were highest in fish fed 25% earthworm meal and lowest in fish fed the control diet. Food conversion ratio was lowest in fish fed 50% earthworm meal and highest in fish fed 100% earthworm meal. These results indicate that replacement of fish meal with earthworm meal at 50 to 75% inclusion level is suitable for optimal growth performance and nutrient utilization in Heteroclarias fingerlings.

Key words: *Eudrilus eugeniae*, culture substrates, Heteroclarias, growth rate.

INTRODUCTION

In fish farming, artificial feeds are normally used in order to increase yield. *Eudrilus eugeniae is* an earthworm species indigenous to Africa but extensively bred in the USA, Canada, Europe and Asia for the fish bait market, where it is commonly called the African night crawler (Dominguez et al., 2001). It is a large worm that grows extremely rapidly and is reasonably prolific (Madge, 1969; Neuhauser et al., 1979; Viljoen and Reinecke, 1989; Reinecke et al., 1992; Reinecke and Viljoen, 1993), and it is furthermore protein-rich and highly palatable to fish (Mason et al., 1992). The rising cost of fish meal has necessitated the commercial production of vermi-meal (processed earthworm) as a potential substitute for fish meal in fish feeds. Unlike the production of fish meal which relies mainly on the exploitation of wild fish, vermi-meal is produced from worm cultures which are based on the recycling of wastes. This study was carried out to investigate the suitability of different culture media for rearing of the earthworm; *E. eugeniae,* and to determine the effects of replacing fish meal with earthworm meal on the growth of the hybrid mud catfish Heteroclarias, (♂*Heterobranchus longifilis* X ♀*Clarias gariepinus*) which is popularly cultured in Nigeria.

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Table 1. Composition of ingredients for the three substrates.

Material	Soil substrate (%)	Cellulose substrate (%)*	Cellulose and soil substrate (%)
Sandy soil	60.0	-	30.0
Loamy soil	30.0	-	15.0
Plant materials (harvest remains materials)	10.0	-	5.0
Saw dust	-	30.0	15.0
Rice bran	-	20.0	10.0
Mushroom (*Pleurotus sajor caju)*	-	20.0	10.0
Centro-leaves (*Centrosema pubescens*)	-	15.0	7.50
Poultry droppings	-	15.0	7.50
Total	100.0	100.0	100.0

Source: *****Sogbesan et al. (2006).

MATERIALS AND METHODS

E. eugeniae cultures

Composition and preparation of culture substrates

Three rearing substrates reported by Sogbesan et al. (2006) for earthworm cultures were used: Soil, cellulose, or a mixture of soil and cellulose (Table 1).

Soil substrate (control)

Soil substrate used was moist loamy-sandy soil, the conventional substrate used for earthworm culture (Dynes, 2003) and it served as the control. The soil sample was collected from natural earthworm habitat within the premises of the University of Ibadan, Ibadan, Nigeria using a spade and carried to the laboratory in a bucket.

Cellulose substrate

The composition and preparation of the cellulose substrate was carried out following Razon-Arceno et al. (1981) as modified by Sogbesan and Madu (2003). Appropriate quantities of rice bran (collected from local rice sellers in Bodija Market, Ibadan), saw dust (collected from Bodija Saw Mill, Ibadan) and mushroom (*Pleurotus sajor caju*) obtained from the Forestry Research Institute of Nigeria (FRIN), Ibadan, were thoroughly mixed with water in a glass aquarium of 1.0 × 1.0 × 0.5 m using a wooden stick. The mixture was covered with a nylon bag and allowed to ferment indoors for four weeks. Freshly collected poultry droppings from the Poultry Farm of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan were mixed separately with water to form a paste in another glass aquarium (1.0 × 1.0 × 0.5 m) and covered with a polythene bag to protect it from houseflies. This was also left indoors for four weeks to ferment.

During the fermentation period, the fermenting mixtures were thoroughly stirred with the wooden stick every other day in the first week; twice in the second week and once in the third and fourth weeks. During each mixing process, adequate water was added to the pastes to activate the fermentation process. Two days before the end of the fourth week, Centro leaves (*Centrosema pubescens*) were soaked in water overnight and added to the mixture of saw dust, rice bran and mushroom. At the end of the fourth week, the fermented composts were separately sun-dried. The poultry droppings formed chunks, which were crushed into powdery form using pestle and mortar, and the composts were then mixed together.

Cellulose and soil substrate

This substrate was made of equal proportions of soil and cellulose substrates mixed in 1:1 ratio (by weight).

Collection of earthworms and stocking of culture boxes: Two hundred and seventy adult *E. eugeniae* of mean weight and mean length of 2.1 ± 0.9 g and 20.0 ± 5.0 cm, respectively, were collected from Ajibode Area of the University of Ibadan, Ibadan, Nigeria. The earthworms were randomly collected from moist loamy-sandy soil by digging with a spade. The worms were hand-picked into a plastic container and transported to the experimental site. The earthworms were reared in wooden boxes (0.9 × 0.6 × 0.3 m each) in triplicates following the procedure described by Sogbesan et al. *(*2006). The base of each culture box was divided into three equal parts of 0.9 × 0.2 m each. The two side partitions were made with wooden materials to support the weight of the substrate and the bottom sealed with wood. The centre partitions (of open bottom) had nylon net of 3 mm mesh size at the bottom to prevent the box from becoming waterlogged and the earthworms from escaping. The lids of the boxes were screened with 3 mm mesh size nylon net in order to protect the earthworms from predators. The boxes were placed outdoors in the spade. Each box was first lined with banana leaves, followed by old newspaper and then covered with each substratum to about 5 cm height. Then 30 adult earthworms of known weight and length were introduced into each box and covered with substrate to a height of 15 cm. The substrates were kept moist by sprinkling with water twice a day during the dry season and once a day during the rainy season. The cultures were run for 12 weeks.

Feeding of earthworms: The worms were fed 10% of their body weight twice a week with fermented poultry dung (Dynes, 2003). The feed was spread on top of the substrate and water was sprinkled on it. The quantity of feed was adjusted based on the changes in the fortnightly body weight of the earthworms throughout the duration of the culture experiment.

Sampling of earthworms: The worms were sampled fortnightly. Wetting of the substrates was stopped a day before sampling to make collection of the worms easier as reported by Sogbesan and Madu (2003). Collection was done by hand-picking. Ten worms were randomly picked, and, their weights and lengths were measured. The worms were then returned back into the substrates.

Table 2. Percentage composition (% Dry Weight) of experimental diets.

Diet	1	2	3	4	5
Fish meal	49.05	38.57	27.0	14.21	0
Earthworm meal	0	12.85	27.0	42.63	60.00
Corn meal	40.95	38.57	36.0	33.16	29.99
*Vitamin and mineral premix	0.50	0.50	0.50	0.50	0.50
Soybean oil	1.50	1.50	1.50	1.50	1.50
Cassava starch	8.0	8.0	8.0	8.0	8.0
Total	100	100	100	100	100
Percentage levels of earthworm meal(earthworm meal +fish meal)	0	25	50	75	100

*Vitamins and minerals premix: 2.5 kg of premix contained: Vitamin A – 12,500,000.00 I.U, Vitamin D_3 – 2,500,000.00 I.U, Vitamin E – 40,000.00 mg, Vitamin K3 – 2,000.00 mg, Vitamin B1 – 3,000.00 mg, Vitamin B2 – 5,500.00 mg, Niacin – 55,000.00 mg, Calcium pantothenate – 11,500.00 mg, Vitamin B6 – 5,000.00 mg, Vitamin B12 – 25.00 mg, Folic Acid – 1,000 mg, Choline chloride – 500,000.00 mg, Biotin – 80.0 mg, Manganese – 120,000.00 mg, Iron – 100,000.00 mg, Zinc – 80,000.00 mg, Copper – 8,500.00 mg, Iodine – 1,500.00 mg, Cobalt – 300.00 mg, Selenium – 120.00 mg, Anti-oxidant – 120,000.00 mg.

Harvesting of earthworms: At the end of the culture period, total harvest of the earthworms was done by the introduction of wet Neem (*Azadirachta indica*) leaves into the cultured boxes a day prior to harvesting, following the method of Jameson and Venkataramanujam (2002). The earthworms were found under the leaves, hand-picked and weighed.

Processing of earthworms: The harvested worms were thoroughly rinsed in water and kept in a bowl for 30 min to evacuate their guts (Akpodiete and Okagbare, 1999). The worms were then weighed again, blanched in hot water and oven-dried at 80°C for 3 h (Sogbesan et al., 2006) and cooled. They were then processed into powdery form as earthworm meal using pestle and mortar. The earthworm meal was packed in an air-tight plastic container and stored at 0°C till when needed.

Feeding trial with Heteroclarias

Experimental diet

Five dry feeds were formulated based on the protein content of the major feed ingredients namely fish meal, earthworm meal and corn meal using Pearson's Square Method described in Eyo (1996). Fish meal was replaced in the diets with increasing inclusion levels of earthworm meal at 0% (Diet 1: Control Diet); 25% (Diet 2); 50% (Diet 3), 75% (Diet 4) and 100% (Diet 5) earthworm meal. Table 2 shows the percentage composition of experimental diets.

Fish meal, corn meal and vitamins and mineral premix were purchased from Adom Feeds, Orogun, Ibadan, Nigeria, while starch (binder) and soybean oil were purchased at Bodija Market, Ibadan, Nigeria. Appropriate quantities of ingredients in each diet were weighed and mixed thoroughly in a bowl before adding gelatinized starch (Akinwande et al., 2002). The feeds were oven-dried at 50oC for six hours as crumbs, after which cooling was allowed. The feeds were packed in air-tight plastic bags and stored in deep freezer. Proximate analyses of earthworm meal, fish meal, corn meal and the compounded diets were carried out using the methods of AOAC (2005).

Experimental procedure

Fingerlings of Heteroclarias were randomly assigned to the tanks at a stocking density of 15 fingerlings per tank, giving a total of 75 fingerlings per experimental diet, after measuring the standard length and weight of each fingerling. Total and average lengths and weights for each tank were calculated and recorded as initial lengths and weights. The tanks were monitored every day for mortality. The length and weight of the five randomly selected fingerlings from each tank were measured weekly to access growth rate and to calculate feed rations from estimated total fingerling weight in the tank. The fish were fed diet corresponding to 5% of their body weight daily for ten weeks; half of the ration was fed at 9.00 h and the other half at 18.00 h. Feeding was done manually at a particular point in each tank and visual observations of the fingerlings were made during this process. At the end of the 10 weeks experiment, final lengths and weights of all fish left in each tank were measured, and total and average final lengths and weights were calculated.

Evaluation of growth performance and nutrient utilization

The following growth and nutrient utilization parameters and survival of fish as reported by Akinwande et al. (2002) were computed as follows:

$$\text{Weekly Growth Rate (\%)} = \frac{W_f - W_i}{100\ n} \times$$

Where W_i = initial average weight (g) ,W_f = final average weight (g) n = number of weeks

$$\text{Relative Growth Rate (\%)} = \frac{W_f - W_i}{W_i} \times 100$$

$$\text{Specific Growth Rate (\%)} = \frac{\log W_f - \log W_i}{t} \times 100$$

Where, Log_e = the natural logarithm; t = duration of experiment in days

$$\text{Food Conversion Ratio} = \frac{\text{Total food supplied to fish (g)}}{\text{Total weight gain by fish (g)}}$$

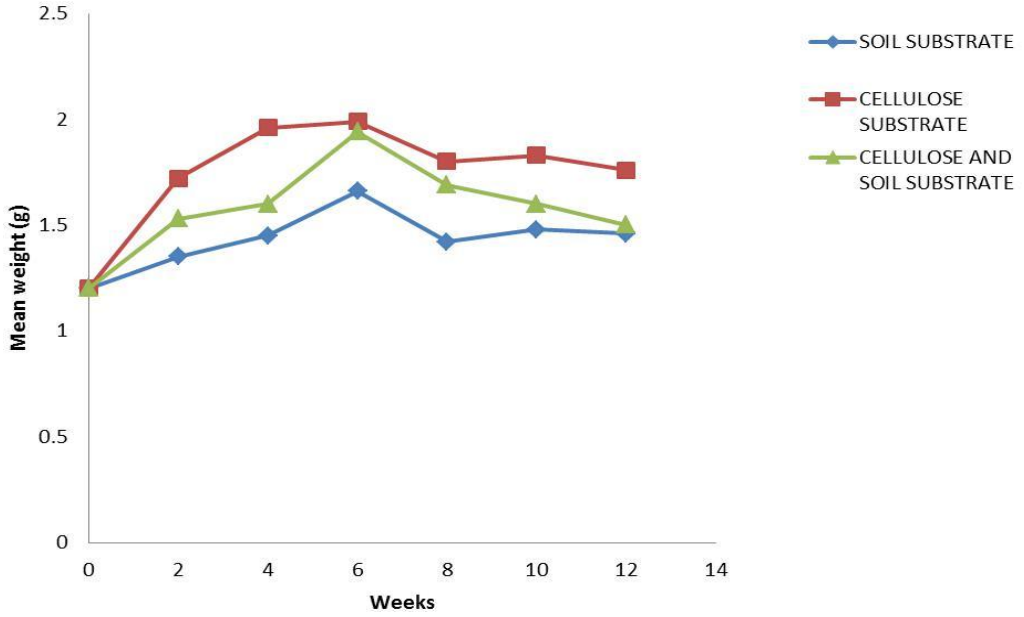

Figure 1. Average weight of *E. eugeniae* in the different substrates over the experiment.

Protein Intake: = Food supplied (g) x % crude protein of feed

$$\text{Protein Utilization Efficiency} = \frac{\text{Mean weight gain by fish (g)}}{\text{Mean protein intake (g)}}$$

$$\text{Survival (\%)} = \frac{Nf}{Ni} \times 100$$

Where, Ni = Number of fish at the beginning of the experiment, and Nf = Number of fish at the end of the experiment**.**

Statistical analysis

All data collected were subjected to descriptive statistics, student's t-test and Analysis of Variance (ANOVA).

RESULTS

Culture of earthworm

There was a gradual and continuous increase in the growth of the earthworms up until the sixth week, after which the growth dropped sharply to the eighth week and gradually by the twelfth week (Figure 1).

Earthworms cultured in cellulose substrate had the highest total weight gain of 228.8 g/kg of substrate while the lowest value, 183.6 g/kg of substrate was recorded for the control (that is, soil substrate) (Table 3). There was significant difference ($p<0.05$) between the weight gains of the earthworms cultured in all the three substrates. Relative and specific growth rates were highest, 635.6% and 2.4%/day respectively in earthworms cultured in cellulose substrate while the lowest (510.0 and 2.2%/day respectively) were in earthworms cultured in soil substrate. There was significant difference (p 0.05) between the relative and specific growth rates at all levels of inclusion. The result of the proximate composition of earthworm produced showed that crude protein content was 55.80%; crude lipid, 7.85%; crude fibre, 1.94% and ash content, 8.95%.

Trial with Heteroclarias

The crude protein content of the five experimental diets ranged from 43.2 to 43.6% and was not significantly different ($p>0.05$) (Table 4). The lipid content differed significantly and was highest (11.0%) in the control diet and the lowest (10.2%) in the 100% earthworm meal diet ($p<0.05$).

The weekly changes in weight of the fingerlings fed the different diets are illustrated in Figure 2. There was continuous and progressive weight gain of fish fed all the experimental diets up to the ninth week, after which growth rates decreased at the 10^{th} week except for fingerlings fed 75% earthworm meal diets where growth was steady until the end of the experimental period. Results on the growth and nutrient utilization of fingerlings are shown in Table 5. The relative and specific growth rates were highest, 1002.0 and 1.50% in fish fed 25% earthworm meal diet and lowest, 579.6 and 1.2% respectively in fish fed the control diet. Differences in growth rates between the diets were significantly different ($p<0.05$). The food conversion ratio was highest (3.1) in fish fed 100% earthworm meal diet, while the lowest (1.6) was in fish fed 50% earthworm meal diet and the difference was significant (p 0.05). The highest protein

Table 3. Growth performance of *E. eugeniae* cultured in the different substrates for 12 weeks.

Parameter	Soil substrate	Cellulose substrate	Cellulose and soil substrate
Initial total weight of worms (g)	36.0	36.0	36.0
Final total weight of worms (g)	219.6^{a}	264.8^{b}	240.3^{c}
Total weight gain of worm (g/)	183.6^{a}	228.8^{b}	204.3^{c}
Relative growth rate (%)	510.0^{a}	635.6^{b}	567.8^{c}
Specific growth rate (%/ day)	2.15^{a}	2.40^{c}	2.26^{b}

Values on the same row with different superscript are significantly different ($p<0.05$).

Table 4. Proximate composition of experimental diets (% dry weight).

Nutrients	Diet 1 (Control, 0% EWM)	Diet 2 (25% EWM)	Diet 3 (50% EWM)	Diet 4 (75% EWM)	Diet 5 (100% EWM)
Crude protein	43.5^{a}	43.3^{a}	43.5^{a}	43.3^{a}	43.2^{a}
Crude lipid	11.0^{a}	10.5^{b}	10.8^{a}	10.5^{b}	10.2^{c}
Crude fibre	3.2^{b}	3.4^{b}	3.5^{a}	3.9^{a}	4.0^{a}
Ash	8.9^{a}	8.8^{a}	7.5^{c}	7.2^{a}	6.8^{b}

EWM=Earthworm meal. Values on the same row with different superscript are significantly different ($p<0.05$).

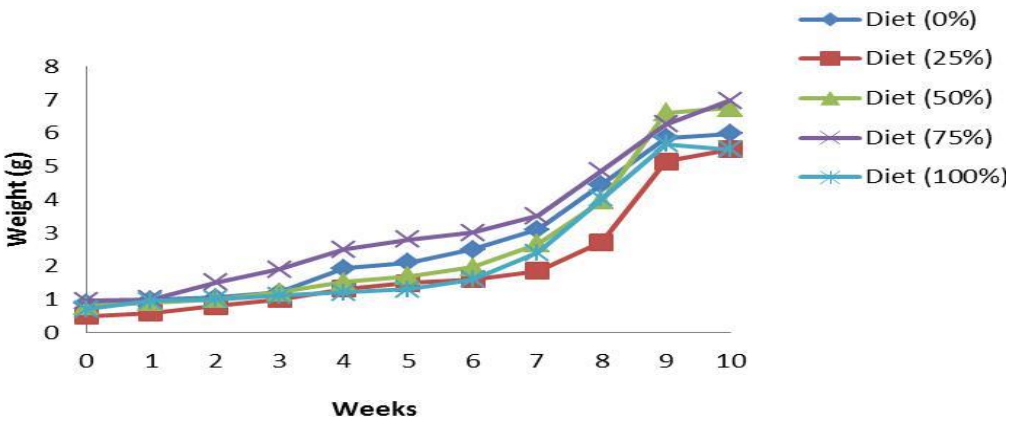

Figure 2. Weekly growth patterns of Heteroclarias fingerlings fed the various experimental diets.

efficiency ratio (1.0) was obtained in fish fed 25% earthworm meal diet, while the lowest value (0.5) was in fish fed 100% earthworm meal diet and the differences were significant ($p<0.05$). Survival was high (72.0 to 73.3%) between fish fed 25 to 75% earthworm meal diets, while it was lower in the control (66.7%) and least in 100% (62.7%) earthworm meal diet. The difference between the control and 100% earthworm meal diets was not significant but they were significantly different ($p<0.05$) from survival of fish fed 25 to 75% earthworm meal diets.

DISCUSSION

Feeding trial with Heteroclarias

Fagbenro et al. (1992) and Eyo (1996) reported that the optimum protein requirement of the mud catfishes (*Clarias gariepinus*, *Heterobranchus bidorsalis* and Heteroclarias) falls within 40.0 to 42.5%, which is close to the crude protein of diets in this present study. Progressive weight gain of Heteroclarias recorded in all the dietary treatments throughout the duration of the experiment is an indication that the fish responded positively to all the diets in terms of growth and that the protein content of the experimental diet was likely adequate for growth of the fish. It also implies that none of the experimental diets contained anti-nutritional growth factors.

The results obtained from this present study show that fingerlings fed with 75% earthworm meal inclusion has the highest mean weight gain. This is contrary to the work of Sogbesan and Ugwumba (2008), who recorded the highest mean weight gain in 50% earthworm meal. This

Table 5. Growth and nutrient utilization of Heteroclarias fed earthworm meal diets for 10 weeks.

Parameter	Diet 1 (control) 0% EWM	Diet 2 25% EWM	Diet 3 50% EWM	Diet 4 75% EWM	Diet 5 100% EWM
Duration (days)	70	70	70	70	70
No. of fish stocked	75	75	75	75	75
Number of fish left	50	55	54	54	47
Initial mean length (cm/fish)	3.0^{a}	3.7^{c}	3.8^{c}	3.6^{b}	3.9^{c}
Final mean length (cm/fish)	11.2^{c}	8.9^{a}	9.3^{b}	9.0^{b}	9.4^{b}
Relative length gain (cm/fish)	9.7^{b}	5.1^{a}	5.5^{a}	5.5^{a}	5.5^{a}
Initial mean weight (g/fish)	0.9^{c}	0.5^{a}	0.8^{c}	0.9^{c}	0.7^{b}
Final mean weight (g/fish)	5.6^{b}	5.5^{a}	6.7^{c}	6.9^{c}	5.5^{a}
Mean weight gain (g/fish)	5.1^{b}	5.1^{a}	5.9^{c}	6.0^{c}	4.8^{a}
Weekly growth rate (g/fish)	0.5^{a}	0.5^{a}	0.6^{b}	0.6^{b}	0.5^{a}
Total feed intake (g/fish)	9.8^{b}	8.4^{a}	9.8^{b}	11.2^{c}	14.7^{d}
Relative growth rate (%)	579.6^{a}	1002.0^{b}	745.0^{c}	633.7^{d}	687.1^{e}
Specific growth rate (%)	1.2^{a}	1.5^{b}	1.3^{c}	1.2^{a}	1.3^{c}
Food conversion Ratio	1.9^{a}	1.7^{a}	1.6^{a}	1.9^{a}	3.1^{b}
Protein intake	6.0^{a}	4.9^{a}	6.1^{a}	6.9^{b}	9.0^{c}
Protein utilization efficiency	0.8^{b}	1.0^{c}	0.9^{c}	0.9^{c}	0.5^{a}
Survival (%)	66.7^{a}	73.3^{b}	72.0^{b}	72.0^{b}	62.7^{a}

EW M=Earthworm meal. Values with different superscripts on the same row are significantly different ($p< 0.05$).

could possibly be due to the fact that Sogbesan and Ugwumba (2008) worked with Hyperiodrilus euryaulos.

The result of this study however, is in accordance with that recorded by Guererro (1983), who recorded the highest mean weight gain in 75% inclusion level of earthworm (Perionyx excavatus) meal.

Furthermore, food conversion efficiency was highest in fish fed 50% earthworm meal, while protein utilization efficiency was best in fish fed 25% earthworm meal diet and fingerlings on this diet had the best relative and specific growth rates closely followed by those fed 50 and 75% earthworm meal diet. Survival was generally high and was not significantly different ($p>0.05$) between fingerlings fed 50 and 75% earthworm meal diets, further indicating that these diet were most suitable for Heteroclarias fingerlings.

Earthworm culture

The three cultured substrates investigated supported the growth of *E. eugeniae* and this indicates that they are good culture media for the earthworm. However, cellulose substrate was the best medium for the culture of *E. eugeniae* compared to soil and the mixture of cellulose and soil substrates. Aston (1984) reported that a possible explanation for best growth and production of earthworms from cellulose substrate could be the production of bacteria involved in the breaking down of cellulose and possibly biochemical by-products activated by the presence of cellulase. Mushrooms are important in the biodegradation of organic matter. The mushroom incorporated in the cellulose substrate may have contributed in the degradation of the substrate, making the nutrients available for the earthworms. Centrosema also incorporated into the cellulose substrate have been reported to enrich and improve soil nutrients since it is a leguminous plant (Teitzel and Burt, 1976). This may have further enhanced the availability of nutrients in cellulose substrate and hence enhanced the growth of the worms. Cellulose substrate has also been reported to be the best substrate for the culture of other worms like Hyperiodrilus euryaulos (Sogbesan et al., 2007). The drop in growth of earthworm from the sixth week in this present study may likely be due to the depletion of nutrients in the substrates.

The crude protein of earthworm recorded in this study is higher than 46.57% for the wild caught Perionyx excavatus reported by Hasanuzzaman et al. (2010) but lower than 69.8% for cultured P. excavatus (Guerrero, 1983) and 63.04% for cultured Hyperiodrilus euryaulos (Sogbesan et al. 2007). The crude lipid of worms in this present study is higher than 5.8% of P. excavatus (Guerrero, 1983) and 5.9% for H. euryaulos (Sogbesan et al., 2007). The ash content was also higher than that of H. euryaulos (8.9%) (Sogbesan and Ugwumba, 2008) but lower than for the wild Lumbricus terrestris (45.7%) (Barker et al., 1998). Variations in proximate compositions have been reported to be likely associated with specific-ecology, food, seasons, life stages and reproductive states of earthworm (Mason et al., 1990; Pennino et al., 1991). In this present study, proximate

composition of the worms was determined regardless of life stages or reproductive stages.

This study showed that earthworm meal can completely replace fish meal in the diet of Heteroclarias fingerlings. However, though replacement of fish meal with earthworm meal at 25 to 50% is most suitable in the diet of mud catfish fingerlings, for optimum growth and nutrient utilization; replacement of fish meal with earthworm meal at 50 to 75% inclusion level is recommended. E. eugeniae is best produced from cellulose substrate.

REFERENCES

Akinwande AA, Ugwumba A, AA, Ugwumba OA (2002) - Effects of replacement of fishmeal with maggot meal in the diets of *Clarias gariepinus* (Burchell, 1822) fingerlings. Zoology 1(2):41–46.

Akpodiete OJ, Okagbare GN (1999). Feed accessories from animal production. In: *Issues on Animal Sciences. A compendium of ideas, fact and methods in science and technology of animal agriculture* (Omeje, S.I. Ed.), Ran Kennedy. Benin City, Nigeria. pp. 71-82.

AOAC (Association of Official Analytical Chemists) International. (2005). *Official Methods of Analysis of AOAC International, 18th Edition*. Gaithersburg, Maryland, USA, AOAC International. p. 201.

Aston RA (1984) - The culture of *Brachiura sowerbyi* (Tubificidae, Oligochaeta) using cellulose substrate. Aquaculture 40:89-94

Barker D, Fitzpatrick MP, Dierenfeld ES (1998). Nutrient composition of selected whole invertebrates. Zoobiology 17:123-134

Dominguez J, Edwards CA, Ashby J (2001). The biology and population dynamics of *Eudrilus eugeniae* (Kinberg) (Oligochaeta) in cattle waste solids. Pedobiologia 45:341-353.

Dynes RA (2003). Earthworms-Technological information to enable the development of earthworm production. *RIRDC Publication No. 03/085*, 23 September 2003. pp. 1-3.

Eyo AA (1996). Feed Formulation and Feeding Practices in Fish Culture. Fish Feed Formulation National Institute for Freshwater Fisheries Research (NIFFR) Extension Guide Series No. 4. In collaboration with German Technical Cooperation Agency (GTZ) and National Agricultural Research Extension and Liaison Services (NAERLS).

Fagbenro OA, Balogun AM, Anyanwu CN (1992). Optimal dietary level for *Heterobranchus bidorsalis*fingerlings fed compounded diets. Nig. J. Appl. Fisheries Hydrobiol. 1:41-45.

Guererro RD (1983). The culture and use of *Perionyx excavatus* as protein resource in the Philippines. In: *Earthworm Ecology*. J.E. Satchell (ed.) Chapman and Hall, London. pp. 309-319.

Hasanuzzaman AFMD, Hossian SKZ, Das M (2010). Nutritional potentiality of earthworm (*Perionyx excavatus*) for substituting fishmeal used in local feed company in Bangladesh. Mesopot. J. Mar. Sci. 25(2):25-30

Jameson JD, Venkataramanujam K (2002). Low cost system for producing worms and isopods. Fish Farmer 16:29-30

Madge DS (1969). Field and laboratory studies on the activities of two species of tropical earthworms. Pedobiologia 9:184–214.

Mason LJ, Johnson SJ, Woodring JP (1990). Influence of age and season on whole body lipid content of *Plathypena scabra* (Lepidoptera: Noctuidae). Environ. Entomol. 19:1259-1262.

Mason WT, Rottmann RW, Dequine JF (1992). Culture of Earthworms for Bait or Fish Food. Publication #CIR1053 of the Department of Fisheries and Aquatic Sci., Florida Cooperative Extension Service, Institute of Food Agric. Sci. University of Florida. 1053. p. 4.

Neuhauser EF, Kaplan DL, Hartenstein R (1979). Life history of the earthworm, *Eudrilus eugeniae. Revue* d'Ecologie et Biologie du Sol 16:525–534.

Pennino M, Dierenfeld ES, Behler JL (1991). Retinol, α-tocopherol, and proximate nutrient composition of invertebrates used as feed. *International Zoo Yearbook* 30:143-149.

Razon-Arceno C, Beningo E, Rason E (1981). *How to raise red earthworms profitably*. Vermin-Farms (Rosemarie de los Reyers Ed.) Philippine Inc., Makati, Philippines. p. 42.

Reinecke AJ, Viljoen SA (1993). Effects of worm density on growth and cocoon production of the African

Nightcrawler, *Eudrilus eugeniae* (Oligochaeta). Eur. J. Soil Biol. 29:29–34.

Reinecke AJ, Viljoen SA, Saayman RJ (1992). The suitability of *Eudrilus eugeniae, Perionyx excavatus* and *Eisenia fetida* (Oligochaeta) for vermi-composting in Southern Africa in terms of their temperature requirements.Soil Biol. Biochem. 24:1295–1307.

Sogbesan AO, Madu CT (2003). Vermiculture: A Practical technique for the mass production of earthworm (*Hyperiodrilus sp.*) as fish food. Paper presented at the *Joint Fisheries Society of Nigeria/ F.A.O. National Special Programme for Food Security, National Workshop on fish feed development and feeding practices in Aquaculture*. (Eyo AA Ed.) Held at National Institute for Freshwater Fisheries Research, New Bussa. 15th-19th September 2003. p. 9.

Sogbesan OA, Ugwumba AAA (2008). Nutritional values of some non-conventional animal protein feedstuffs used as fishmeal supplement in aquaculture practices in Nigeria. Turkish J. Fisheries Aquat. Sci. 8:159-164.

Sogbesan AO, Ugwumba AAA, Madu CT (2006). Productivity potentials and nutritional values of semi-arid zone earthworm (*Hyperiodrilus euryaulos*; Clausen, 1967) cultured on organic wastes as fish meal supplement. Pak. J. Biol. Sci. 10(2):409-414.

Sogbesan OA, Ugwumba AAA Madu CT, Eze SS, Isa J (2007). Culture and utilization of Earthworm as Animal Protein Supplement in the Diet of Heteroclarias longifilis Fingerlings. J. Fisheries Aquat. Sci. 2(6):375-386.

Teitzel JK, Burt RL (1976). *Centrosema pubescens* in Australia. *Tropical Grasslands* 10(1):1-10

Viljoen SA, Reinecke AR (1989). Life-cycle of the African nightcrawler, *Eudrilus* eugeniae (Oligochaeta). South. Afr. J. Zool. 24:27-32.