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Culture and Utilization of Earthworm as Animal Protein Supplement in the Diet of *Heterobranchus longifilis* Fingerlings

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Abstract: The potential of soil and agro-allied waste substrates in vermiculture was assessed in terms of their efficiency for growth, reproductive performance and zoomass production of cultured earthworms (*Hyperiodrilus euryaulos*). Four wooden boxes were stocked in duplicates with 50 matured *H. euryaulos* of average weight 1.94±0.2 g and cultured for 12 weeks. Harvested earthworms were dried, used to formulate five 42.5% isoproteic diets and 1900 kJ/100 g isocaloric diets and fed to fingerlings of *Heterobranchus longifilis* for 70 days. In both vermireactors, the earthworm grew very well with significantly different ($p<0.05$) mean weight gain, 304.25 and 208.15 g ffom agro-allied and soil substrate respectively. Significantly ($p<0.05$) higher specific growth rate of 0.73% day^{-1} and reproductive performance of 2,120 worms kg^{-1} of substrate were from agro-allied substrate compared to 0.59% day^{-1} and 1,914 worms kg^{-1} of substrate, respectively from soil substrate worms. The highest percentage weight gain, 400.5% fish^{-1} and specific growth rate of 0.999% day^{-1} were in fish fed control diet. The lowest feed conversion rate, 1.51; highest protein efficiency ratio, 1.52 and apparent net protein utilization, 52.48% were from 25% earthworm meal diet. The highest daily energy gain 3.34 kJ fish^{-1} day^{-1} was from the control diet. There was significant differences ($p<0.05$) between the growth and feed utilization indices. Haematocrit level, haemoglobin concentration and leucocyte count improved with earthworm inclusion levels. The highest profit index, 9.33; lowest incidence of cost, 1.17 and highest cost benefit, 2.38 were from 25% earthworm meal diet. Based on results from this study agro-allied waste substrate could be a better culture substrate for *H. euryaulos* than soil substrate and 7.5 to 25% earthworm meal inclusion is recommended in the diet of *H. longifilis* fingerlings for profitable and sustainable aquaculture practices.

Key words: Vermiculture, fishmeal, growth, nutrient utilization, haematology, cost benefits

INTRODUCTION

Earthworm production is gaining much interest globally as an effective and environmentally sound method of increasing the rate of decomposition of organic waste and as a potential valuable product used as aqua and livestock feed. Many fish farmers face difficulty in getting live foods during the dry season. Some people hesitate to collect live foods such as worms, *Chironomids*, *Daphnia*, *Moina*, maggots and rotifers from dirt's, rotten wastes, poultry and livestock wastes, pools, ditches and so on because it is labour intensive and time consuming. Also, the above methods have not been resourceful, hence there is a need to culture some of these live foods especially earthworm which availability is seasonal but utilization as fish foods, bait by anglers and in sport fishing cannot be underestimated (Guerrero, 1983; Sogbesan *et al*., 2006a, 2007a).

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Production of earthworm from waste materials either from plants or animals origin is a biodegradation process, which has the advantage of producing useful animal protein (earthworm) used in feeding ornamental fishes in aquarium, as bait in capture and sport fishing and as a replaceable animal protein source in the fish feed (Sogbesan *et al*., 2007a) and useful organic manure (scum and vermicast) that could be used to fertilize farms for crop production (Sogbesan *et al*., 2006a). Ramu (2001) appraised the presence of prostaglandins and related compounds in worms and these hormones are possible inducers of gonad maturation in fin and shell fish. Thus modifications in the ratio of food as fish meal supplement could have the growth, fecundity of broodstock, improving maturation and eggs sizes (Ramu, 2001) in fish fed. *Hyperiodrilus euryaulos* is one of the commonest earthworm in the semi-arid zone of Nigeria 48 (Segun, 1989) and has prolific reproductive capacity of about 200 worms from one egg capsule of a mature worm within 3 weeks (Sogbesan and Madu, 2003).

The high cost and scarcity of fishmeal in formulated feeds has led to the use of other alternative protein sources such as Toad meal (Annune, 1990), Tadpole meal (Ayinla *et al*., 1994; Sogbesan *et al*., 2007b), Fermented fish silage (Fagbenro and Jauncey, 1995), Maggot meal (Ugwumba *et al*., 2001; Sogbesan *et al*., 2005), Poultry dung meal (Fasakin *et al*., 2000) and Garden snail meal (Sogbesan and Ugwumba, 2006a, b) to mention a few.

Heterobranchus longifilis, a highly priced fish due to its good taste, flavour, high growth rate and hardy is one of the major mud catfish species in Nigeria that inhabits freshwater bodies (Idodo-Umeh, 2003). It feeds on any available food, including plankton, insects, fish, benthic invertebrates (annelids), tadpoles and detritus (Olufeagba, 1999) in the wild. The optimum protein requirement for the fingerling stage as reported by Fagbenro *et al.* (1992) is 42.5% which is the most expensive nutrient in feed formulation (Eyo and Olatunde, 2001). The Nigerian fish farmers have not been able to meet the demand for the species by the populace based on the high feeding cost (Faturoti and Lawal, 1986; Olomola, 1990). Hence, there is a need to boost the production of this highly demanded cultured fish with low-cost balanced diet feed for aquaculture sustainability and food security in Nigeria.

MATERIALS AND METHODS

The experimental was carried out between April-September, 2006 in the Hatchery complex and Fish Nutrition Laboratory of Aquaculture and Biotechnology Department of the National Institute for Freshwater Fisheries Research (NIFFR), New-Bussa, Nigeria.

Culture of Earthworm (*Hyperiodrilus euryaulos*)

Two different culturing substrata (Soil substrate (control) and Agro-allied waste substrate) were investigated for their vermicompost efficiency and production capacity in culturing the earthworm (*H. euryaulos*).

The soil substrate used was moist loamy-sandy soil. This was the conventional substrate used for earthworm culture (Dynes, 2003) and it served as the control. The composition and preparation of the agro-allied substrate was carried out following Sogbesan and Madu (2003) method. The materials were allowed to ferment for four weeks. At the end of the fourth week, the compost was separately sun dried, chunks formed were crushed into powdery form using pestle and mortar.

Four wooden boxes of dimension 0.9×0.6×0.3 m were used for the culture experiment. The culture of each substrate was duplicated. The boxes were initially lined first with banana leaves, followed by old newspaper and covered with each substratum to about 5 cm forming a windrow then each substrate was added into the boxes separately to two-third of their depth and placed outdoor of the hatchery complex of NIFFR.

Three hundred and twenty matured adult earthworms (*H. euryaulos*) of weight and length range 1.8-3.3 g (mean = 2.65±0.01 g) and 15.0-30.0 cm (mean = 22.5±7.5 cm), respectively were collected from the wild at Awuru village of Borgu Local government area of Niger-State, Nigeria by digging the muddy soil with spade and hand sorting of the worms into sampling plastic bottles and transported to the experimental site. Fifty Adult earthworms of known weights and lengths were introduced into each box and covered with the substrate to a height of 15 cm. Wetting was done by sprinkling of water twice a day during the dry season and once a day during the rainy season to maintain the moist medium. The worms were fed 10% of their body weight twice a week with fermented poultry dung. The worms were sampled fortnightly. Wetting of the substrata was stopped a day before the sampling day to make the collection of the worm easier which is in accordance with the report of Sogbesan and Madu (2003).

At the end of the experimental period (84th day) the total harvest of the earthworm was done. Harvesting of earthworms was done following the methods of Jameson and Ventakaramanujam (2002). Bi-weekly weight of the worms were measured, recorded and used in determining the growth and productivity indices according to Dynes (2003).

Processing of the Earthworm Meal

At the end of the culture period, the harvested worms were thoroughly rinsed in water and kept in a bowl for 30 min for them to evacuate the residual undigested contents in their guts (Akpodiete and Okagbare, 1999). The worms were then weighed, blanched in hot water, re-weighed fresh and oven-dried at 80°C for 3 h. After drying, the worms were weighed, then milled with Hammer milling machine into powdered form, packed as dried earthworm meal in an airtight plastic bowl and stored at 0-20°C till when needed.

Feeding Experiment

Diet Preparation

A completely randomized design was used, with each treatment. Five experimental diets which were isonitrogenous at about 42.5% crude protein were formulated using algebraic method along with Least Cost Formulae (LCF) of Falayi (2003). In the diets earthworm meal was used to replace fishmeal as animal protein source at various inclusion levels namely 0% (control), 25, 50, 75 and 100%. The diets were coded EM1 (control) to EM5. The percentage composition of the ingredients and production costs in the diets is shown on Table 1.

After formulation, maize, groundnut cake, fish meal, soybean (mild heated), salt and palm oil purchased from Monday market, New-Bussa, Nigeria; blood meal, bone meal, chromic oxide and vitamin premix purchased from Rexton feed miller, Ilorin, Nigeria were measured using electric sensitive weighing balance (OHAUS-LS 2000 Model), milled into fine particles (<0.25 mm) using hammer machine and mixed thoroughly in a bowl for 30 min to ensure homogeneity of the ingredients. Starch was prepared with hot water into a paste and mixed with the other ingredients as binder. The dough was pelleted wet using hand pelleting machine (Kitchen hand Cranker Pelletizer). The pelleted dough was collected in flat trays and sun-dried to constant weight after which the feeds were crushed into crumbs with pestle and mortar (for easy ingestion by the fish). They were packed in jute bags, labelled and stored at -20°C.

Experimental Set-up, Fish and Feeding

Five experimental sets in triplicates were used for this experiment. A total of 15 indoor mini-flow through system at a rate of 25.8 L h^{-1}, 0.25 m depth and 0.55 m diameter circular plastic tanks of 50 L capacity each were used for the trials. Water was supplied to each tank from 30,000 L head tanks. Each unit had a control for comparison. The plastic tanks were cleaned, disinfected and allowed to dry for 24 h, after which water was supplied to two-third of the size of the tank and were covered with a net of mesh size 3 mm to protect the fish from jumping out of the tanks. A constant photoperiod of 12 h light and 12 h dark was maintained.

Table 1: Feed formulation (% dry matter) and production cost (N/kg) of ingredients in earthworm meal diets for the feeding trial

	Experimental diets				
Ingredients	EM1 (control)	EM2	EM3	EM4	EM5
Fish meal	30.00	22.50	15.00	7.50	0.00
Earthworm meal	0.00	7.50	15.00	22.50	30.00
Yellow maize	28.70	26.90	26.10	23.50	18.70
Groundnut cake	11.70	12.60	13.40	14.20	16.20
Soy bean meal	12.60	13.50	13.50	15.30	18.10
Blood meal	10.00	10.00	10.00	10.00	10.00
Vitamin/Mineral premix*	2.00	2.00	2.00	2.00	2.00
Palm oil	2.00	2.00	2.00	2.00	2.00
Common salt	0.50	0.50	0.50	0.50	0.50
Bone meal	1.00	1.00	1.00	1.00	1.00
Cassava starch binder	1.50	1.50	1.50	1.50	1.50
Total	100.00	100.00	100.00	100.00	100.00
Calculated crude protein (%)	42.50	42.50	42.50	42.50	42.50
Calculated gross energy (kJ/100 g)	1900.00	1900.00	1900.00	1900.00	1900.00
Inclusion levels of earthworm meal (%)	0.00	25.00	50.00	75.00	100.00
Production cost (N/100 g)	82.29	77.73	69.37	63.74	56.32

*Vitamin and Minerals: Vitamin A- 10,000,000 I.U.; D3- 2,000,000 I.U.; E- 23,000 mg; K3- 2,000 mg; B1- 3000 mg; B2- 6000 mg; Nacin- 50,000 mg; Calcium Pathonate- 10,000 mg; B6- 5000 mg; B12- 25.0 mg; Folic acid 1,000 mg; Biotin- 50.0 mg; Choline chloride- 400,000 mg; Manganese- 120,000 mg; Iron- 100,000 mg; Copper- 8,500 mg; Iodine-1500 mg; Cobalt- 300 mg; Selenium- 120 mg; Anti-oxidant- 120,000 mg; EM = Earthworm Meal

A total number of 250 fingerlings of *H. longifilis* of weight range 1.69-2.45 g (mean = 1.98±0.083 g) and total length range of 6.2-7.2 cm (mean = 6.5±0.08 cm) were purchased from the Hatchery Unit of NIFFR. They were acclimatized for one week in plastic holding tanks of 2.0×0.5×0.4 m aerated with Erckman Electric Aerator and fed a compounded NIFFR feed of 35% crude protein in the Laboratory.

Fingerlings were sorted, weighed, randomly stocked into the experimental tanks at the rate of 15 fingerlings per tank. They were starved overnight before the commencement of the feeding trials. Fish were offered 5% of their body weight meal per day; administered in two equal portions between 8.00-9.00 and 18.00-19.00 h. The quantity of feed was adjusted based on the weight of fish for previous week throughout the 10 weeks duration of the feeding trials. The fish were monitored for mortality daily. Dead fish were removed, counted, recorded and not replaced.

The length and weight of each fingerling in each tank was measured at the commencement of the experiment. Subsequently, 5 fingerlings were taken randomly from each tank once a week and weighed with beam balance to access the growth rates. At the end of the experiment, all fingerlings in each tank were measured. Survival rate was determined from the number left at the end of the experiment relative to the number stocked. The weekly weights, feed supplied and feaces collected by stripping of the belly of the fish after 7 h of feeding were used to compute the growth, nutrient utilization, apparent digestibility and economic evaluations following the methods of Morais *et al*. (2001), Aksnes *et al*. (1996) and New (1989).

Biochemical and Statistical Analysis

Water temperature was taken daily with thermometer while dissolved oxygen and pH were measured weekly using Boyd (1990) method and pH meter (E251), respectively. At the end of the experiment, the blood sample of the fish was gently collected following the methods of Stosskopf (1992) and Alada *et al*. (2004) to determine haematocrit (%), haemoglobin (g dL^{-1}) and leucocyte ($\times 10^3$ dL^{-1}).

Proximate composition of processed earthworm meal and fish meal, experimental diets, fish feaces, fish carcasses before and after the experiments were analysed for crude protein, crude fibre, crude lipid, ash, nitrogen free extracts and gross energy according to Association of Analytical Chemist Methods (AOAC, 2000). The minerals in the ash of each diet was brought into solution by wet digestion using Conc. HNO_3 (63%), Perchloric acid (60%) and Sulphuric acid (98%) in the ratio of 4:1:1 (Harris, 1974). Potassium and Sodium was determined using flame photometer (Allen, 1974). Phosphorus was determined using spectronic 20E, while Calcium by Perkin Elmer Atomic Absorption Spectrophotometer Model 2900.

All data collected were subjected to analysis of variance (ANOVA). Comparisons among treatment means were carried out by one way analysis of variance followed by Tukey's test (0.05). Least Significance Differences (LSD) was used to determine the level of significance among treatments at $p = 0.05$. Correlation and regression analysis was carried out to determine the relationship between the treatments based on the parameters using SPSS 7.5 Windows 2000 and Graph pad Instat packages. The broken line model (Robbins, 1986) was used to estimate the relationship between the specific growth rate and earthworm meal inclusion levels using second degree polynomial analysis as established by (Snedecor and Cochran, 1967).

RESULTS

The result revealed that there was progressive increase in the growth of the earthworms in the two substrata with time (Fig. 1). Earthworms cultured in soil substrate had total initial weight of 98.15 g and total final weight of 306.3 g while agro-allied substrate cultured worms had 96.35 and 400.6 g as total initial weight and total final weight, respectively. The total weight gain of 304.25 g and 2,150 mean individual worms were harvested from agro-allied substrate while the total weight gain of 208.15 g and 1,974 mean individual worms harvested from soil substrate (Table 2). A significantly ($p<0.05$) high positive correlation of $R = 0.9644$ and $R = 0.9704$ exists between the weight gain and the experimental period for soil and agro-allied substrate cultured worms, respectively. The daily weight gain, relative growth rate and specific growth rate of 3.62 g day^{-1}, 315.78%, 0.73% and 2.48 g day^{-1}, 212.07%, 0.59% which are significantly different at $p<0.05$ were recorded from agro-allied substrate and soil substrate culture worms, respectively.

The crude protein content of fishmeal and earthworm were significantly different ($p<0.05$) while that of the feed were not ($p>0.05$) (Table 3). The highest crude lipid, 11.21% was in 100% earthworm meal diet while lowest crude lipid, 5.9% was in earthworm meal diet. The gross energy values of fishmeal and earthworm were significantly higher ($p<0.05$) than that of each experimental diet. A gradual rise in the line graph of EM1 from week 0 till week 6 when there was a slight decrease and rise

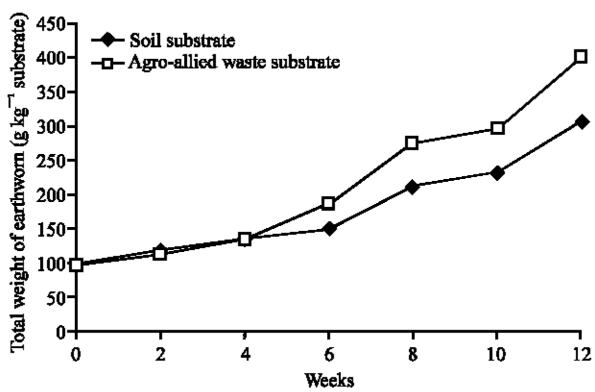

Fig. 1: Growth pattern of *Hyperiodrilus euryaulos* cultured for 84 days

Table 2: Growth performances of *Hyperiodrilus euryaulos* cultured from two different substrata for 84 days

Parameters	Soil substrate	Agro-allied waste substrate
Total weight stocked (g)	98.15^{a}	96.35^{a}
Mean weight stocked (g individual^{-1})	1.96^{a}	1.93^{a}
Total individual number harvested	$1,945^{b}$	$2,150^{a}$
Total number of individual produced/kg of substrate	$1,914^{b}$	$2,120^{a}$
Net reproductive rate worm^{-1} week^{-1}	3.19^{b}	3.53^{a}
Total weight harvested (g)	306.3^{b}	400.60^{a}
Percentage weight gain (%)	212.07^{b}	315.78^{a}
Specific growth rate (% day^{-1})	0.59^{b}	0.73^{a}
No. of days reared	84.00	84.00
No. stocked	50.00	50.00

All values on the same row with different superscripts were significantly different at $p<0.05$. Data without superscript were insignificantly different at $p>0.05$

Table 3: Proximate composition of earthworm meal, fish meal and experimental diets (g/100 g dry matter) used for experiment

Parameters	Fish meal (clupeids)	Earthworm meal	EM1 (control)	EM2	EM3	EM4	EM5
Dry matter (%)	90.21^{a}	91.40^{a}	84.28^{b}	84.41^{b}	84.53^{b}	84.56^{b}	84.83^{b}
Crude protein (%)	71.46^{a}	63.04^{b}	43.53^{c}	43.44^{c}	43.32^{c}	43.18^{c}	43.59^{c}
Crude lipid (%)	7.97^{c}	5.90^{d}	10.63^{b}	10.42^{b}	10.65^{b}	11.09^{a}	11.21^{a}
Crude fibre (%)	1.18^{b}	1.90^{b}	3.36^{b}	3.51^{b}	3.67^{b}	3.82^{b}	4.03^{b}
Ash (%)	7.33^{b}	8.90^{a}	8.42^{a}	7.65^{b}	6.68^{c}	6.08^{c}	5.38^{c}
Nitrogen free extract (%)	3.17^{e}	11.76^{d}	18.33^{c}	19.39^{b}	20.03^{a}	20.39^{a}	20.62^{a}
Sodium (g/100 g)	0.91^{a}	0.43^{b}	0.53^{b}	0.49^{b}	0.46^{b}	0.42^{b}	0.38^{b}
Calcium (g)	3.53^{a}	0.53^{c}	1.47^{b}	1.25^{b}	1.03^{b}	0.81^{bc}	0.59^{c}
Potassium (g)	0.96	0.62	0.72	0.71	0.69	0.69	0.71
Phosphorus (g)	2.40^{a}	0.94^{b}	0.95^{b}	0.85^{b}	0.74^{b}	0.64^{bc}	0.55^{c}
Gross energy (kJ/100 g)	2075.00^{c}	1942.00^{b}	1876.00^{a}	1883.00^{a}	1892.00^{a}	1903.00^{b}	1913.00^{b}

All values on the same row with the different superscripts are significantly difference $p<0.05$. Data without superscript are not significantly difference $p>0.05$, EM = Earthworm Meal

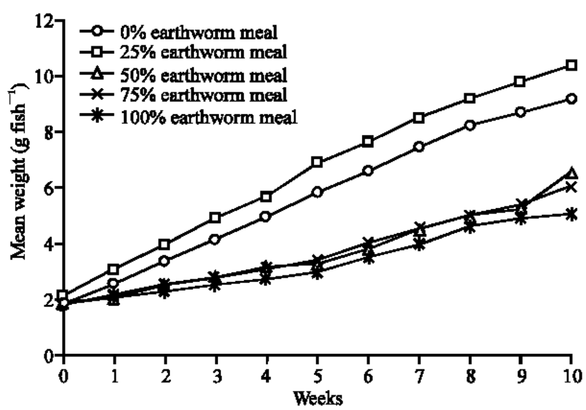

Fig. 2: Growth pattern of *Heterobranchus longifilis* fed earthworm meal diets for 70 days

again in week 7 till the end of the experimental period (Fig. 2). EM2 showed a gradual increase in the weekly weight with rise in the slope of graph line. The growth pattern recorded in EM5 slowly increased from week 1 till the end of the experiment. Highest mean weight gain, 8.22 g fish^{-1} was from EM2 while the lowest, 3.20 g fish^{-1} was from EM5 (Table 4). The highest SGR, 0.999% day^{-1} was from control, followed by 0.981% day^{-1} while the lowest SGR, 0.621% day^{-1} was from EM5. The second degree polynomial analysis gave a quadratic prediction equation of $y = -0.0423x^2 - 0.3581 \pm 1.0183$, $R = 0.970$, $p<0.05$ and broken line analysis gave $y_{max} = 0.985\%$ at $x_{max} = 7.5\%$

Table 4: Growth performances, feed efficiency and economic benefits of *Heterobranchus longifilis* fingerlings fed earthworm meal based diets for 70 days

Indices	EM1	EM2	EM3	EM4	EM5
Mean initial weight (g $fish^{-1}$)	1.83	2.13	1.93	1.84	1.86
Mean weight gain (g $fish^{-1}$)	7.33^{a}	8.22^{a}	4.64^{b}	4.19^{bc}	3.20^{c}
Percentage weigh gain (% $fish^{-1}$)	400.50^{a}	385.90^{a}	240.40^{b}	227.80^{b}	172.00^{c}
Specific growth rate (%)	0.999^{a}	0.98^{a}	0.78^{b}	0.736^{b}	0.621^{c}
Survival rate (%)	93.30^{a}	93.30^{a}	93.30^{a}	86.70^{b}	86.70^{b}
Acceptability index (% sec^{-1})	24.39^{a}	16.39^{b}	13.70^{b}	11.24^{bc}	9.26^{c}
Daily feed Intake (g day^{-1})	2.63^{a}	2.36^{b}	2.22^{b}	2.01^{c}	1.42^{d}
Feed conversion rate	1.60^{d}	1.51^{d}	1.79^{c}	2.06^{b}	2.59^{a}
Protein efficiency rate	1.33^{b}	1.52^{a}	1.29^{b}	1.12^{c}	0.88^{d}
Apparent net protein utilization (%)	39.92^{c}	52.48^{a}	47.37^{b}	37.96^{cd}	33.76^{d}
Apparent net lipid utilization (%)	20.87^{d}	24.75^{c}	33.67^{b}	34.91^{b}	44.87^{a}
Daily energy gain (kJ $fish^{-1}$ day^{-1})	3.34^{a}	3.01^{ab}	2.84^{b}	2.79^{b}	1.98^{c}
Apparent protein digestibility (%)	90.84^{a}	90.62^{a}	89.63^{a}	86.08^{b}	84.08^{b}
Haematocrit (%)	27.00^{bc}	30.00^{b}	38.00^{a}	33.00^{ab}	40.00^{a}
Haemoglobin (g dL^{-1})	10.67	11.43	12.67	11.67	13.39
Leucocycte count ($\times 10^{3}$ dL^{-1})	2.80^{a}	2.60^{a}	2.00^{b}	1.40^{c}	2.30^{ab}
Expenditure (N)	421.30^{a}	402.67^{a}	386.16^{b}	371.78^{b}	344.86^{c}
Total sales (N)	958.73^{a}	872.91^{a}	777.66^{ab}	713.06^{b}	547.67^{c}
Profit index	7.20^{c}	9.33^{b}	9.03^{b}	9.93^{b}	12.21^{a}
Incidence of cost	1.43^{a}	1.17^{d}	1.24^{c}	1.31^{c}	1.83^{a}
Benefit Cost Ratio (BCR)	2.07^{b}	2.38^{a}	2.01^{bc}	1.92^{c}	1.59^{d}

All values on the same row with the different superscripts are significantly difference $p<0.05$. Data without superscript are not significantly difference $p>0.05$. EM = Earthworm Meal

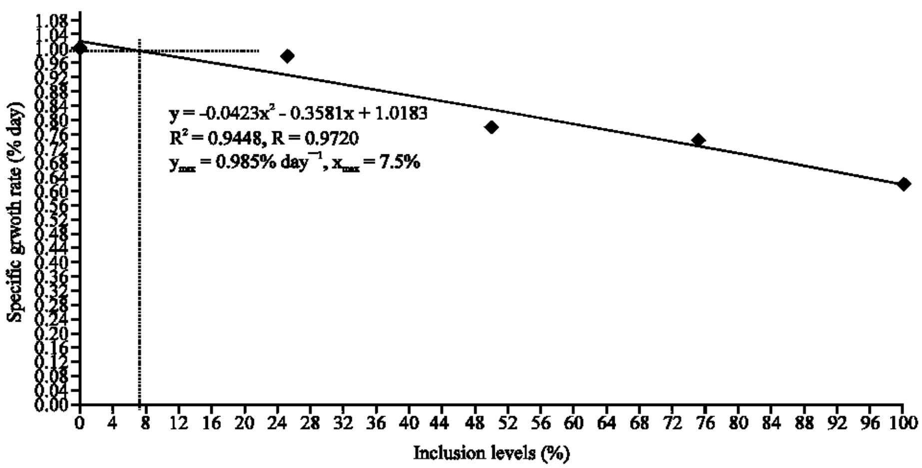

Fig. 3: Effect of earthworm meal inclusion levels on specific growth rate (% day^{-1}) of *Heterobranchus longifilis*

inclusions of earthworm meal (Fig. 3). All growth indices were significantly different ($p<0.05$). The FCR 1.51, 1.73, 1.79, 2.06 and 2.59 were recorded from EM2, EM1, EM3, EM4 and EM5 which were not significantly different ($p>0.05$) between EM1 and EM2 but significant ($p<0.05$) between others. EM2 had the highest PER of 1.52 while EM5 had the lowest PER, 0.88 and these were significantly different ($p<0.05$). Haemoglobin and haematocrit significantly ($p<0.05$) increased as along with earthworm meal inclusions while there was reduction in the leucocyte. The highest correlation ($R = 0.997$; $p<0.05$) was recorded between relative weight gain and specific growth rate while the lowest ($R = -0.31869$ ($p>0.05$) was recorded between acceptability index and incidence of cost of fish fed 25% earthworm meal diet.

DISCUSSION

The higher growth rates and the production of earthworms in the agro-allied substrate could be attributed the production of the bacteria involved in the break down of cellulose from saw dust and rice bran and possibly the biochemical by-products was probably activated by the presence of cellulase in agro-allied substrate culture (Aston, 1984). Guerrero *et al*. (1984) had also credited saw dust in improving earthworm meal.

Animal waste (Poultry and cattle dung) are major component of agro-allied substrate and have been reported to be rich in bacteria (Akpodiete and Okagbare, 1999) and these had been reported as the basic food of earthworms (Patrick and Loutit, 1976). Bacteria have also been identified in the diet of tubificid worms and Oligochaete worms from other families (Aston, 1984). Suzuki and Kurihara (1981) demonstrated that *Aelosoma hemprichi* could feed exclusively on bacteria, showing remarkably rapid population growth on this diet. The high correlation between the number of earthworms stocked and the harvested recorded in this study implies that the two media favoured the culture of earthworm and this agrees with the study of Aston (1984), Guerrero *et al*. (1984) and Jameson and Ventaramajanum (2002). Higher percentage weight gain in agro-allied which contained saw dust as on of the components (Sogbesan and Madu, 2003) compared to soil substrate could be attributed to higher organic content, increase in decomposition rate as a result of higher cellulose from cellulose present in the saw dust (Aston, 1984; Guerrero *et al*., 1984) which activated higher conversion of the substrate to worm tissue (Ramu, 2001). The net reproductive rate range of 3.53-3.19 worms week^{-1} recorded for *H. euryaulos* from the two substrates in this study is lower to 6.7 worms week^{-1} and higher than 1.4 worms week^{-1} for *Eudrilinus eugeniae* and *Dendrobaena veneta,* respectively. The high reproductive rate of the tropical earthworms make them ideally suited for worm meal production (Dynes, 2003). It has also been observed that earthworms secret enzymes, including protease, lipases, amylases, cellulose and chitinases which bring about rapid biochemical conversion of cellulosic and proteinous materials in a variety of organic wastes which help to enhance the speed of decomposition activities of bacteria and other microbes, converting them into worm tissues (Sinha *et al*., 2002; Williams *et al*., 2004; Sogbesan *et al*., 2006c).

The physiological parameters monitored were within the suitable range for tropical fish indicating that environmental conditions of the fish during the experimental period were adequate. The fact that weekly weight increase was recorded in all the treatments showed that none of the experimental diets contain anti-growth factors (Sogbesan *et al*., 2006d). In the present study, the inclusion levels of dry earthworm meal appeared to be an important factor in influencing growth, acceptability index, feed utilization, digestibility, physiology and the cost benefits of *H. longifilis* fed than each index determined. This observation was corroborate with that of Stafford and Tacon (1985) that weight gain reduced as earthworm inclusions increased in practical diets of rainbrow trout. Similar reports were made by Guerrero (1983), Guerrero *et al*. (1984), Alegbeleye and Oresegun (1998) and Sayed (1999) who recorded adverse effect on the productivity of tilapia when fed earthworm supplemented diets.

7.5% inclusion level of earthworm reported in this study based on the broken line analysis to give the highest specific growth rate is not significantly ($p>0.05$) higher 5% inclusions reported by Pascual 1983 (Guerrero, 1983) which gave significantly increased weight of prawn compared with fishmeal diet. Though, it is significantly ($p<0.05$) lower than 15% reported by Guerrero *et al*. (1984) for tilapia fed earthworm meal. It was observed that these authors did not make their projection on the broken line analysis rather the inclusion level which gave 25% inclusion in this study. Hence, this implies that *H. longifilis* will utilize earthworm meal better than tilapia since it is a known voracious carnivore (Fagbenro, 1989).

The emphasis on the specific growth rate (daily incremental weight gain) than mean weight gain in this study agreed with the report of Hassan *et al*. (1995) and Adikwu (2003). The reduction in the acceptability index reported from this study could have been as a result of secretion of eleocytic cells in the epithelium. This similar observation of reduction in weight and acceptability was made by Alegbeleye and Oresegun (1998) when they substituted three lumbrid worms namely for trash fish (on weight basis) as protein sources in *Oreochromis niloticus* feed. Alegbeleye and Oresegon (1998) linked the poor growth and nutrient utilization of *O. niloticus* fed complete earthworm meal to unpalatable tendency of the worms due to secretion of eleocyte cells in its epithelium. Despite this, Cardinete *et al*. (1991) had also reported reduction in food intake and nutritive utilization of protein in rainbow trout fingerlings fed earthworm meal diet and linked this to the present of ceolmic fluid in the earthworm hence fish will definitely perform better at low earthworm meal inclusions in their diet.

Differences in the quality of protein fed to the fish reveled from specific growth rate and protein utilization may have been influenced by the digestibility, indispensable amino acids composition, availability (biological value), palatability and presence of anti-nutritional factor in the experimental diets (Scott *et al*., 1976; Sogbesan *et al*., 2006d). The apparent net protein utilization, a factor of digestibility, utilization and quality of the protein, significantly correlated $R = 0.89$; $p<0.05$ with protein efficiency rate which appraised the protein quality of each of experimental diets. Inclusion of dry earthworm meal at the lowest treatment level showed a better ANPU compared to both the control and other (different inclusion levels) which may indicate the presence some proteolitic factors that limits its utilization at higher inclusion levels (Stafford and Tacon, 1985). Increased in apparent net lipid utilization along with earthworm meal inclusion could attributed higher inclusion of this animal protein supplement with increase in fat deposition in fish muscle which could lead to production of fatty fish.

Better improvement in the haematology parameters observed from this study indicated a positive contribution of earthworm proteins in blood formation and improving immunity. Several protein-rich diets have been shown to increase both haematocrit levels and haemoglobin concentrations in animal fed (Alada *et al*., 2004; Bolarinwa *et al*., 1991). Apart from the high quantity of protein in earthworm, the protein is also of high quality, consisting of most of the essential amino acid present in fishmeal (Sogbesan *et al*., 2007a). Earthworm meal is also rich in minerals which are well known hemantinics and are essential in the formation of red blood cell (Gannong, 1993).

The substitution of dry earthworm meal for fish meal lowers the cost of diet production (Table 1) which is an indication of a more cost efficient and cheaper non-conventional ingredient in relative to the fishmeal (Table 4). Consequently, the farmer will benefit economically through the utilization of this cheaper ingredient at 7.5 to 25% inclusion of dry earthworm meal to raise *H. longifilis* without reduction in specific growth rate.

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