

Full Length Research Paper

Fish meal Substitution by earthworm meal (*Libyodrilus violaceus*) in diets for African catfish, (*Clarias gariepinus*)

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A seven-week feeding trial was conducted to examine the possibility of replacing fish meal with earthworm meal in the diets of the African catfish, *Clarias gariepinus* fry. Fish meal protein was replaced by earthworm meal at 0% (D0); 15% (D15); 25% (D25); 35% (D35); and 50% (D50). The diets were isonitrogenous (54%) and fed thrice daily to triplicate groups of African catfish fry at 5% body weight. Diet had a significant effect on growth performance and feed utilization ($p \leq 0.05$). Final weight, weight gain, daily weight gain, and specific growth rate, were highest in fish fed diet D25. A similar pattern of growth was observed for length measurements. Fish fed diets exceeding 25% replacement of fish meal with earthworm meal had depressed growth. Feed conversion ratio was highest in fish fed D35. Mortality was not dependent on diet. It is concluded that fish meal can be substituted with earthworm meal up to 25% in the diet of *C. gariepinus* fry without adverse effects on growth and nutrient utilization.

Key words: Earthworm meal, fish nutrition, African catfish (*Clarias gariepinus*), *Libyodrilus violaceus*.

INTRODUCTION

Fish meal is the most common source of protein for aqua feeds. However, the cost of fish meal is on the rise, because of its competing use as feed ingredient by other animals (livestock, ruminants etc.). It is therefore necessary to find alternatives to fish meal in the preparation of animal diets. Various workers have attempted to use other locally available cheap protein sources (e.g. plant protein, agricultural by-products, fishery by-products, terrestrial animal by-products, grain legumes, oil seed plants etc.) in animal feeds. Plant protein sources have been used as alternatives in the diets of fish with some measure of success particularly grain legumes (Zhou et al., 2005; Emre et al., 2008;

Monentcham et al., 2010; Lim et al., 2011). A major setback in the use of plant proteins however, is the presence of antigrowth factors. Other workers have also used other animal sources such as poultry by-product (Tucker et al., 2005; Shapawi et al., 2007), meat and bone meal (Gimenez et al., 2009) with favourable results in some cases. The use of earthworms has also been documented. Earthworms are abundant in most parts of Africa and their nutritional values have been determined. They possess amino acid profiles similar to fish (Dedeke et al., 2010a) and have been used as protein supplements in the diets of fish (Hilton, 1983; Sogbesan et al., 2007; Monebi and Ugwumba, 2013).

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Stafford and Tacon (1985) showed that replacing fishmeal with earthworm meals at low levels of inclusion in the diet for rainbow trout did not have any adverse effect on the growth performance or feed utilization efficiency of the species. This study was aimed at evaluating the potential of earthworm (*Libyodrilus violaceus*) meal as an alternative to fish meal in *Clarias gariepinus* fry diets.

MATERIALS AND METHODS

Fish larvae production

The larvae were spawned from brood stock obtained from Zartech Farms, Ibadan, Oyo State. They were fed with *Artemia* nauplii for three days after the yolk had been absorbed and afterwards placed on a commercial feed (0.2 mm) for four weeks until they attained a mean weight of 0.19 g (2.63–2.7 cm) before they were subjected to experimental diets.

Collection of earthworms

The earthworms were collected between the months of August and October, 2006 during the rainy season when the adult stages of earthworms were available. Collections were made along the banks of streams and flood plains or limicolous environment in Ago-Iwoye, Ijebu North Local Government area, Ogun State, Nigeria, lying on longitude 4°32' E, latitude 7°30' N at an altitude of 76m above sea level with mean annual rainfall of 1,779 mm and mean annual temperature of 27°C. Digging and hand sorting method (Owa, 1992) was employed. The collected earthworms were transferred in plastic containers filled with humus to the laboratory for further sorting and identification.

Diagnostic features of *Libyodrilus violaceus*

It has a pale albinoid zone around the clitella region. The male pore is unpaired and the penial setae are simple. The spermathecal diverticulum and receptaculum are paired. The spermathecal atrium is paired and circumenteric. The setal distance is $ab = cd$ and the euprostate is baggy and laterally placed. Identification was done using the method of Owa (1992).

Experimental diets

Five experimental diets (Table 1) were formulated to replace fish meal protein with earthworm meal protein (EW) at 0% (D0), 15% (D15), 25% (D25), 35% (D35) and 50% (D50). The diets were isonitrogenous (54%).

Feeding trials

The experimental feeding trials were conducted in a recirculation system, using a Completely Randomized Design (CRD) with five treatments and three replicates consisting of fifteen plastic tanks (each tank measured 0.6 m long × 0.3 m wide × 0.3 m deep with a total volume of 0.054 m³ water occupying 50% of the total volume). The larvae were randomly distributed into the plastic tanks at a stocking density of 50 fry/tank. The diets were fed to the fry on the basis of 5% body weight (dry matter) per day thrice daily at 06.30, 12.30 and 18.30 h.

Data collection

During the experimental period water temperature was measured using hand held Hg thermometer (24.3 to 24.7°C). pH was measured using a Mettler Toledo digital pH meter with glass electrode (7.4–7.5). Dissolved oxygen (DO) was measured using a Jenway Dissolved Oxygen Meter, Model 970 with a % Oxygen saturation determination of -5 to 25% (9.8 to 10.6 mg/L). The fry were batch weighed and length measurements taken at ten day intervals. The weights (W) of the fry were determined by a top loading electronic Mettler balance to the nearest 0.01 g while the lengths (L) of the fry were determined with a ruler to the nearest 0.01 cm. The specific growth rate (SGR) was determined by the formula $SGR (\%/day) = 100 \times (\ln W_t - \ln W_o) / t$ where $\ln W_t$ is natural logarithm of final weight, $\ln W_o$ in natural logarithm of initial weight and t is days of experiment. The daily weight gain (DWG) (g/day) = $(W_t - W_o) / t$. Daily length gain (DLG) (cm/day) = $(l_t - l_o) / t$ where l_t is the final length and l_o is the original length. The survival rate (X%) of fry was determined by the equation $X\% = (N_t / N_o) \times 100$ where N_o is initial number of fry and N_t is the final number of fry. The feed conversion ratio (FCR) was determined by weight of the feed fed to the fry/live weight gained.

Determination of proximate, mineral and amino acid profiles of earthworm and diets

The proximate composition of earthworm and formulated diets was determined according to the AOAC (1993) method (Table 2). Dry matter (moisture content) was determined by differential weighing of dried and fresh samples. Crude protein was determined by the macro-kjedahl method. Ash content was by drying, ashing at 500°C in a muffle furnace and weighing. Ether extract or crude fat determination was by ether extraction method. Crude fibre was determined by acid digestion of residues from the ether extraction and loss in weight on ignition. Five macro (Calcium, Potassium, Sodium, Phosphorus and Magnesium) and four micro minerals (Iron, Zinc, Copper and Manganese) were determined using Atomic Absorption Spectrophotometer at International Institute of Tropical Agriculture (IITA), Ibadan. The amino acid concentrations of the earthworm and experimental diets were analyzed using the High Performance Liquid Chromatography (Technichon TSM-1, technosequential multisample analyzer) at the Department of Zoology, University of Jos, Nigeria.

Statistical analysis

Statistical analysis of data was done using SPSS version 13. Data were analysed using the one way analysis of variance (Steel and Torrie, 1981); and mean differences compared using the Duncan's multiple range tests ($p \leq 0.05$).

RESULTS

The proximate composition of the experimental diets is shown in Table 1. The nutrient values were similar and reflected the pattern in the ingredients.

L. violaceus had a protein composition greater than 60%, a value lower than that of Danish fish meal (72%) used in this study (Table 2).

Table 3 shows the amino acid profile of the earthworm and formulated diets. All the essential amino acids were represented and the values are comparable. Arginine had the highest value while Threonine had the least value

Table 1. Ingredient and proximate composition of the experimental diets.

| Ingredient composition (%) | D0 | D15 | D25 | D35 | D50 |
|------------------------------|------------|------------|------------|------------|------------|
| Fishmeal (72% crude protein) | 45.00 | 38.25 | 33.75 | 29.25 | 22.50 |
| EW meal | 0.00 | 6.75 | 11.25 | 15.75 | 22.50 |
| SBM | 30.00 | 33.00 | 33.50 | 40.00 | 40.00 |
| FFS | 15.00 | 13.00 | 11.50 | 9.30 | 9.00 |
| FH | 2.65 | 3.65 | 2.65 | 3.35 | 3.65 |
| Corn Starch | 7.00 | 5.00 | 5.00 | 2.00 | 2.00 |
| Mineral/vitamin premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Dicalcium phosphate | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Proximate composition | | | | | |
| Dry matter | 92.35 | 91.89 | 93.02 | 93.45 | 94.33 |
| Crude protein | 54.20 | 54.24 | 54.36 | 54.41 | 53.88 |
| Crude fibre | 2.10 | 1.89 | 3.43 | 3.21 | 3.03 |
| Crude fat (EE) | 7.94 | 8.06 | 7.96 | 7.69 | 7.49 |
| Ash | 13.83 | 11.42 | 12.72 | 11.52 | 11.18 |
| NFE | 18.48 | 11.42 | 12.72 | 11.52 | 11.18 |
| Ca | 0.11 | 0.88 | 0.79 | 0.77 | 0.63 |
| P | 0.11 | 0.11 | 0.99 | 0.93 | 0.82 |

SBM, Soybean meal; FFS, full fat soya; FH, fish hydrolysate; EW, earthworm; NFE, nitrogen free extract.

Table 2. Proximate composition and mineral profile of *L. violaceus*.

| Proximate composition | Mean±SD (N=10) |
|------------------------|----------------|
| %Moisture content | 6.95±1.55 |
| %Ash | 39.53±13.66 |
| %Crude protein | 60.46±14.30 |
| %Crude fat | 4.68±0.36 |
| %Crude fibre | 0.50±0.08 |
| Mineral profile | |
| %Ca | 0.40±0.09 |
| %Mg | 0.12±0.18 |
| %K | 0.028±0.002 |
| Na (ppm) | 23.12±9.96 |
| Mn (ppm) | 21.23±6.74 |
| Fe (ppm) | 18.24±3.68 |
| Cu (ppm) | 8.05±2.02 |
| Zn (ppm) | 28.22±6.68 |
| % P | 0.51±1.10 |

among the essential amino acids. In terms of non essential amino acids, Aspartic acid had the highest value. The mineral profile of the earthworm indicated Phosphorus as the dominant element (Table 2). Fish fed on the treatment diets throughout the experiment and diet had a significant effect on growth performance and feed utilization (Table 4). Final weight, weight gained, daily

weight gained, specific growth rate(weight) were highest in fish fed diets in which fish meal was substituted at 25%. A similar pattern was observed for length measurements. Feed conversion ratio was highest in fish fed diet D (35), indicating poor utilization. Fish fed diets exceeding 25% earthworm meal substitution showed a depressed growth performance and nutrient utilization. Survival was similar in all the treatments (Table 4).

DISCUSSION

The percentage crude protein found in earthworm meal compared favourably with fish meals produced by menhaden, tuna and catfish (BOANR, 1993), which have been used by other workers. The mineral profile of *L. violaceus* is also consistent with those found in literature. Dedeke et al. (2010b) observed that five macrominerals (calcium, magnesium, potassium, sodium and phosphorus) were well represented in five earthworms examined (*Hyperiodrilus africanus*, *Eudrilus eugena*, *Libyodrilus vilaceus* and *Alma mansonii*). The present study showed that earthworm meal can be a suitable substitute for fish meal in the diet of the African catfish, *C. gariepinus*. Earthworm meal is a high protein feedstuff and contains a balanced amino acid profile similar to that found in fish meal (Hilton, 1983; Dedeke et al., 2010a). Lysine and methionine which are limiting amino acids in most feedstuffs were adequately present in *L. violaceus*. However, there are contrasting results in the literature on the nutritional effects of fish meal

Table 3. Amino acid concentration(g/100g crude protein) in *L. violaceus* and formulated diets.

| Amino acid | <i>L. violaceus</i> | Control diet | Diets containing varied levels of <i>L. violaceus</i> | | | |
|----------------------|---------------------|-------------------|---|-------|-------|-------|
| | (g/100 g protein) | (g/100 g protein) | (g/100 g protein) | | | |
| | | D0 | D15 | D25 | D35 | D50 |
| Essential | | | | | | |
| Lysine | 5.50 | 5.02 | 6.01 | 5.90 | 6.05 | 6.51 |
| Histidine | 3.36 | 2.05 | 2.16 | 2.45 | 2.61 | 3.02 |
| Arginine | 8.01 | 5.58 | 6.12 | 6.50 | 7.02 | 6.95 |
| Threonine | 1.02 | 2.01 | 2.41 | 2.55 | 2.62 | 2.50 |
| Valine | 4.00 | 3.81 | 3.20 | 4.10 | 4.51 | 4.31 |
| Methionine | 2.30 | 2.80 | 3.00 | 3.20 | 3.20 | 2.99 |
| Isoleucine | 4.80 | 3.21 | 3.51 | 3.05 | 3.65 | 3.59 |
| Leucine | 6.71 | 5.01 | 4.91 | 5.79 | 6.21 | 6.56 |
| Phenylalanine | 4.40 | 4.30 | 4.62 | 4.46 | 5.03 | 5.51 |
| Non-essential | | | | | | |
| Aspartic acid | 10.68 | 10.54 | 8.97 | 9.65 | 11.15 | 12.00 |
| Serine | 4.03 | 2.40 | 3.20 | 3.07 | 2.99 | 3.26 |
| Glutamic acid | 11.80 | 12.03 | 11.92 | 13.00 | 13.18 | 13.11 |
| Proline | 2.56 | 2.09 | 2.56 | 2.36 | 2.31 | 2.18 |
| Glycine | 0.85 | 3.05 | 3.02 | 2.61 | 3.21 | 2.91 |
| Alanine | 3.50 | 3.00 | 3.31 | 3.56 | 4.02 | 3.80 |
| Cystine | 0.80 | 0.88 | 0.96 | 0.90 | 1.00 | 0.88 |
| Tyrosine | 3.18 | 3.04 | 2.92 | 3.31 | 3.32 | 3.18 |

Table 4. Growth performance and survival of *Clarias gariepinus* fingerlings fed with experimental diets.

| Parameter | Diets | | | | |
|--------------------------------------|-------------------------|-------------------------|------------------------|------------------------|-------------------------|
| | D(0) | D(15) | D(25) | D(35) | D(50) |
| Initial weight (g) | 0.21±0.08 ^a | 0.19±0.07 ^a | 0.19±0.06 ^a | 0.21±0.07 ^a | 0.18±0.06 ^a |
| Initial length (cm) | 2.67±0.34 ^a | 2.74±0.35 ^a | 2.67±0.31 ^a | 2.80±0.25 ^a | 2.67±0.39 ^a |
| Final weight (g) | 2.48±1.09 ^{bc} | 2.89±1.07 ^b | 3.53±1.59 ^a | 1.60±0.74 ^d | 1.97±0.79 ^{cd} |
| Final length (cm) | 6.78±1.08 ^b | 7.08±0.96 ^{ab} | 7.45±0.98 ^a | 5.93±1.06 ^c | 6.18±0.75 ^c |
| Weight gain (g) | 2.26±1.09 ^{bc} | 2.65±1.07 ^b | 3.34±1.59 ^a | 1.39±0.72 ^d | 1.79±0.81 ^{cd} |
| Daily weight gain (g/day) | 0.06±0.03 ^{bc} | 0.07±0.03 ^b | 0.08±0.04 ^a | 0.04±0.02 ^d | 0.05±0.02 ^{cd} |
| Specific growth rate (%/day) | 2.63±0.61 ^b | 2.94±0.57 ^b | 3.11±0.58 ^a | 2.18±0.53 ^d | 2.59±0.61 ^c |
| Length gain (cm) | 4.05±0.92 ^b | 4.29±1.03 ^{ab} | 4.78±0.98 ^a | 3.13±1.07 ^c | 3.51±0.96 ^c |
| Daily length gain (cm/day) | 0.10±0.02 ^b | 0.11±0.03 ^{ab} | 0.12±0.03 ^a | 0.08±0.03 ^c | 0.09±0.02 ^c |
| Specific growth rate (%/day) | 0.99±0.16 ^b | 1.02±0.21 ^{ab} | 1.11±0.18 ^a | 0.80±0.21 ^c | 0.92±0.20 ^b |
| Feed Conversion Ratio (kgDM/kg gain) | 6.19 ^b | 4.42 ^{bc} | 3.83 ^c | 10.08 ^a | 5.95 ^b |
| Survival rate (%) | 62.0 ^d | 61.3 ^d | 70.0 ^d | 70.0 ^d | 67.0 ^d |

Means with the same superscript in the same row are not significantly different ($p>0.05$).

substitution with earth worm meal. Tacon et al. (1983) reported that trout fed frozen earthworms (*Allobophora longa* and *Lumbricus terrestris*) grew as well or better than fish fed a commercial trout pellet.

In contrast the frozen earthworm, *Eisenia foelida* fed to trout was totally unpalatable and showed little or no growth (Tacon et al., 1983), while a growth depression was also observed in trout fed on worm digest meals (Hilton, 1983). The author observed that protein digestibility was

high and could not have been the cause of growth depression. He attributed the poor dietary response to a lack of some unidentified essential component in high worm meal diets. Poor growth performance and nutrient utilization beyond a certain level of inclusion of earthworm meal as observed in this study has also been documented by other workers. Sogbesan et al. (2007) observed growth depression in *H. longifilis* fingerlings when fed with earthworm meal beyond 25% substitution

level, while highest growth was found in fish fed control diet. In other studies on fish meal substitution with other protein sources, it has also been reported that there have been depressed growths, when fish meal protein was substituted beyond a certain level. Ng et al. (2001) reported that African catfish fed meal worm (larvae of the beetle, *Tenebrio molitor*) as a replacement of fish meal up to 40% substitution showed comparative growth, while catfish fed solely meal worms showed growth depression. These have been attributed to a number of factors such as poor nutrient digestibility, mineral deficiencies, amino acid deficiencies and anti-nutritional factors (Storebakken et al., 2000).

In other studies in which fish meal has been substituted with other protein sources, a trend has been observed in which beyond a certain level of inclusion, there are growth depressions. Such studies include the use of soybean meal (Zhou et al., 2005; Lim et al., 2011), soybean and cotton seed meals (Monentcham et al., 2010), and poultry by-product meal (Shapawi et al., 2007) as substitutes for fish meal. The decline in food conversion efficiency also observed beyond a certain level could be due to decreased food intake and digestibility of protein and energy (Storebakken et al., 2000).

It is concluded that fish meal can be substituted by earthworm meal in the diet of *C. gariepinus* up to 25%, without adverse effects on growth and nutrient utilization.

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