Apparent nutrient digestibility and performance of *Heterobranchus longifilis* fed with *Libyodrilus violaceus* meal supplemented-diets

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Abstract

Apparent digestibility coefficients (ADCs) of nutrients is a useful tool for fish diet formulation, which gives the right estimation of growth, thereby reducing waste products. The ADCs of crude protein, energy and dry matter of processed earthworm, *Libyodrilus violaceus* meal by *Heterobranchus longifilis* fingerlings (1.06±0.01g) was evaluated at 0 to 100 %. Eleven dry isonitrogenous (40% crude protein) diets were formulated at levels of 0% (control) to 100%. All experimental diets contained chromic oxide at 0.5% as an indigestible marker. The feeding experiment was carried out in 40 L plastic tanks in triplicate treatments using randomized block design. Fingerlings were fed 5% of their body weight twice daily for 12 weeks and faecal samples were collected seven hours after the first feeding of the day. The digestibility coefficients of nutrients was calculated using proximate analyses of feeds and faeces carried out for crude protein, energy and dry matter. Apparent digestibility of protein and energy were highest, 82.0±0.20% and 81.50±0.32% respectively, in fish fed with 60% *L. violaceus* meal diet and lowest, 78.40±0.40% and 76.20±0.62%, in those fed with the control diet respectively. These values were significantly ($p \leq 0.05$) different between the treatments. Apparent digestibility coefficient of dry matter was lowest (82.28±0.09%) in fish fed with the control diet and highest (86.21±2.0%) in those fed 100% diet, and differed significantly ($p \leq 0.05$) from other treatments. These findings suggests that replacement of fishmeal with *L. violaceus* meal at 60% inclusion level in the diet of *H. longifilis* fingerlings was most suitable for optimum growth, nutrient utilization and protein digestibility.

Keywords: *Heterobranchus longifilis*; *Libyodrilus violaceus;* digestibility; growth.

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Introduction

Aquaculture is the fastest growing food production sector worldwide (66.6 million tonnes) (Food and Agriculture Organization of the United Nations, 2014) and one of the available feasible options for Nigeria to meet the demand for fish for its population. It has the potential of bridging the wide gap between fish demand and supply in Nigeria due to insufficient production from capture fisheries (Ugwumba and Ugwumba, 2003; FAO, 2013). The lack of readily available and affordable fishmeal, which is scarce and expensive, is a major constraint to the development of aquaculture in Nigeria. The future growth of the aquaculture sector largely depends on the availability of suitable and economical feeds (Anetekhai *et al* 2004). This has therefore, led to the search for cheaper and adequate non-conventional animal protein source as fish feed ingredient. *L. violaceus*, a common earthworm species in Nigeria is rich in proteins with a balanced array of essential amino acids, and its use as protein supplements in fish has been reported by Dedeke *et al* (2010; 2013).

Fagbenro (2001) reported that digestibility is one of the most important aspects in evaluating the efficiency of animal feedstuffs and a basic requirement for formulating fish diets. It is also important in the selection of feed ingredients for feed formulation (Falaye *et al* 2014). A feedstuff may appear from its chemical composition to be an excellent source of nutrients but will be of minute actual value unless it can be digested and absorbed in the targeted fish species. Bioavailability of nutrients fed to fish should be defined mainly by their digestibility (NRC, 1993; Goddard and Mclean, 2011) and is one of the most important aspect in evaluating the efficiency of feedstuffs (Hasan, 2001). This study was carried out to investigate the apparent digestibility coefficients (ADCs) of crude protein, energy and dry matter in processed *L. violaceus* in the diets of the fingerlings of *H. longifilis.*

Materials and methods

Collection and identification of Libyodrilus violaceus

Libyodrilus violaceus (Beddard, 1891) is a semi-aquatic endogeic earthworm species collected from the shoreline of Awba Reservoir, University of Ibadan, Ibadan. They have little pigmentation, and generally construct horizontal, deep-branching burrow systems that fill with cast material as they move through the organic-mineral layer of the soil (Edwards and Bohlen, 1996). They are native to West Africa (Dedeke *et al* 2010). *L. violaceus* has pale-grayish flesh above and an orange cuticle with slight bluish-green iridescence below. Body shape is quadrangular; body length ranged from 106-132 mm, diameter ranged between 4 to 10 mm and has 159 to 179 segments. The clitellum is well-ringed, extending over four segments (XIV to XVII) and multi-layered; it is violet in colour and its cuticle had bluish-green iridescence with pink reflections.

Processing of fish feed ingredients

Following the method of Akpodiete and Okagbare (1999) and Monebi and Ugwumba (2016), *L. violaceus* was processed into powdery form as *L. violaceus* meal. Eleven dry isonitrogenous diets were formulated based on the nutrient composition of the protein contents of feedstuffs using Pearson's square method (Pearson, 1976) (Table 1). Fishmeal, corn meal, vitamins and mineral premix and other ingredients were obtained from commercial sources in Ibadan, Nigeria. Chromic oxide was used as an inert marker at a concentration of 0.5% in all the treatments. Appropriate quantities of ingredients in each diet were weighed and mixed thoroughly in a bowl before adding gelatinized starch. The diets were oven-dried at 60^{o}C to a constant weight, after which the feeds were crushed into crumbs with pestle and mortar to enable easy ingestion by the fingerlings. The dried-crumbs were packed and sealed in well-labelled air-tight plastic bowls and kept at room temperature. Proximate composition of *L. violaceus* meal, fishmeal, corn meal and the formulated diets were carried out following the methods of AOAC (1990). Chromic oxide in the diets and faeces were determined by acid digestion method of Furukawa and Tsukahara (1966). Energy contents of faeces and diets were determined according to Guillaume *et al* (2001).

Experimental design and procedure

A completely randomized block design in triplicate treatment was designed for the feeding trial experiment, using a total of thirty-three plastic tanks of 40 L capacity each. The tanks were washed, filled with dechlorinated tap water up to half their volume and covered with nylon netting of 2 mm mesh size to prevent the fish from leaping out of the tanks. Fingerlings of *H. longifilis* were acclimatized to laboratory condition for seven days during which they were fed with fishmeal. They were thereafter starved for 24 hours before the commencement of the feeding trial experiment.

A total of 20 fingerlings (average weight = 1.06±0.01g) were distributed in triplicates randomly into plastic tanks. The fish were fed diets corresponding to 5% of their body weight twice daily at 08.00 hr and 17.00 hr for twelve weeks. The quantity of feed was adjusted based

Table 1. Percentage composition of ingredients (%) in *Libyodrilus violaceus* meal diets.

Ingredients	LVM 1 (0%, control)	LVM 2 (10%)	LVM 3 (20%)	LVM 4 (30%)	LVM 5 (40%)	LVM 6 (50%)	LVM 7 (60%)	LVM 8 (70%)	LVM 9 (80%)	LVM 10 (90%)	LVM 11 (100%)
Fishmeal	44.63	40.84	36.93	32.87	28.68	24.33	19.82	15.14	10.30	5.24	0.0
LVM	0.0	4.54	9.23	14.09	19.12	24.33	29.73	35.33	41.15	47.18	53.47
Corn meal	44.87	44.08	43.34	42.54	41.71	40.85	39.95	39.02	38.06	37.07	36.03
*Vitamin and mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Soybean oil	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Cassava starch	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated crude protein	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Levels of LVM inclusion	0.0	10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0
Calculated gross energy (kcal/g)	484.09	485.08	486.50	487.69	489.04	490.40	491.71	493.10	494.70	496.12	497.78
Calculated gross energy:crude protein	12.10	12.13	12.16	12.19	12.23	12.26	12.29	12.33	12.37	12.40	12.44

LVM = *Libyodrilus violaceus* Meal.
*Vitamin and mineral premix: 2.5 kg of premix contained: Vitamin A – 12,500,000.00 I.U, Vitamin D3 – 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.

on the new weekly weight of fish. Supplementary aeration was provided throughout the duration of the experiment by means of aquarium pump (Cosmos model 1500 Aquarium air pump) and complete changing of water was done twice a week to prevent pollution.

Collection of faecal samples began after six weeks dietary adaptation period and lasted for another four weeks; the faecal samples were collected twice weekly by slightly pressing the anal region of the fish seven hours after feeding of the day (Monebi and Ugwumba, 2016). The samples collected were weighed, and dried at 60°C for 24 hours. After drying, the samples were reweighed, ground in a mortar, and sealed in well-labelled polythene bags, according to each treatment and kept at 4°C prior to determination of the crude protein, dry matter and the amount of chromic oxide following AOAC (1990) methods. Water temperature and pH were measured daily between 07.00-8.00 am using combined digital pH meter (pH-009 (III), UK), while dissolved oxygen was determined weekly with dissolved oxygen meter (PDO-520, UK). Fish mortality was recorded daily and used to calculate percentage survival for each treatment. Initial, weekly and final weights of experimental fish were recorded for each treatment, and were used to determine the growth and nutrient utilization. Initial and final proximate analyses of carcass of the experimental fish were carried out according to AOAC (1990) methods.

Determination of apparent digestibility coefficients

The ADCs of protein, energy and dry matter for all treatments were determined using the method described by Aksnes *et al* (1996):

ADC protein (%)

$$= \frac{1-(\text{dietary chromic oxide})}{\text{faecal chromic oxide}} \times \frac{\text{faecal protein}}{\text{dietary protein}} \times 100$$

ADC energy (%)

$$= \frac{1-(\text{dietary chromic oxide})}{\text{faecal chromic oxide}} \times \frac{\text{faecal energy}}{\text{dietary energy}} \times 100$$

ADC dry matter (%)

$$= \frac{1-(\text{dietary chromic oxide})}{\text{faecal chromic oxide}} \times \frac{\text{faecal dry matter}}{\text{dietary dry matter}} \times 100$$

Determination of growth performance and nutrient utilization of fish

Mean weight gain/fish (g/fish) = ($W_f - W_i$).

Where W_f = final weight of fish at the end of the experiment.

W_i = initial weight of fish at the beginning of the experiment.

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

$$\text{Specific growth rate (\%)} = \frac{\text{Log}_e W_f - \text{Log}_e W_i}{t} \times 100$$

Where Log_e = the natural logarithm of the fish.
t = experimental period in days.

$$\text{Survival (\%)} = \frac{N_f}{N_i} \times 100$$

Where: N_i = number of cultured fish stocked at the beginning of the experiment.
N_f = number of fish alive at the end of the experiment.

$$\text{Food conversion ratio} = \frac{\text{mean feed supplied (g)}}{\text{mean weight gain (g)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{mean weight gain (g)}}{\text{mean protein intake (g/fish)}}$$

$$\text{Protein productive value (\%)} = \frac{B - B_0}{\text{PI}} \times 100$$

Where B = final body protein (at the end of experiment), B_0 = initial body protein (at the beginning of experiment) and PI= protein intake.

Statistical analysis

Data were analysed using descriptive statistics, one-way ANOVA and Duncan's multiple range test (Duncan, 1955) to determine significant (p<0.05) differences among the means using the SPSS^{Ò} version 19 (SPSS Inc. IBM, IL, USA).

Results

Proximate, mineral and energy compositions of Libyodrilus violaceus meal diet

The proximate, mineral and energy compositions of *L. violaceus* meal (LVM) diets are presented in Table 2. The percentage crude protein ranged from 44.30±0.05% in 100% LVM to 44.52±0.05% in the control diet, and was not significantly ($p \geq 0.05$) different. Percentage ash ranged between 6.26±0.01% and 11.05±0.02% in the control and 100% LVM diets, and these values differed significantly ($p \leq 0.05$). Calcium was highest (1.68±0.12%) in the control diet, and lowest (1.58±0.01%) in 100% LVM diet, and was not significantly ($p \geq 0.05$) different. Phosphorus was highest (1.08±0.01%) in 70% LVM diet and lowest (0.94±0.06%) in the control diet, and the differences were significant ($p \leq 0.05$). Energy content was highest (376.35±0.05kcal/g) in 20% LVM diet and lowest (369.29±0.05kcal/g) in the control diet. There was a significant difference ($p \leq 0.05$) between energy content of the control and all the LVM diets.

Growth and feed utilization of Heterobranchus longifilis fed Libyodrilus violaceus meal diet

Water temperature, pH and dissolved oxygen were

Table 2. Proximate, mineral and energy compositions of *Libyodrilus violaceus* meal diets (% dry weight).

Parameter	DIET1(Control, 0% LVM)	DIET 2 (10% LVM)	DIET 3 (20% LVM)	DIET 4 (30% LVM)	DIET 5 (40% LVM)	DIET 6 (50% LVM)	DIET 7 (60% LVM)	DIET 8 (70% LVM)	DIET 9 (80% LVM)	DIET 10 (90% LVM)	DIET 11 (100% LVM)
CP (%)	44.52 ±0.05^{a}	44.48 ±0.04^{a}	44.46 ±0.02^{a}	44.42 ±0.02^{a}	44.40 ±0.05^{a}	44.37 ±0.08^{a}	44.39 ±0.02^{a}	44.36 ±0.10^{a}	44.35 ±0.05^{a}	44.32 ±0.04^{a}	44.30 ±0.05^{a}
CL (%)	5.03 ±0.02^{a}	4.81 ±0.04^{b}	4.72 ±0.02^{b}	4.70 ±0.22^{b}	4.50 ±0.01^{bc}	4.40 ±0.10^{c}	4.39 ±0.03^{c}	4.38 ±0.03^{c}	4.32 ±0.05^{c}	4.30 ±0.03^{c}	4.22 ±0.02^{c}
Ash (%)	6.26 ±0.01^{e}	9.72 ±0.10^{d}	10.02 ±0.05^{c}	10.43 ±0.20^{b}	10.44 ±0.10^{b}	10.55 ±0.03^{b}	9.30 ±0.05^{e}	9.70 ±0.02^{d}	10.05 ±0.02^{c}	11.0 ±0.10^{a}	11.05 ±0.02^{a}
CHO (%)	12.05 ±0.02^{c}	14.31 ±0.02^{bc}	14.50 ±0.05^{b}	14.52 ±0.04^{b}	14.60 ±0.03^{b}	14.68 ±0.02^{b}	14.82 ±0.01^{b}	14.85 ±0.04^{b}	15.20 ±0.02^{a}	15.30 ±0.10^{a}	15.60 ±0.03^{a}
Ca (%)	1.68 ±0.12^{a}	1.66 ±0.01^{a}	1.65 ±0.01^{a}	1.64 ±0.01^{a}	1.63 ±0.02^{a}	1.62 ±0.02^{a}	1.66 ±0.01^{a}	1.65 ±0.02^{a}	1.64 ±0.03^{a}	1.62 ±0.02^{a}	1.58 ±0.01^{a}
P (%)	0.94 ±0.06^{b}	0.95 ±0.02^{b}	0.97 ±0.01^{b}	0.98 ±0.01^{b}	1.01 ±0.05^{a}	1.03 ±0.02^{a}	1.05 ±0.10^{a}	1.08 ±0.01^{a}	1.06 ±0.03^{a}	1.05 ±0.02^{a}	1.03 ±0.05^{a}
Mg (%)	0.75 ±0.05^{b}	0.77 ±0.03^{b}	0.78 ±0.01^{b}	0.80 ±0.02^{ab}	0.82 ±0.01^{ab}	0.85 ±0.06^{a}	0.86 ±0.05^{a}	0.87 ±0.04^{a}	0.88 ±0.05^{a}	0.89 ±0.02^{a}	0.81 ±0.01^{ab}
K (%)	1.08 ±0.02^{e}	1.12 ±0.06^{e}	1.22 ±0.05^{d}	1.28 ±0.02^{d}	1.30 ±0.04^{d}	1.42 ±0.10^{c}	1.68 ±0.02^{a}	1.62 ±0.02^{b}	1.60 ±0.01^{b}	1.56 ±0.05^{ab}	1.50 ±0.03^{b}
Na (%)	0.96 ±0.02^{b}	0.98 ±0.01^{b}	1.02 ±0.05^{ab}	1.04 ±0.03^{ab}	1.07 ±0.06^{ab}	1.10 ±0.04^{a}	1.16 ±0.02^{a}	1.12 ±0.03^{a}	1.10 ±0.01^{a}	1.10 ±0.01^{a}	1.10 ±0.01^{a}

Values on the same row with the different superscripts are significantly different ($p \leq 0.05$). LVM = *Libyodrilus violaceus* meal, CP = Crude protein,
CL = Crude lipid, CHO = Carbohydrate.

maintained at optimum range of 25-28°C, 6.6-7.2 and 5.5-5.7 mg/L respectively. The weekly growth of *H. longifilis* fed all the experimental diets from the first to the twelfth week was continuous (Figure 1). Mean weight gain was highest (14.96±0.01g/fish) in fish fed 60% LVM diet, and lowest (10.61±0.02g/fish) in those fed 100% LVM diet (Table 3), and these were significantly ($p \leq 0.05$) different from all the other diets. Highest relative growth rate of 1438.46±0.03%/fish was from fingerlings fed 60% LVM diet, while the lowest (1013.21±0.04%/fish) was from those fed 90% LVM diet, and these values were significantly ($p \leq 0.05$) different. Survival ranged from 90.0±2.20% to 92.0±2.55%, and was not significantly ($p \geq 0.05$) different between the diets, though higher values were recorded in fish fed 50-70% LVM diets.

The highest food conversion ratio of 4.35±0.02 was

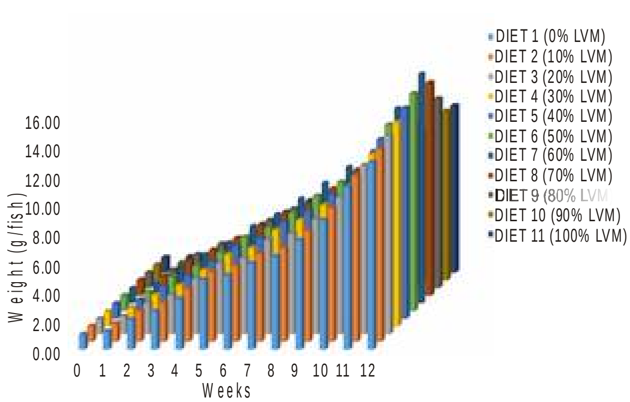

Figure 1. Weekly growth of *Heterobranchus longifilis* fingerlings fed with *Libyodrilus violaceus* meal (LVM) diets.

from fish fed 80% LVM diet, while the lowest (3.59±0.02) was from those fed 60% LVM diet. It was not significantly ($p \geq 0.05$) different between the control and 70% LVM diets, but significantly different ($p \leq 0.05$) in the other diets. Protein efficiency ratio was highest, 4.74±0.06%, in fish fed 60% LVM diet and lowest, 3.47±0.20%, in those fed 100% LVM diet, and was significantly ($p \leq 0.05$) different.

Apparent digestibility of Heterobranchus longifilis fed with Libyodrilus violaceus meal diet

The ADCs of protein and energy were highest, 82.0±0.20% and 81.50±0.32% respectively, in fish fed 60% LVM diet, and lowest, 78.40±0.40% and 76.20±0.62%, in fish fed the control diet (Table 4), and these values were significantly ($p \leq 0.05$) different. Highest ADC of dry matter, 86.21±2.0%, was in fish fed 100% LVM diet, while the lowest, 82.28±0.09%, was from those fed the control diet, and were significantly ($p \leq 0.05$) different.

Carcass composition of Heterobranchus longifilis fed Libyodrilus violaceus meal diet

The carcass compositions (initial and final) of *H. longifilis* fed *L. violaceus* meal (LVM) diets are shown in Table 5. There were significant ($p \leq 0.05$) differences between the crude protein, lipid, ash and dry matter contents of the initial and final carcass compositions. The highest final carcass crude protein, 77.82±0.20%, was from fish fed 60% LVM diet, while the lowest, 75.21±0.02%, was from the control diet and was significantly ($p \leq 0.05$) different from the initial value of 72.51±0.94%.

Table 3. Growth indices and nutrient utilization of *Heterobranchus longifilis* fingerlings fed with *Libyodrilus violaceus* meal diet.

Parameter	Diet 1 (Control, 0% LVM)	Diet 2 (10% LVM)	Diet 3 (20% LVM)	Diet 4 (30% LVM)	Diet 5 (40% LVM)	Diet 6 (50% LVM)	Diet 7 (60% LVM)	Diet 8 (70% LVM)	Diet 9 (80% LVM)	Diet 10 (90% LVM)	Diet 11 (100% LVM)
Initial MW (g/fish)	1.05 ±0.01^{a}	1.08 ±0.02^{a}	1.06 ±0.01^{a}	1.03 ±0.02^{ab}	1.05 ±0.01^{a}	1.07 ±0.01^{a}	1.04 ±0.02^{ab}	1.08 ±0.02^{a}	1.05 ±0.01^{a}	1.06 ±0.02^{a}	1.04 ±0.01^{ab}
Final MW (g/fish)	13.08 ±0.01^{d}	13.45 ±0.01^{d}	13.86 ±0.01^{d}	14.25 ±0.01^{c}	14.70 ±0.01^{c}	15.18 ±0.01^{b}	16.0 ±0.01^{a}	14.86 ±0.0^{c}	13.20 ±0.01^{d}	11.80 ±0.01^{e}	11.65 ±0.01^{e}
MWG (g/fish)	12.03 ±0.15^{c}	12.37 ±0.02^{c}	12.80 ±0.02^{c}	13.22 ±0.01^{b}	13.65 ±0.01^{b}	14.11 ±0.01^{a}	14.96 ±0.01^{a}	13.78 ±0.02^{b}	12.15 ±0.02^{c}	10.74 ±0.02^{d}	10.61 ±0.02^{d}
Survival (%)	90.0 ±2.20^{a}	90.0 ±2.26^{a}	90.0 ±2.30^{a}	90.0 ±2.34^{a}	90.0 ±3.0^{a}	92.0 ±2.40^{a}	92.0 ±2.55^{a}	92.0 ±2.0^{a}	90.0 ±2.88^{a}	90.0 ±3.25^{a}	90.0 ±3.60^{a}
RGR (%/fish)	1145.71 ±0.05^{e}	1145.31 ±0.04^{e}	1207.55 ±0.06^{d}	1283.50 ±0.04^{c}	1300.0 ±0.05^{b}	1318.70 ±0.06^{b}	1438.46 ±0.03^{a}	1275.93 ±0.04^{c}	1157.14 ±0.06^{e}	1013.21 ±0.04^{f}	1020.19 ±0.05^{f}
SGR (%)	1.30 ±0.02^{c}	1.30 ±0.02^{c}	1.33 ±0.01^{bc}	1.36 ±0.02^{b}	1.36 ±0.01^{b}	1.37 ±0.03^{b}	1.41 ±0.02^{a}	1.36 ±0.01^{b}	1.31 ±0.02^{c}	1.25 ±0.02^{d}	1.25 ±0.01^{d}
FCR	3.69 ±0.02b^{c}	3.68 ±0.03^{c}	3.66 ±0.02^{c}	3.64 ±0.02^{c}	3.62 ±0.03^{c}	3.62 ±0.02^{c}	3.59 ±0.02^{c}	3.64 ±0.03^{c}	4.35 ±0.02^{a}	3.90 ±0.03^{b}	3.82 ±0.02^{b}
PER	4.01 ±0.05^{f}	4.0 ±0.06^{f}	4.14 ±0.04^{e}	4.25 ±0.08^{d}	4.38 ±0.10^{c}	4.51 ±0.09^{b}	4.74 ±0.06^{a}	4.38 ±0.12^{c}	3.93 ±0.07^{g}	3.49 ±0.16^{h}	3.47 ±0.20^{h}
PPV (%)	90.0 ±0.02^{h}	87.70 ±0.01^{i}	122.65 ±0.02^{e}	129.90 ±0.01^{d}	131.09 ±0.02^{c}	159.10 ±0.01^{b}	168.57 ±0.01^{a}	129.30 ±0.02^{d}	112.94 ±0.02^{f}	106.82 ±0.01^{g}	91.18 ±0.01^{h}

Values on the same row with the different superscripts are significantly different ($p \leq 0.05$).
LVM = *Libyodrilus violaceus* meal, MW = Mean Weight; MWG = Mean weight gain; RGR = Relative growth rate; SGR = Specific growth rate, FCR = Food conversion ratio, PER = Protein efficiency ratio, PPV = Protein productive value.

Table 4. Apparent digestibility coefficient of *Heterobranchus longifilis* fingerlings fed with *Libyodrilus violaceus* meal diet.

Apparent digestibility Coefficient	Diet 1 (Control, 0% LVM)	Diet 2 (10% LVM)	Diet 3 (20% LVM)	Diet 4 (30% LVM)	Diet 5 (40% LVM)	Diet 6 (50% LVM)	Diet 7 (60% LVM)	Diet 8 (70% LVM)	Diet 9 (80% LVM)	Diet 10 (90% LVM)	Diet 11 (100% LVM)
ADC protein (%)	78.40 ±0.40^{e}	78.65 ±0.25^{e}	78.72 ±0.30^{e}	79.56 ±0.22^{d}	80.0 ±0.26^{d}	80.30 ±0.36^{c}	82.0 ±0.20^{a}	81.50 ±0.30^{b}	80.60 ±0.45^{c}	80.50 ±0.60^{c}	78.50 ±1.10^{e}
ADC energy (%)	76.20 ±0.62^{e}	79.50 ±0.40^{d}	80.10 ±0.18^{c}	80.32 ±0.72^{bc}	80.64 ±0.50^{b}	80.70 ±0.38^{b}	81.50 ±0.32^{a}	81.30 ±0.26^{ab}	80.05 ±0.85^{c}	79.60 ±1.20^{d}	79.24 ±1.60^{d}
ADC dry matter (%)	82.28 ±0.09^{d}	84.05 ±0.15^{c}	84.30 ±0.21^{c}	84.42 ±0.30^{c}	84.52 ±0.36^{c}	84.68 ±0.42^{c}	85.40 ±0.56^{b}	85.60 ±0.64^{b}	85.90 ±1.28^{b}	86.0 ±1.82^{ab}	86.21 ±2.0^{a}

Values on the same row with the different superscripts are significantly different ($p \leq 0.05$).
LVM = *Libyodrilus violaceus* meal, ADC = Apparent Digestibility Coefficient.

Table 5. Initial and final carcass composition of *Heterobranchus longifilis* fingerlings fed with *Libyodrilus violaceus* meal diet.

Parameter	Initial	Final										
		Diet 1 (Control, 0% LVM)	Diet 2 (10% LVM)	Diet 3 (20% LVM)	Diet 4 (30% LVM)	Diet 5 (40% LVM)	Diet 6 (50% LVM)	Diet 7 (60% LVM)	Diet 8 (70% LVM)	Diet 9 (80% LVM)	Diet 10 (90% LVM)	Diet 11 (100% LVM)
CP	72.51 ±0.94^{d}	75.21 ±0.02^{c}	75.22 ±0.07^{c}	76.30 ±0.05^{b}	76.55 ±0.06^{b}	76.60 ±0.60^{b}	77.49 ±0.8^{a}	77.82 ±0.20^{a}	76.57 ±0.90^{b}	76.0 ±0.51^{c}	75.80 ±0.30^{bc}	75.30 ±1.03^{c}
CF	1.85 ±0.04^{g}	2.18 ±0.01^{f}	2.20 ±0.02^{f}	2.24 ±0.01^{f}	2.40 ±0.01^{e}	2.65 ±0.02^{d}	2.72 ±0.02^{c}	2.88 ±0.01^{c}	3.00 ±0.02^{b}	3.02 ±0.30^{b}	3.08 ±0.50^{a}	3.10 ±0.04^{a}
CL	8.47 ±0.90^{f}	10.05 ±0.01^{c}	10.30 ±0.02^{b}	10.50 ±0.01^{a}	10.45 ±0.02^{a}	10.00 ±0.05^{g}	9.60 ±0.01^{d}	9.00 ±0.02^{e}	8.68 ±0.03^{f}	8.55 ±0.05^{f}	8.30 ±0.06^{f}	8.00 ±0.01^{g}
Ash	12.04 ±0.15^{g}	12.77 ±0.20^{f}	12.80 ±0.45^{f}	13.02 ±0.18^{e}	13.24 ±0.09^{d}	13.36 ±0.10^{d}	13.50 ±1.02^{c}	13.74 ±0.27^{c}	14.05 ±0.40^{b}	14.38 ±1.0^{ab}	14.50 ±0.57^{a}	14.65 ±2.38^{a}
*DM	28.60 ±0.18^{a}	24.80 ±0.36^{b}	24.96 ±0.56^{b}	23.84 ±0.72^{d}	23.73 ±0.60^{d}	23.50 ±0.80^{d}	23.22 ±0.50^{e}	22.40 ±0.21^{f}	22.76 ±0.68^{f}	23.80 ±0.42^{d}	23.66 ±0.54^{d}	24.05 ±0.65^{c}

*Calculated as % wet weight.
Values on the same row with the different superscripts are significantly different ($p \leq 0.05$).
LVM = *Libyodrilus violaceus* meal diet, CP = Crude protein, CF = Crude fibre, CL = Crude lipid, DM = Dry Matter.

Discussion

The optimum protein requirement of the mud catfishes falls within 40.0 to 42.5 % (Kiriratnikom and Kiriratnikom, 2012), which is close to the crude protein of diets in the present study. Percentage values of the ADC of crude protein revealed that *L. violaceus* meal protein was highly digested by the fingerlings of *H. longifilis*. The increase in the weekly growth of *H. longifilis* fingerlings fed *L. violaceus* meal in all treatments throughout the duration of the feeding trial 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 sufficient for growth of the fish. The highest mean weight gain of fish from these inclusion levels in different LVM diets could be as a result of the extent of the digestibility of the diets which was similar to the findings of Nwanna (2003) and Farinu *et al* (1992) who both recorded significant mean weight gain of experimental fish with higher digestibility values in their studies.

Fingerlings of *H. longifilis* fed with the experimental diets exhibited significantly higher digestibility values for both protein and energy and consequently significantly higher feed utilization potentials when other growth and nutrient utilization parameters were considered. Best growth rate of fish in this inclusion level is an indication that they were most efficient in converting their food to body growth. Also, the best nutrient utilisation in *H. longifilis* fed 60% LVM diet is related to an improvement in diet digestibility (Olivia-Teles and Rodrigues, 1993).

Highest protein digestibility in *H. longifilis* fed 60% LVM diet is an indication of protein bioavailability and utilization for optimum body protein increase and growth. Carcass protein, also highest for these dietary inclusion levels may be attributed to the ability of *H. longifilis* to convert and utilize the crude protein in their diets for body growth. Highest apparent digestibility coefficient of protein observed for *H. longifilis* fed 60% LVM diet in the present-study may be indicative of nutrient availability and utilization of this diet over all the other diets.

Protein efficiency ratio and protein productive value which were also highest in *H. longifilis* fed with 60% LVM diet was indicative of conversion of protein into energy in this diet. The final carcass protein of *H. longifilis* fed with 60% LVM diet was higher in all dietary inclusion levels than the initial, indicating higher protein retention in these diets and the ability of these fishes to convert and utilize the crude protein in their diets for body growth. Survival, which was generally high for all the experimental fish in the different treatments during the experiment, further indicates the suitability of *L. violaceus* meal diets for *H. longifilis* fingerlings.

Physico-chemical parameters

The monitored water physico-chemical parameters during the feeding trial at all inclusion levels were within the suitable range for tropical fish indicating that the environmental conditions of fish during the experimental period were adequate. According to Santhosh and Singh (2007) the temperature range of 24 and 30^{o}C, pH range of 6.5 to 9.0 and dissolved oxygen range of 5.0 to 9.0 mg/L were suitable for fish culture. Boyd and Lichtkoppler (1979) associated better growth of fish, survival and other physiological activities of fish to good water quality. Therefore, good water quality is very essential for survival and growth of fish (Bhatnagar and Devi, 2013). A sharp drop or an increase within these limits has adverse effects on the body functions of fish (Davenport, 1993; Kiran, 2010).

Conclusion

This study shows that replacement of fishmeal with *L. violaceus* meal at 60% inclusion level in the diet of *H. longifilis* fingerlings was most suitable for optimum growth, nutrient utilization and protein digestibility.

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