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Full Length Research Paper

Effects of increasing dietary protein levels on growth, feed utilization and body composition of *Heterobranchus longifilis* (Valenciennes, 1840) fingerlings

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The effect of different dietary protein levels on growth performance and on feed utilization of catfish (*Heterobranchus longifilis*) fingerlings was carried out in aquaria. Five dietary protein levels 25, 30, 35, 40 and 45% were tried in triplicates. The result shows that 25% protein is too limited to ensure good growth and also the growth rate, weight gain and nitrogen metabolism increased progressively with dietary protein level to a maximum at 40%. However, there was no significant difference in feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), daily weight gain (DGW) and the daily feed intake between fish fed with 30% protein and those fed with 35% protein. There was no significant difference in PER and nitrogen loss (NL) between fish fed with 25 to 40% protein. Above 40% protein, nitrogen metabolism and PER decreased, showing the low utilization of dietary protein. The moisture, ash and gross energy content did not vary between dietary treatments. However, lipid and protein body content varied between treatments.

Key words: Dietary protein, growth, nutrient utilization, body composition, *Heterobranchus longifilis*, fingerlings.

INTRODUCTION

In formulating diets for fish, it is relevant to meet all requirements of nutrients for optimum growth. Lack of good quality feed for economic production adversely affects growth rates, disease manifestation and total harvest of fish (Alatise et al., 2006).

The African catfish, *Heterobranchus longifilis*, is one of the most used species in sub Saharan Africa. It is fresh-water specie with great aquaculture potentials and is widely accepted by fish farmers and consumers (Legendre, 1991) because of its taste, fast growth rate and moderate price (Babalola and Apata, 2006). Studies have been carried out to better understand its nutritional requirements and to improve and develop practical diet formulations for use in aquaculture (Lenient et al., 2008). Olufeagba (1999) recommended 45% for triploid *H. longifilis*, while Fagbenro et al. (1992) reported 42.5% dietary protein requirement for *Heterobranchus bidorsalis*. According to Kerdchuen (1992), the best growth has been obtained with diets containing 43% protein and 15% lipid in fish of 60 g. This author also reported that 45% protein and 14% lipid can allow the best growth in fish of 13 g weight. However, manage-

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Abbreviations: FCR, Feed conversion ratio; **PER,** protein efficiency ratio; **SGR,** specific growth rate; **DGW,** daily weight gain; **NL,** nitrogen loss.

Table 1. Proximate composition of the feed ingredients (% dry matter).

Ingredient	Protein	Lipid	Ash	Fiber
Fish meal	60.30	8.55	20.25	-
Soybean meal	45.25	4.30	5.95	5.22
Wheat bran	17.89	3.80	5.26	9.56
Corn meal	10.85	5.23	1.67	3.31

ment, environmental factors and fish size can affect dietary nutrient levels for optimum performance (Jamabo and Ockiya, 2008). In a previous study carried out by the Oceanological Research Center in Abidjan using fingerlings of *H. longifilis* with varying levels of dietary lipids (5 to 20%), we found that that a diet containing 14% fat and 35% protein improved growth performance of juveniles of *H. longifilis* and promoted protein-sparing effect of lipids (Submitted). More also, most studies reported that the protein requirements of catfish ranged from 35 to 45% (Fagbenro et al., 1992; Eyo, 1995; Olufeagba, 1999; Jamabo and Ockiya, 2008). Hence, it is important to define an optimal protein level in diet containing palm oil for *H. longifilis* fingerlings.

The aim of the present study was to investigate the effects of varying protein levels on growth and survival of *H. longifilis* fingerlings.

MATERIALS AND METHODS

Fish and rearing conditions

Catfishes (*H. longifilis*) were bred at the hatchery of the Oceanological Research Center (ORC) in Abidjan using the method described by Slembrouck and Legendre (1988). After resorption of the yolk sac, larvae were fed with *Artemia salina nauplii* for two weeks, then with a diet containing 35% protein from the Aquaculture Station of Layo before the beginning of the experiment.

Fingerlings (0.76 ± 0.08 g) were collected from the fish hatchery and acclimated to the experimental conditions for two weeks. During this period, fish were maintained on the diet previously used in the hatchery. Six hundred fishes were randomly distributed into 15 tanks at the rate of 40 fishes per tank and 3 replicate tanks were assigned to each of 5 dietary treatments. Glass tanks (50 L containing 45 L tap water that has undergone a natural dechlorination of 24 h before adding fish) were in a closed water system. An electric motor pump ensured a constant flow (1.5 L/min) of well-aerated water. Water was filtered by setting at 30% daily exchange. It was totally replaced weekly. Every day at 08:00 before feeding, temperature (29.06 to 29.45°C) and dissolved oxygen (7.60 to 8.11 mg/l) were measured with an oxymeter coupled with a thermal probe (CRISON Oxi 330). The pH (6.90 to 7.03) was also measured with a pH meter (WTW 330).

Experimental diets and methods

Five experimental diets containing 14% lipid and different levels of protein ranging from 25 to 45% DM were prepared using a pellet press with a 2 mm diameter (Table 2). The protein in the diet was supplied by fish and soybean meal. Corn and wheat bran were used as a source of carbohydrate to make up the energy to the same level and also to have total ingredients up to 100 during formulation. Crude palm oil was used as lipid source. The cassava starch was used like binder (Slembrouck et al., 1991). The composition of these raw materials is reported in Table 1. The fish meal was obtained from the tuna processing company, REAL (Research and Expansion of the Animal feeds).The soybean and the other ingredients were obtained from the usual suppliers of ORC.

The diets were dried at 37°C and stored at 20°C throughout the experimental. The fish were fed till apparent satiation in a completely randomized design, twice daily for 56 days. To quantify the exact feed intake, refused feed was siphoned out immediately, dried, and weighed. Body weights of fish from each tank were recorded weekly and tanks were cleaned. Dead fish were removed from culture tanks per day. The experiment was conducted under artificial light with a dark light cycle ratio of 12:12 h. At the beginning of the experiment, an initial sample of 40 fishes were taken and kept frozen (-20°C) for subsequent whole-body proximate analysis. At the end of the experiment, the weight and the length of each fish were recorded. All fishes were killed and samples from a single tank were immediately frozen at -20°C for further determination of whole body composition.

Proximate compositions of diets and fish were determined as followed: Dry matter after drying at 105°C for 24 h, fat by petroleum ether extraction (Soxtherm, Gerhardt, Germany), protein content (N × 6.25) by the Kjeldahl method after acid digestion, ash by combustion at 550°C in a muffle furnace to a constant weight; and crude fibre by acid/alkali digestion. Gross energy contents were calculated according to 38.9 $KJ.g^{-1}$ for crude fats, 22.2 $KJ.g^{-1}$ for crude protein and 17.2 $KJ.g^{-1}$ for carbohydrate (NFE) (Luquet and Moreau, 1989).

Biological evaluation

Daily weight gain (DWG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), protein gain (PG), lipid gain (LG), nitrogen loss (NL), gross energy (GE) and nitrogen metabolism (NM) were calculated as follows:

- $DWG = (W_2 - W_1) / t$

- $SGR = 100 (LnW2 - LnW1) / t$; where W_1 is the Initial body weight, W_2, the Final body weight and t the duration of the experiment.

- FCR = Dry feed consumed / Wet weight

- PER = Wet weight gain / Protein consumed.

- Protein gain or Lipid gain = Final carcass nutrient content - Initial carcass nutrient.

- GE = 22.2 x protein content + 38.9 x lipid content + 17.2 x carbohydrate content, (Luquet and Moreau, 1989)

- NL= Nitrogen intake - Nitrogen gain

Table 2. Formulation and composition of the experimental diets (% dry weight).

Ingredient (g/100g)	Diet (%)				
	25	30	35	40	45
Fish meal	07.00	15.00	23.00	35.00	45.00
Soybean meal	35.00	35.00	35.00	35.00	35.00
Wheat bran	05.00	16.00	18.50	10.00	4.00
Corn meal	39.00	20.00	09.50	06.00	2.00
Cassava starch	1.00	1.00	1.00	1.00	1.00
Crude palm oil	9.00	9.00	9.00	9.00	9.00
Mineral mixture[1]	1.50	1.50	1.50	1.50	1.50
Vitamin mixture[2]	1.50	1.50	1.50	1.50	1.50
Phosphate dicalcium	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Proximate analysis					
Dry matter (DM) (%)	93.45	94.48	95.56	95.46	94.59
Crude protein (% DM)	25.35	30.12	35.25	39.95	44.81
Total fat (% DM)	13.72	13.70	13.79	13.84	14.00
Ash (% DM)	4,61	6.39	7.98	9.42	11.41
Crude fiber (% DM)	3,02	4.10	3.88	2.94	2.19
Nitrogen free extract (NFE) (% DM)	46.75	40.18	35.15	39.31	22.18
Digestible energy (ED) (kJg^{-1})[3]	15.22	15.60	15.87	16.12	16.32
[4]P/E (mg protein kJ^{-1} ED)	16.65	19.30	22.21	24.78	27.45

[1]Composition for 1 kg of premix: vitamin A, 1.760000 IU; vitamin D3, 880000 IU; vitamin E, 22.000 mg; vitamin B1, 4400 mg; vitamin B2, 5280 mg; vitamin B6, 4400 mg; vitamin B, 1236 mg; vitamin C, 151 000 mg; Vitamin K, 4400 mg; vitamin PP, 35 200 mg; folic acid, 880 mg; choline chloride, 220 000 mg; pantothenic acid, D-14 080 mg. [2]Composition for 1 kg of premix: cobalt 20 mg, iron 17 600 mg, iodine 2000 mg, copper 1600 mg, zinc 60.000 mg, manganese10000mg, selenium 40 mg. Nitrogen-free extract (NFE) = 100 - (% protein + % lipid + % moisture + % ash + % fiber) Digestible energy (ED) = 18.8 × protein content + 37.7 × lipid content + 11.3 × carbohydrate content. [3]Smith, 1971; Page and Andrew, 1973. [4]P/E: Protein/Energy ratio.

- Nitrogen metabolism = [(0.54) (b - a) h] / 2; where, *a* is the initial weight; *b* is the final weight; *h* is the experimental period (days) and 0.54 is the experimental constant (Jamabo and Alfred-Ockiya, 2008).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using statistical software for Windows (release 7.1). Percentage data were arc-sine transformed prior to ANOVA (Zar, 1984). Treatment means were compared by one-way analysis of variance followed by Duncan's test (0.05) (Duncan, 1955).

RESULTS

Data on growth performance are reported in Table 3. At the end of 56 days of feeding, the survival rate ranged from 75 to 80%. Final body weight (FBW) and specific growth rate (SGR) ranged from 3.93 to 5.56 g and 2.92 to 3.55% day^{-1}, respectively with the highest values in fish fed with 40% protein. Daily feed intake and feed conversion ratio (FCR) were highest in fish fed with 25% protein. In addition, protein efficiency ratio (PER) varied from 1.46 to 1.91; PER of fish fed with 45% protein was significantly different from those of fish fed with 25, 30, 35 and 40% protein ($p < 0.05$). Nutrients utilization parameters are reported in Table 4. Daily protein gain (PG) and lipid gain (LG) ranged from 2.88 to 3.10 g $kg^{-1}day^{-1}$ and 0.93 to 1.46 g $kg^{-1}day^{-1}$, respectively; these values were affected by dietary treatments. Although, the highest nitrogen metabolism level was found in fish fed with the diet containing 40% protein, there was not a direct relationship between nitrogen metabolism level and change in dietary protein. However, nitrogenous losses varied from 1.64 to 2.33 g $kg^{-1}day^{-1}$, with the significantly highest value ($p < 0.05$) being observed in fish fed with 45% protein.

Furthermore, no significant differences were observed in the amount of protein needed to produce 1 kg of fish with diets containing 25, 30, 35 and 40% protein. In contrast, the amount of lipids needed to produce the same amount of fish decreased significantly with increasing dietary protein from 25 to 40%. At the end of the experimental period, the whole-body moisture, gross energy and ash content of fish did not differ significantly between the experimental treatments (Table 5). However, the whole-body protein and lipid content were significantly affected by the dietary protein level ($p > 0.05$).

Table 3. Growth performances of *Heterobranchus longifilis* fed with different levels of protein for 56 days.

Parameter	Diet (%)				
	25	30	35	40	45
IBW (g)	0.76 ± 0.08^{a}	0.76 ± 0.08^{a}	0.76 ± 0.08^{a}	0.76 ± 0.08^{a}	0.76 ± 0.08^{a}
FBW (g)	3.93 ± 0.53^{a}	4.76 ± 0.38^{b}	4.76 ± 0.25^{bc}	5.56 ± 0.45^{c}	5.05 ± 0.21^{bc}
DWG (g d^{-1})	0.07 ± 0.01^{a}	0.09 ± 0.01^{b}	0.09 ± 0.00^{b}	0.10 ± 0.01^{c}	0.09 ± 0.00^{bc}
SGR (% d^{-1})	2.92 ± 0.23^{a}	3.27 ± 0.14^{b}	3.27 ± 0.09^{b}	3.55 ± 0.15^{b}	3.38 ± 0.07^{b}
Daily feed intake (g $fish^{-1}$ day^{-1})	0.13 ± 0.00^{b}	0.13 ± 0.01^{ab}	0.12 ± 0.00^{a}	0.12 ± 0.01^{ab}	0.12 ± 0.00^{a}
FCR	2.25 ± 0.25^{c}	1.74 ± 0.05^{b}	1.54 ± 0.17^{ab}	1.33 ± 0.08^{a}	1.54 ± 0.12^{ab}
PER	1.76 ± 0.20^{b}	1.91 ± 0.05^{b}	1.86 ± 0.22^{b}	1.90 ± 0.11^{b}	1.46 ± 0.12^{a}
Survival	74.67±4.62^{ab}	72.00 ±4.00^{a}	81.33 ±2.31^{b}	78.67 ±4.62^{ab}	80.67 ±2.08^{b}

Values are means ± SD (n = 3). Means in the same row having different superscripts are significantly different (P < 0.05), while values in the same row with same superscript are not significantly different (P > 0.05). IBW, Initial body weight; FBW, final body weight.

Table 4. Nutrient utilization of *Heterobranchus longifilis* fed with different levels protein for 56 days.

Parameter	Diet (%)				
	25	30	35	40	45
PG (g $kg^{-1}day^{-1}$)	2.88 ± 0.20^{ab}	2.75 ±0.31^{ab}	2.77 ±0.08^{a}	3.07±0.13^{b}	3.10 ±0.17^{b}
LG (g $kg^{-1}day^{-1}$)	1.20 ±0.03^{b}	1.46 ±0.14^{c}	1.16 ±0.02^{b}	0.93±0.01^{a}	1.28 ±0.08^{b}
NL (g $kg^{-1}day^{-1}$)	1.64 ±0.18^{a}	1.64 ±0.14^{a}	1.75 ±0.21^{a}	1.74 ±0.1^{a}	2.33 ±0.17^{b}
Protein intake (g) / 1 kg of fish	623.71± 76.91^{a}	569.42 ±5.10^{a}	585.01±59.94^{a}	562.02 ±36.18^{a}	734.63 ±53.14^{b}
Lipid intake (g) / 1 kg of fish	337.86 ±41.75^{c}	259.73 ±2.29^{b}	228.78 ±23.44^{ab}	193.83 ±12.48^{a}	275.36 ±19.92^{c}
Nitrogen metabolism	47.97 ±7.971^{a}	65.55 ±5.82^{b}	65.52±5.82^{bc}	72.62±6.75^{c}	64.89±3.19^{bc}

Values are means ± SD (n = 3). Means in the same row having different superscripts are significantly different (P < 0.05), while values in the same row with same superscript are not significantly different (P > 0.05).

Table 5. Body composition of *Heterobranchus longifilis* fed with different levels of proteins (on% fresh basis)[1].

Parameter	Diet (%)				
	25	30	35	40	45
Moisture (%)	76.87 ± 0.75^{a}	77.43 ± 2.77^{a}	78.03 ± 0.23^{a}	77.47 ± 1.08^{a}	77.09 ± 0.98^{a}
Crude protein	13.01 ± 0.43^{b}	11.86 ± 1.46^{ab}	11.66 ± 0.14^{a}	12.15 ± 0.58^{ab}	12.57 ±0.59^{ab}
Total fat	4.87 ± 0.19^{b}	5.58± 0.65^{c}	4.45± 0.05^{b}	3.51 ± 0.02^{a}	4.76 ± 0.30^{b}
Ash	2.75 ± 0.25^{a}	2.84 ± 0.54^{a}	3.04± 0.28^{a}	2.86 ± 0.34^{a}	3.07 ± 0.24^{a}
Gross energy ($KJ.g^{-1}$)	6.14 ± 0.20^{a}	5.85 ± 0.71^{a}	5.52 ± 0.06^{a}	5.52 ± 0.13^{a}	5.95 ± 0.29^{a}

Values are means ± SD (n=3). Means in the same row having different superscripts are significantly different (P < 0.05) and values in the same row with same superscript are not significantly different (P > 0.05). [1]Composition of fish slaughtered at the beginning of the experiment (moisture, 82.71%; crude protein, 10.95%; fat, 2.14%; ash, 3.16% and gross energy, 3.26 $KJ.g^{-1}$).

DISCUSSION

Under the conditions of this study, the survival rate was at least 70% in all fish groups and high (80%) in groups of fish fed with diets containing 35 and 45% protein. However, this value is less than 90% observed by Babalola and Apata (2006) for *H. longifilis* (0.83 g of weight) fed with different dietary protein level (25 to 40%) and fat level (7 to 18%) from animal sources. This difference may be related to the lipid source and experimental conditions. These aforementioned authors used cod liver oil (animal oil), while palm oil (vegetal oil) has been used in this study. Moreover, the physicochemical variables measured in this experiment are within the recommended ranges for fish culture (Viveen et al., 1985; Boyd, 1990). The mortalities can be attributed to handling during control periods as observed by Davies et al. (2006).

This study shows that the growth performance of *H. longifilis* was influenced by dietary protein levels as observed in juveniles and larvae respectively by Kerdchuen (1992) and Babalola and Apata (2006).

Increasing the protein content gradually improves fish growth. Under the experimental conditions, fish fed with diet containing 40% protein had high growth with low food intake and feed conversion ratio of 1.33. This indicated the capacity of this species to accept and utilize compounded diet as reported by Jamabo and Alfred-Ockiya (2008). The feed conversion ratio of 1.3 obtained in our study was close to that observed (1.28) in *Clarias gariepinus* of 6.37 g weight fed with 40% protein and 13.76% fat (palm oil) (Sotolu, 2010). Jamabo and Alfred-Ockiya (2008) reported that the growth rate and weight gain increased progressively with dietary protein level to a maximum at 40% in *H. bidorsalis*. Jana et al. (2006) also reported high growth in terms of live weight gain and specific growth rate in milkfish (*Chanos chanos*) fry fed at 40% protein level. For juveniles of *H. longifilis* (15 g), the optimal growth was obtained when the protein content range from 33.5 to 60.7% (Kerdchuen, 1992), while Degani et al. (1989) showed that among the tested diets (23 to 40% protein) in *Clarias gariepinus* (10 to 12 g), the high growth rate was obtained when the diet contained 40% protein. In *Heteropneutres fossilis,* fingerlings of 0.8 g weight, the optimal growth was observed when the diets contained 27.7 to 35.4% protein (Akand et al., 1989). In addition, Alatise et al. (2005) reported that catfish fingerlings (8.34 g) fed with 40% protein gave the best growth (SGR: 1.06% day^{-1} and FCR: 0.62). Interspecific differences noted, however, can partly be explained by the variety of methodologies used (feed formulation and feeding rate tests).

Fish meal is a highly digestible protein source, containing all the essential amino acids. The quality and quantity of fish meal corresponding to the fish needs can be reached at 40% for a best utilization. The fish fed with 40% protein need low food intake to satisfy the growth as indicated by a low feed conversion ration. In spite of the high food intake and feed conversion ratio recorded in fish fed with 25% protein, growth performances (DWG, SGR) remained low. This suggested that for *H. longifilis* (0.70 g), 25% protein is too limited to ensure good growth. Moreover, for the fish fed with 45% protein, nitrogen metabolism and PER decreased showing the bad utilization of dietary protein. The decrease of growth beyond 40% can be due to the fact that the fish cannot use all of the available protein (Jamabo and Alfred-Ockiya, 2008). Akegbejo-Samsons (1999) reported that excess protein could reduce growth performance due to energy requirement for metabolism, rather than for protein deposition.

The ability to use proteins for growth is the same for fish fed with 25, 30, 35 and 40% protein according to the results of protein efficiency ratio, nitrogenous losses and the amount of protein to produce 1 kg of fish. *H.* longifilis preferably uses proteins for growth, but this capacity depends on the availability of dietary proteins. When the dietary proteins are less available, the lipids which seem to support the growth are misused, resulting in fat depositions in the fish; whereas, with 40% protein, lipids are well metabolized and the fishes are not fat. Also, high protein rate (45%) combined with a bad utilization of lipids may lead to the production of fatty fish.The energy value of lipids being higher than that of proteins could support this similarity of the body crude energy between the various batches of fish. The present study therefore concluded that for *H. longifilis* fingerlings, a good growth was obtained with diet containing 40% protein.

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