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Full Length Research Paper

Effects of dietary protein level on growth and body composition of mudfish, *Heterobranchus longifilis* fingerlings

Reginald I. Keremah* and John F. Alfred-Ockiya

Department of Fisheries and Livestock Production Technology, Niger Delta University, Wilberforce Island, P.M.B 071, Yenagoa, Bayelsa State, Nigeria.

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Heterobranchus longifilis **fingerlings of mean weight 1.648 g were stocked in plastic aquaria of 0.049 m^3 at a rate of 10 fish per aquarium. Fish were fed with diets containing 30, 35 and 40% protein in triplicate for 10 weeks using fish meal as the main protein source. Growth of *H. longifilis* was significantly different (P < 0.05) among treatments with increasing dietary protein levels. Mean weight gain, length increase, relative growth, specific growth rates, survival and feed conversion ratio for fish fed 40% protein diet were 6.99 ± 1.70 g, 43.58 ± 7.64 mm, 469.26 ± 114.28%, 4.05 ± 0.19% day^{-1}, 100% and 1.12 ± 0.06, respectively. Protein efficiency ratio was also significantly higher (P < 0.05) for fish fed diet containing 40% protein compared to fish fed 30 and 35% protein. Percent protein in carcass of fish (22.92%) was significantly higher (P < 0.05) and the fat level was fairly similar with all diets fed. Results of this study indicate that *H. longifilis* fingerlings had the best growth when fed with a 40% dietary protein.**

Key words: *Heterobranchus longifilis,* fingerlings, dietary protein, growth, body composition.

INTRODUCTION

Heterobranchus longifilis, a freshwater Clariid catfish is an indigenous species of importance in fish culture in Nigeria (Eyo, 1999). This fish adapts readily to pond conditions, is acceptable, has high conversion rate of artificial feeds, tolerates crowded conditions and grows fast (Alatise et al., 2005). Despite the rearing interest by culturists and its good culture characteristics, there are few data in the feeding and nutrition of *H. longifilis*. An essential consideration to enhance fish yield is protein concentration in the feed necessary to obtain satisfactory growth and conversion values for *H. longifilis.* Protein is essential in the diet of fishes as a source of amino acids, which are the building blocks for flesh, enzymes, eggs, milt, antibodies, some hormones (Dupree and Huner, 1984), tissue repair and growth (Maynard et al., 1979). A liberal source must be available to fish in its diet throughout life. The weight gain of fish is essentially linear with protein content in the feed and directly proportional to the protein content of the diet at a range of 20 to 40% (Dupree and Huner, 1984). Carnivorous fish have higher protein requirements in their diets than non-carnivorous species in the range of 35 - 70% (Tacon and Cowey, 1985). Inadequate protein in the diet for fish results in rapid reduction, cessation of growth or a loss of weight as protein could be withdrawn from tissues with lesser needs to maintain other vital functions (Wilson, 1984).

Protein is considered to be the most expensive component of a diet and knowledge about protein requirement of *H. longifilis* will be essential for the formulation of nutritious, economical diets (Lazo et al., 1998). Therefore, the present study was conducted to determine the effect of varying protein levels on the growth,

*Corresponding author. E-mail: regreggie5@5yahoo.com. Tel: +2348055311660.

Abbreviations: NFE, Nitrogen free extract; DO, dissolved oxygen; RGR, relative growth rate; CP, crude protein; PER, protein efficiency ratio; ANPU, apparent net protein utilization.

Table 1. Formulation (% composition) and proximate analysis of experimental diets fed to *Heterobranchus longifilis* fingerlings.

Ingredient	Diet		
	1 (30% CP)	2 (35% CP)	3 (40% CP)
Tilapia meal (64.25% protein)	35.22	44.53	53.84
Corn (10.55% protein)	43.18	33.87	24.56
Wheat bran (18.54% protein)	15.00	15.00	15.00
Vitamin and mineral premix[a]	0.50	0.50	0.50
Ascorbic acid[b]	0.10	0.10	0.10
Table salt	0.20	0.20	0.20
Red palm oil	2.80	2.80	2.80
Pellet binder (garri:cassava product)	3.00	3.00	3.00
Total	100.0	100.0	100.0
Proximate composition of diets (%)			
Moisture	5.57	5.73	5.53
Crude protein	30.38	35.25	40.50
Lipid	11.49	9.42	12.94
Crude fibre	6.25	5.86	5.21
Ash	13.34	14.25	13.07
Nitrogen free extract	32.97	29.49	22.75

[a]Optimix vitamin-mineral premix: Vitamins A,D_3, K, B_1, B_2, B_6, B_{12}, niacin, pantothenic acid, folic acid, biotin, choline chloride, antioxidant, manganese, zinc, iron, copper, iodine, selenium and cobalt. Produced by Animal Care® for Animal Services Konsult (Nig.) Ltd. Agege, Lagos. [b]Supplied by L-ascorbyl-2- phosphate (25% activity).

growth, survival and body composition of this important food fish.

MATERIALS AND METHODS

A feeding experiment was carried out between December, 2001 and November, 2002 at the Department of Fisheries of the Rivers State University of Science and Technology, Port Harcourt, Nigeria. Prepared diets containing different protein levels were produced and used in this study.

Experimental diets

Details of composition of diets are in Table 1. The ingredients were weighed out and hand mixed thoroughly in a basin and blended with 10% water to moisten the mixture. A measured quantity of garri (cassava derivative) was treated with hot water to form a paste to blend the ingredients. The dough formed was then extruded through a meat mince machine with a 2 mm diameter to produce strands of feed. The feed was then dried over a heated aluminum sheet for 30 min. The pellets were deliberately broken into smaller bits, forming crumbs. Three diets at 30% (Diet 1), 35% (Diet 2) and 40% (Diet 3) protein levels were produced by this method.

The dried and ground crumbs of experimental diets were analyzed for moisture, crude protein, crude fat (either extract), crude fiber and ash contents according to AOAC (1990). The nitrogen free extract (NFE) was determined by difference as shown below

NFE = 100 - (% protein + % fat + % fiber + % ash + % moisture)

Fish and experimental conditions

Ten *H. longifilis* fingerlings of mean weight 1.648 g and 49.00 mm mean total length were randomly stocked into each plastic aquarium of 46 x 38 x 28 cm^3 in triplicates per diet. Fish were fed daily with crumbs of prepared diet at 5% of fish body weight. Daily rations were divided into two halves and fed to fish at 0800 - 0830 h and 1600 - 1630 h local time. Fish were weighed at the start of experiment and bi-weekly intervals in each aquarium. The amount of diet was also adjusted from recorded weights of fish. Feeding lasted for 10 weeks.

Dissolved oxygen (DO), pH, NH_3-N, NO_2-N and total hardness were measured bi-weekly. Water temperature was measured daily using a laboratory mercury thermometer (0 - 100°C). The DO was determined by Winkler's method, un-ionized NH_3 by Nesslerization and water hardness was monitored titrimetrically. Nitrite was measured by steam distillation method (Boyd, 1979). The pH of water was measured using a portable electronic pH meter (Model Jenway 3150).

Harvest data

Ten (10) fishes were randomly sampled for each diet for carcass analysis. Whole fish (wet) were homogenized in a blender and analyzed for protein, lipid and moisture contents. The protein was determined with macro- kjeldahl, lipid was by ether extraction and moisture by drying 2 g of feed sample in an oven (100°C) until constant weight was achieved (AOAC, 1990). Body weight gain was calculated as the difference between the initial (W_1) and final weight (W_2) values for fish (Okoye et al., 2001). Length increase, L_2 - L_1, where L_2 and L_1 are final and initial body lengths of fish.

Table 2. Mean and range values of water quality parameters for *Heterobranchus longifilis* fingerlings fed diets containing varying protein level.

Parameter	Experimental diet		
	1 (30% CP)	2 (35% CP)	3 (40% CP)
pH	7.5 (7.2 - 7.8)	7.3 (6.9 - 7.4)	7.5 (7.1 - 7.6)
Temperature (°C)	28.0 (27.5 - 29.0)	27.5 (27.1 - 28.0)	27.5 (26.9 - 29.0)
DO (mg/l)	3.5 (3.0 - 4.2)	5.5 (5.52 - 5.7)	4.5 (4.3 - 5.0)
NH_3-N (mg/l)	0.07 (0.06 - 0.08)	0.07 (0.06 - 0.09)	0.05 (0.04 - 0.08)
NO_2-N(mg/l)	0.36 (0.31 - 0.42)	0.45 (0.40 - 0.49)	0.45 (0.42 - 0.50)
Total hardness (mg/l)	30.05 (29.5 - 31.0)	20.70 (19.50 - 21.0)	40.30(38.50 - 43.0)

Figures in brackets are range values of parameters.

Relative growth rate (RGR) = W_t - W_o x 100/W_o (Orisamuko, 2006), W_t as final fish weight (g) at end of experiment and W_o, fish weight (g) at start of experiment. The specific growth rate (SGR), % day^{-1} = l_n W_2 - l_n W_1 x 100/t_2-t_1 (Brown, 1957); where, W_2 = final weight of fish (g), W_1 = initial weight of fish (g), t_2 and t_1 = end of growth period and at time 0 in days, l_n = natural logarithm. Fulton's condition factor (K) = 100w/l^3 (Bagenal and Tesch, 1978) where w and l are the observed total weight (g) and total length (cm) of a fish. Survival of fish (S) = N_i x 100/N_o (Alatise and Otubusin, 2006), N_i = number of fish alive at end of experiment and N_o = number of fish at beginning of experiment. The feed conversion ration (FCR) was calculated as feed intake (g) /weight gain (g) (Utne, 1979); protein efficiency ratio (PER) as wet weight gain of fish (g)/ weight of protein fed (g) (Zeitoun et al., 1973) and apparent net protein utilization (ANPU) = [(P_b - P_a)/ P_1] x 100 (Miller and Bender, 1955 cited by Adikwu, 2003), where P_b = total body protein at end of feeding trial, P_a = total body protein at beginning of feeding trial and P_i = amount of protein consumed over duration of feeding trial.

Data analysis

Data were analyzed using the Statistical Analysis System (SAS) Analysis of Variance (ANOVA) procedure (2003) for significance. Differences between means were determined by Duncan's multiple range test (Duncan, 1955).

RESULTS

Water quality

The mean pH values varied between 7.3 - 7.5 for all diets. Mean water temperature was 28°C (range, 27.5 - 29.0°C) for diet 1 and 27.5°C for diets 2 (range, 27.1 - 28.0°C) and 3 (range, 26.9 to 29.0°C), respectively. Dissolved oxygen level averaged 3.5 mg/l with a range of 3.0 - 4.2 mg/l for diet 1. Highest DO values were obtained with diet 2 (range, 5.2 to 5.7 mg/l). Total NH_3-N averaged 0.07 mg/l for diets 1 and 2, 0.45 mg/l, NO_2-N for diets 2 and 3 while total hardness values were 30.05 mg/l (diet l), 20.70 mg/l (diet 2) and 40.30 mg/l (diet 3), respectively. Values observed for all the water quality parameters fell within acceptable limits for the well-being of warm water fishes including *H. longifilis* investigated upon in this study (Table 2).

Survival

Table 3 shows survival values which varied and increased with dietary protein as 97.0 ± 0.81% (Diet 1), 96.0 ± 0.66 (Diet 2) and 100% (Diet 3). The experimental fish showed excellent adaptability to the aquaria used. Fish remained active from the time of stocking to the end of experiment.

Growth

The mean weight gains of fish were 2.05 ± 0.56 g (Diet 1), 2.88 ± 0.71 g (Diet 2) and 6.99 ± 1.70 g (Diet 3). Despite the initial size variations between the three groups of fish fed with the different protein levels, both the percent body weight gain (range, 65.92 - 82.43%) and length increase (range, 33.72 - 44.16%) showed a similar trend of increase from Diet 1 (30% CP) - diet 3 (40% CP) as in mean weight gain. Progressive increase in performance was also observed in RGR with Diet 3 as best (469.26 ± 114.28%). The SGR values were 2.14 ± 0.20% day^{-1} (diet 1), 2.92 ± 0.06% day^{-1} (diet 2) and 4.05 ± 0.19% day^{-1} (diet 3). However, the condition factor ranged from 0.72 ± 0.01 (Diet 1) - 0.77 ± 0.02 in diet 3 (Table 3).

Feed utilization

Feed conversion ratio was different among test diets fed to *H. longifilis* fingerlings. Feed utilization increased with dietary protein level, indicating the best FCR of 1.12 ± 0.06 with 40% CP diet and least with 2.64 ± 0.74 for the 30% CP diet. Protein efficiency ratio increased with protein levels from 1.61 ± 0.15 (30% CP diet) - 2.28 ± 0.12 (40% CP diet). The ANPU values varied between 1.65 - 1.91 among diets (Table 3).

Body composition

Protein content of the carcass was higher in fish fed diet

Table 3. Performance of *Heterobranchus longifilis* fingerlings fed diets containing different protein levels (±SEM[1]).

Parameter	Diet		
	1 (30% CP)	2 (35% CP)	3 (40% CP)
Sample size per aquarium	10	10	10
Initial weight of fish (g)	3.11 ± 0.56[b]	3.98 ± 0.71[b]	8.48 ± 1.70[a]
Weight gain (g)	2.05 ± 0.56[b]	2.88 ± 0.71[b]	6.99 ± 1.70[a]
Percent weight gain (%)	65.92	72.36	82.43
Total length of fish (mm)	73.07 ± 4.99[c]	79.33 ± 5.98[b]	98.69 ± 7.64[a]
Length increase (mm)	24.64 ± 4.99[c]	30.20 ± 5.98[b]	43.58 ± 7.64[a]
Percent length increase (%)	33.72	38.07	44.16
Relative growth rate (%)	193.49 ± 53.01[c]	265.50 ± 65.84[b]	469.26 ± 114.28[a]
Specific growth rate (%d^{-1})	2.14 ± 0.20[b]	2.92 ± 0.06[b]	4.05 ± 0.19[a]
Condition factor (K)	0.72 ± 0.01	0.74 ± 0.02	0.77 ± 0.02
Percent survival (%)	97.0 ± 0.81[b]	96.0 ± 0.66[b]	100.0 ± 0[a]
Feed conversion ratio	2.64 ± 0.74[a]	1.56 ± 0.04[b]	1.12±0.06[b]
Protein efficiency ratio	1.61 ± 0.15[b]	1.84 ± 0.05[b]	2.28±0.12[a]
Apparent net protein utilization	1.78	1.65	1.91

[1]SEM: Standard error of mean. Means with same letter for a given parameter in same horizontal row are not significantly different (P > 0.05).

Table 4. Proximate body composition (% wet weight) of *Heterobranchus longifilis* fingerlings at start and after feeding graded protein levels.

Experimental diet	Sample size	Moisture (%)	Crude protein (%)	Ether extract (%)	Ash (%)
Fish at start of experiment	10	78.15[a]	15.14[c]	1.15[b]	3.26[a]
Diet 1 (30% CP)	10	71.25[b]	17.71[b]	2.54[a]	3.45[a]
Diet 2 (35% CP)	10	72.58[b]	18.83[b]	2.72[a]	3.06[a]
Diet 3 (40% CP)	10	70.59[c]	22.92[a]	2.18[a]	2.95[a]

Values within the same vertical row with same superscripts are not significantly different (P > 0.05).

3 (22.92%) compared to diets 1 (17.71%) and 2 (18.83%). Fat content values were 2.54% (Diet 1), 2.72% (diet 2) and 2.18% (diet 3). Moisture level in fish fed the different diets (range, 70.59 to 72.58%) were similar but lower than fish at the start of experiment (78.15%) (Table 4).

DISCUSSION

The general trend of increase in growth and nutrient utilization increased with protein level. Diet containing 40% CP produced the best growth when compared to the performance of fish fed lower levels of dietary protein of 30% or 35%. Hence the protein requirement of pure *H. longifilis* fingerlings for optimal growth in this study could be put at 40%. This requirement is similar to that reported for pure *H. longifilis* (Eyo, 1995), *Clarias anguillaris* (Madu and Olurebi, 1987) and *Clarias gariepinus* (Degani et al., 1989).

The dietary protein concentration tested for *H. longifilis* fingerlings in this study did not exceed 40% level. It would have been of interest to observe the effect of a continuous increase of dietary protein above 40% level. Cowey et al. (1972) however reported a decline of PER with further increase in dietary protein beyond the 40% concentration for Plaices and Coho Salmon. Dabrowski (1977) and Falaye and Arogunjo (1989) observed a general decrease of ANPU with increasing dietary protein in grass carp and *Oreochromis niloticus.* These results corroborate the observations made on ANPU in *H. longifilis* fingerlings in this study. Fish meal which is an important ingredient and protein source in the compounded feeds for this experiment is of a high protein quality and palatability (Webster et al., 2000).

It is probable that the amino acid profile of fishmeal and the proximate composition of the diet containing 40% CP was able to meet the nutritional needs of *H. longifilis* fingerlings. This equally influenced the observed greater growth and food utilization of these fingerlings in a conducive culture environment.

The proximate carcass composition of *H. longifilis* fingerlings showed relative differences in growth between the initial fish sample and those under treatments. This suggests that fish growth in this study was also associated with synthesis of tissue protein (Ipinjolu and Faturoti, 1999). Fish fed diet containing 40% had higher protein content in the carcass than fish fed lower dietary protein level. This showed that increase in dietary protein resulted in elevated levels of body protein. This observation could be attributed to the quantity of protein consumed. The authors therefore suggest that feeds for the life stages of mudfish should contain adequate protein content of high quality to enhance good growth and survival.

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