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Growth Performance, Feed Efficiency and Body Composition of Larvae *H. longifilis* Fed Different Beef Brain Meal Dietary Protein Levels

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Abstract: ***The effects of increasing beef brain dietary protein level on growth, nutrient utilization; and biochemical composition of larvae Heterobranchus longifilis were evaluated. Three diets in 25%, 30% and 35% of crude beef brain protein were formulated, to fed triplicate groups of 50 larvae (initial weight 0.0035 g) per aquarium at ad libitum during 49 days. At the end of the experiment, the growth parameters such as final body weight (FBW), final body length (FBL), specific growth rate (SGR), body weight gain (BWG) increased with increasing dietary protein levels. Significant difference ($p<0.05$) existed between treatments in FBW, FBL and SGR. However, in spite of increasing dietary protein level no significant difference was shown among all treatments in condition factor. The feed conversion ratio (FCR) and the protein efficiency ratio (PER) were better in 35% of dietary crude protein. At the end of the experiment, the larvae protein content increased with increasing dietary protein levels while the larvae lipid content decreased. Body mineral composition in sodium and potassium are significantly higher in larvae fed 25 % and 30 % beef brain meal crude protein level. The highest body values of iron, magnesium and phosphorous were obtained in larvae H. longifilis fed 35 % of dietary beef brain protein. From the present results, diets containing 35% of crude protein improve growth of larvae H. longifilis.***

Keywords: Beef brain meal, growth performance, mineral composition, fatty acid composition.

1. Introduction

African catfish *Heterobranchus longifilis* is the major species used in intensive aquaculture in Ivory Coast because of their rapid growth, ready acceptance of artificial diets [1] and resistance to disease. Furthermore, its culture is very economically in tropical regions of Africa [2] where its flesh is highly valued. Feeding larvae *H. longifilis* necessities high protein because of their high protein requirement (ranges to 32-42.5 %). Fishmeal and *Artemia salina* are generally used as the main protein source in small fish nutrition due to its high protein content, balanced amino acid profile, essential fatty acid content, mineral and vitamins content, palatability and highly digestibility to most fresh water and marine fish [3, 1, 4]. However, these feeds foods are not only very expensive but also usually unavailable particularly in the developing countries [1]. This has made the cost of growing fish over a period time to be very high in developing countries where aquaculture is not sufficiently developed.

Several at tempts have therefore been made to find adequate substitutes for fish meal [5] and *Artemia salina*. Recent studies showed that beef brain can substitute *Artemia salina* in rearing *H. Longifilis* larvae [1, 6, 7]. Because it's rich in protein (35 % of crude protein reported by [1]. Moreover, recent study has reported 32.01 % of crude lipid in diet formulated with beef brain meal [7], which could act as protein sparer, and can be used to maximize protein utilization for growth [8, 9, 10].

In this study, beef brain meals were formulated as feeds at different crude protein levels (25%, 30% and 35%) to identify the optimum level of protein for optimum growth of larvae *H. longifilis* in farming.

2. Materials and Methods

2.1. Experimental diets

Three practical diets on crude protein of 25 %, 30 % and 35 % crude protein were formulated with Beef brain meal (36.5 % of crude protein) as the main protein source. Diets were prepared through combination of practical ingredients in different proportions to obtain the desired final protein levels. Ingredients and chemical composition of diets are shown in Table 1. To prepare the diets, Beef brain meal and the other ingredients such as maiz meal, VMD-Aminovit (premix), lysin, methionin, iron, phosphorous chlorine and palm oil were milled together into fine particulate with hammer machine. The milled ingredients were thoroughly mixed dry then added with warm water to obtain homogeneous paste. The paste obtained was collected in fat trays and was dried in electric oven at 60 °C for 48 hours. The dried paste was crushed into powdery with pestle and mortar to obtained meal, then was hydrated by vapor with a combined mechanism saucepan / sieve (diameter = 200 µm).The hydrated meal was stored in a plastic container at -20 °C until utilization.

2.2. Experimental fish and feeding trial

Larvae of African catfish *H. longifilis*, Valenciennes, 1840 used in this experiment were obtained from induced brood stock reared in Layo Aquaculture station (5°19'N, 4°19'W;

Ivory Cost). 3days-age larvae were transferred in aquaria (38.5 x 46.5 x 28 cm^3), capacity of 50 L and acclimated in aquaria system for 4 days prior to the commencement of the growth trial. Water was supplied to each aquarium from 250 L head tank. Fish in each aquarium were weighted, counted and stored at density of 50 larvae per aquarium (1 larva L^{-1}). Three replicate aquaria were constituted for each diet. During 49 days, fish were fed three times a day (08:00, 12:00 and 17:00 hours) *ad libitum*. A total of 450 larvae were used in this experiment. Three times a week, undigested food particles and waste products were siphoned out with rubber hose before feeding fish. Once a week, 10 larvae were randomly sampled in each aquarium to measure growth (total length and wet weight). Total length was measured to the nearest half millimetre. Wet weight was measured on an electronic digital balance SARTORIUS L 6200 S (accuracy of ± 0.01 mg). Subsequently, all larvae were weighed and ration was adjusted to reflect the new weight. After 49 days of rearing, all survival larvae were collected, weighted, and counted from each aquarium. Individual total length and body wet weight were also recorded. At the end of the experiment, survival fish were collected, counted from each replicate. Then 30 larvae were removed from each replicate to chemical composition determination. After collection, samples were stored at -20 °C until use for chemical analyses.

The growth indices and nutrient utilization parameters were calculated for each treatment as follows: specific growth rate (SGR) (%/day) = ln(final body weight) – ln(initial body weight) x 100/Duration of rearing period, body weight gain (BWG) (g) = final body weight - initial body weight, condition of factor (K) = final body weight (g)/final body $lenght^3$ (cm), survival rate (SR) (%) = (final number of larvae/initial number of larvae) x 100, apparent food conversion ratio (AFCR) = dry feed intake (g)/wet weight gain (g) and protein efficiency ratio (PER) = weight gain (g)/protein intake (g)

2.3. Biochemical analysis

Experimental diets and the whole body fish approximate composition were analyzed using standard methods [11] as follows: moisture content after drying in oven at 105 °C for 24 hours until constant weight, ash by incineration at 550 °C in a muffle furnace for 24 hours, crude proteins (nitrogen x 6.25) by the Kjeldahl method after acid digestion, lipid by hexane extraction in soxhlet system, while nitrogen-free extract (NFE) was calculated by difference. The gross energy contents of the diet and fish were calculated on the basis of their crude protein, total fat and carbohydrate contents using the equivalents of 22.2, 38.9 and 17.15 kjg^{-1}, respectively [12] Experimental diets and fish were analyzed for mineral composition (calcium, potassium, sodium, phosphorus, magnesium, iron, zinc, copper, manganese) using microwave digestion and atomic absorption spectrophotometer (Varian SAA 110) air acetylene flame [11]. Proximate and mineral compositions of the experimental diets are given in Table 1. Fatty acid determination in experimental diets and larvae was performed by gas-liquid chromatography (GLC) based the method [13] to separate liquid extract into methyl esters and then fatty acid methyl esters were quantified and identified by using gas-liquid chromatography (HP 6890, GC SYSTEM) equipped with a flame ionization detector (FID) and a 30 m x 0.25 mm fused silica capillary column. The composition of fatty acids of three best experimental formulated diets is presented in Table 2.

2.4. Statistical Analysis

Data on weight and length were transformed in log(x + 1) to have a normal distribution. The specific growth rate, body weight gain, coefficient of variance, Cannibalism rate, and Survival rate were analyzed using one-way analysis of variance (ANOVA). The Duncan's multiple-range tests were used to compare differences among treatment means. Treatment effects were considered significant at $p < 0.05$. The analyses were performed using Statistica 7.1 software

3. Results and Discussion

3.1 Water Quality

During the 49-days experiment period, water quality parameters values remained within ranges allowing for high growth rate and production for *Heterobranchus longifilis* reported by [14] and [15]. Water temperature was maintained at 28.44 ± 0.02 °C, Dissolved Oxygen 5.94 ± 0.14 mg/L, pH 7.21 ± 0.14, ion ammonium-N ranged from 0.33 to 0.45 mg/L, nitrite-N at 0.63 ± 0.04 mg/L and phosphate-D at 0.18 ± 0.02 mg/L.

Table 1: Ingredients proximate and mineral compositions (dry matter basis) of the experimental diets

	Diets (Protein level, %)		
Parameters	*25*	*30*	*35*
Ingredients (g/100g)			
Beef brain meal	60.06	79.31	89.26
Maiz meal	31.1	10.95	1.00
Palm oil	2.00	2.00	2.00
Lysin	2.13	2.13	2.13
Methionine	1.61	1.61	1.61
Premix[1]	2.00	2.00	2.00
Phosphorus	0.67	0.67	0.67
Iron	0.67	0.67	0.67
Chlorine	0.66	0.66	0.66
Total	*100*	*100*	*100*
Proximate analysis (%)			
Moisture	11.42	12.61	11.53
Crude protein	25.07	30.96	35.02
Total fat	27.42	31.91	35.21
Ash	4.02	4.46	5.48
Crude fiber	0.86	0.44	0.83
Nitrogen free extract	31.21	19.62	11.93
Gross energy	21.77	22.66	23,52
P/E $(g.kJ^{-1})^4$	1.15	1.37	1.49
Cost (CFA kg^{-1})	3018	3737.06	4226.6
Mineral composition			
Calcium	30.10	33.85	35.88
Phosphor	13228.91	13020.5	13805.2
Potassium	118.31	155.1	217.18
Sodium	3058.61	3999.30	4885.98
Magnesium	30.45	30.82	30.11
Iron	6.64	11.63	42.99
Zinc	4.54	6.16	6.07
Manganese	29.55	23.46	26.31
Copper	6.64	11.63	42.99

[1]Composition for 1 kg of premix : Vitamin A = 10000 UI, Methionine = 50.0 mg, Vitamin D3 = 1000 UI, Vitamin E = 10.0 mg, VitaminB1= 2.0 mg, Vitamine B2 = 4.0 mg, pantothenic Calcium = 10.0 mg, Vitamin B6 = 1.5 mg, Vitamin C = 25.0 mg, Vitamin K3 = 1.5 mg, Acide folique = 0.5 mg, Nicotinamide = 20.0 mg, Biotine = 15.0 µg, Lysin HCl = 50.0 mg, Alanin = 12.96 mg, Arginin = 15.6 mg, Aspartic Acid = 27.8 mg, Cystine = 1.9 mg, Glutamic Acid = 85.0 mg, Glycin = 8.0 mg, Histidin = 11.8 mg, Isoleucin = 23.6 mg, Leucin = 35.4 mg, Phenylalanin = 19.0 mg, Prolin = 392 mg, Serin = 24.0 mg, Threonin = 18.6 mg, Tryptophane = 6.4 mg, Valin = 27.4 mg ; [2] Nitrogen-free extract (NFE) = 100 - (% protein + % lipid + % moisture + % ash + % fiber) ; [3] Gross energy = % protein x 22.2 kJ/g + % lipid x 38.9 kJ/g + % Nitrogen-free extract x 17.2 kJ/g 4 ; [4]P/E = Protein to energy ratio in g protein / kJ gross energy

3.2. Growth performance and feed efficiency

Final body weight (FBW), final body length (FBL), specific growth rate (SGR), body weight gain (BWG), condition factor (CF), survival rate (SR), feed conversion ratio (FCR) and protein efficiency ratio (PER) of larvae *H. longifilis* are presented in Table 2. Growth parameter (FBW, FBL, SGR and BWG) increased with the increasing of dietary protein level (to 25 % at 35 % of protein level) in larvae *H. longifilis* fed with beef brain based diets. Contrary, FCR decreased with the increasing dietary protein level to 25 % at 35 %. FCR in the diets containing 35 % (0.67 ± 0.05) protein levels were significantly lower ($p< 0.05$) than in the diets containing 25 % (1.02 ± 0.02) and 30 % (0.90 ± 0.09) protein levels. The highest significant values ($p< 0.05$) of PER (4.87 ± 0.08, 4.98 ± 0.06) was observed for *H. longifilis* larvae fed the diet containing 25 % and 35 % protein level, while the lowest values were recorded for larvae fed diets containing 30 % (4.49 ± 0.07) protein levels. The best survival rate was observed in larvae fed with diet containing 30 % (56.07 ± 1.16) and 35 % (54.66 ± 1.75) protein level. Diet containing 25 % protein level shown lowest survival value (51.33 ± 1.21). Increase of dietary protein level did not affect significantly ($p> 0.05$) condition factor in larvae fed with crude beef brain protein.

Table 2: Growth performance, feed utilization of larvae *H. longifilis* fed with maggot meal and beef brain meal at different dietary protein level

	Dietary protein level		
Parameters	*25*	*30*	*35*
FBW (g)	1.94 ± 0.24^{a}	2.75 ± 0.42^{ab}	3.5 ± 0.34^{b}
FBL (g)	58.32 ± 1.12^{a}	64.70 ± 3.41^{ab}	$70,06 \pm 1,03^{b}$
SGR (%/j)	12.93 ± 0.05^{a}	13.07 ± 0.14^{ab}	13.44 ± 0.19^{b}
BWG (g)	1.94 ± 0.11^{a}	2.74 ± 0.02^{b}	3.49 ± 0.34^{b}
CF	1.08 ± 0.12^{a}	1.42 ± 0.23^{a}	1.02 ± 0.15^{a}
SR (%)	51.33 ± 1.21^{a}	56.07 ± 1.16^{b}	54.66 ± 1.75^{ab}
AFCR	1.02 ± 0.02^{b}	0.90 ± 0.09^{b}	0.67 ± 0.05^{a}
PER	4.87 ± 0.08^{b}	4.49 ± 0.07^{a}	4.98 ± 0.06^{b}

IBW = Initial body weight (g), FBW = Final body weight (g), FBL = Final body length (g), SGR = Specific growth (%/j), BWG = Body weight gain (g), CF = Condition factor. SR = Survival (%), AFCR = Feed conversion ratio, PER = Protein efficiency ratio. Means with different superscript letters within a row are significantly different ($P < 0.05$).

3.3. Proximate composition of larvae *H. longifilis*

Body composition data are shown in table 3. No significant difference in moisture content, crude protein content and gross energy of the larvae *H. longifilis* was observed between the treatments. In contrast, fish ash and lipid content were significantly ($p< 0.05$) affected by the levels of beef brain dietary protein. The lowest ($p< 0.05$) ash content was recorded for the diet containing 25 % protein level while the highest values were recorded for larvae fed diets containing 30 % protein level. Whole lipid content significantly ($p<0.05$) decreased with increasing of dietary protein levels. The highest significant ($p< 0.05$) value of lipid content (3.53 ± 0.17 %) was observed for *H. longifilis* larvae fed the diet containing 25 % protein level, while the lowest values (2.88 ± 0.11 and 2.51 ± 0.18 %) were recorded for larvae fed diet containing 30 % and 35 % protein level (respectively).

Table 3: Body composition of larvae *H. longifilis* fed with beef brain meal at different dietary protein level (wet matter basis)

	Dietary protein level		
Composition (%)	*25*	*30*	*35*
Moisture	78.07 ± 2.57^{a}	77.99 ± 1.22^{a}	76.40 ± 2.72^{a}
Ash	4.67 ± 0.13^{a}	4.95 ± 0.04^{b}	4.69 ± 0.12^{ab}
Crude protein	13.73 ± 0.39^{a}	14.18 ± 0.27^{a}	14.40 ± 0.44^{a}
Crude lipid	3.53 ± 0.17^{b}	2.88 ± 0.11^{a}	2.51 ± 0.18^{a}
Gross energy (KJ/g)	4.42 ± 0.15^{a}	4.27 ± 0.10^{a}	4.17 ± 0.17^{a}

Means with different superscript letters within a row are significantly different ($P < 0.05$).

3.4. Mineral Composition

At the end of feeding trial, the mineral composition of larvae *H. longifilis* fed with experimental diets are shown in Table 4. There are significant differences ($p<0.05$) in sodium (Na), potassium (K), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), magnesium (Mg), Calcium (Ca) and phosphorus (P) between all groups of larvae fed with different crude beef brain protein level. Body potassium decreased while iron and manganese contents in larvae increased significantly as dietary beef brain protein increased (Table 5). The highest body sodium, zinc, calcium and copper values had been noted with larvae *H. longifilis* fed 30 % dietary protein level. However, phosphorus was significantly higher in larvae fed with 35 % dietary crude protein, followed by larvae fed 25 % and 30 % dietary protein level.

Table 4: Body mineral composition of larvae *H. longifilis* fed with beef brain meal at different dietary protein level

	Dietary protein level		
Minerals	*25*	*30*	*35*
Soduim (ppm)	1068.56 ± 25.1^{b}	1255.08 ± 31.9^{c}	515.97 ± 8.1^{a}
Potassium (ppm)	52.07 ± 6.1^{c}	28.94 ± 1.3^{b}	18.97 ± 1.44^{a}
Iron (ppm)	31.62 ± 2.5^{a}	47.36 ± 3.4^{b}	192.61 ± 9.6^{c}
Copper (ppm)	1.15 ± 0.07^{a}	6.99 ± 0.3^{c}	4.78 ± 0.7^{b}
Zinc (ppm)	0.07 ± 0.01^{a}	3.08 ± 0.08^{c}	1.06 ± 0.02^{b}
Manganese (ppm)	2.95 ± 0.06^{a}	7.05 ± 0.17^{b}	10.77 ± 1.21^{c}
Magnesium (ppm)	455.98 ± 9.4^{c}	48.51 ± 0.98^{a}	66.46 ± 1.51^{b}
Calcium (ppm)	46.98 ± 4.22^{a}	112.94 ± 2.88^{b}	43.79 ± 2.4^{a}
Phosphor (ppm)	9005.9 ± 85.7^{b}	6690.5 ± 63.06^{a}	10001.8 ± 118.2^{c}

Means with different superscript letters within a row are significantly different ($P < 0.05$).

4. Discussion

At the end of the experiment, the results on the specific growth rate (12.93-13.44) recorded with the experimental diets largely higher than 3 %/j reported by [16] as good growth values of specific growth rate for most species reflect the best quality and palatability of the three formulated diets. In the present study, the proportion of dietary protein appeared to be an important factor influencing fish growth, feed utilization and body biochemical composition. This observation was in agreement with [17], [18], [2], [19], [20] who observed in catfish *Heterobranchus bidorsalis* and *Chrysichthys walker* increasing growth with the dietary protein level increasing. In fact, because protein is the most essential component in the carnivorous fish diets, dietary protein must be in accordance to the protein requirements for larvae *H.longifilis* to improve growth. Similarly, a diet with inadequate protein content can result in reduced weight gain because the fish cannot eat enough feed to satisfy their nutriments requirements for growth. In this experiment larval growth was better at diet formulated 35 % crude beef brain protein. The best fish growth performance observed from diet formulated 35 % protein level is due to its high crude protein content (35.02 %) ranged to 35-56 % recommended by [8] to fish fry for its good growth. Dietary protein is not only very important nutrient for the growth of fish, but it is used by fish for their energy and body maintenance [21]. More, lipid, ash and gross energy contained in diet formulated 35 % protein level higher than those contained in others diets give it an advantage for good fish growth. The feed conversion ratio (FCR) values were significantly influenced by the dietary protein level. Increasing dietary protein level from 25 % to 35 %, the FCR values decreased significantly from 1.02 to 0.67 lower than the FCR value 1.00 reported by [22] for an improved feed outcome and good nutritional quality. The FCR value 0.67 largely lower than 1.00 reflects the good nutritional quality of the nutriments and the good biological value of dietary protein contained in experimental diet formulated 35 % dietary protein. This explains the good growth performance and feed utilization noted from *H. longifilis* larvae fed at 35 % protein level.

Biochemical analysis of larval body *H. longifils* showed that the increase protein level in beef brain diets not influence fish moisture, protein and gross energy content. But, these values of protein content increase insignificantly from 13.73 ± 0.39 % (25 % dietary beef brain protein) to 14.40 ± 0.44 % (35 % dietary beef brain protein); inversely, the body lipid content decreases significantly with increasing dietary protein level. This relationship was also noted in others studies by [23] on grass carp, [24] on Sarotherodon mossambicus, [25] on guppy, *Poecilia reticulate*, [26], [27] on hybrid tilapia (*O. niloticus* X *O. aureus*), [28], [29] on Nile tilapia (*O. aureus*), [30] in juvenile monosex Nile tilapia. The increase of muscle protein and decrease of lipid content with increasing dietary protein level may be attributed to their high carbohydrate and low protein content in the diet of the low protein level. The excess carbohydrate in the diet may be converted into body fat for storage [25].

In teleost, calcium and phosphorus are the main constituents of the mineral fraction of the bone [31]. Bone calcium may originate from both water and diet, whereas bone phosphorous originates from the diet [32, 33, 34, 35]. During the experiment, the good representation of phosphorus and calcium in all experimental diets contributed to improve the growth performance of larvae. The high P^{2-} values observed in larvae fed 35 % of crude beef brain protein and the important Ca^{2+} content shown that those minerals were well removed by organism to improve growth performance. Indeed, it is well documented that in fish, Calcium and phosphorus are directly involved in the development and maintenance of the skeletal system and participate in several physiological processes [36]. Ca^{2+} is complexed with P^{2-} in hydroxyapatite to form the principal crystalline material of bone [37]. The results of the analysis of body calcium and phosphorus showed that the rates of these two minerals are not directly related to the level of protein in formulated diets. This is because generally, for Ca^{2+}, the requirement of most fish is met by absorption from the water or from feed ingredients of practical and purified diet [38]. In contrast, the decrease in potassium and increasing concentrations of iron and manganese in larvae fed with feed containing beef brain with increasing dietary protein content shows that the composition of fish in these minerals would be bound by the protein content of food distributed. Indeed, these elements are interacting to form chelates with other chemicals (protein) complexes that facilitate their transport into the organs. Increasing levels of dietary protein was reduced or increased availability of Mn^{2+}, Fe^{2+}, and K^{+} in diets-based beef brains.

In conclusion, this study indicates that diets containing 35% dietary crude protein seems to be most appropriate and economical for the growth of larvae *H. longifilis*.

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