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A Preliminary Study on the Dietary Protein Requirement of *Parachanna obscura* (Günther, 1861) Larvae

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Abstract

Effects of increasing dietary protein on growth parameters, feed utilization were studied in *Parachanna obscura* larvae with initial body weight 0.12 ± 0.01 g. The experiment was conducted during 28 days in re-circulating system composed of 15 tanks containing 20 L of water each. 50 larvae were stocked per tank. Five semi – purified isoenergetic experimental diets were formulated with different protein level (35, 45, 50, 55 and 60 g/100 g of diet). Each diet was tested in triplicate. Growth parameters and feed utilization were significantly influenced by dietary protein ($P<0.05$). Specific Growth Rate varied from 8.86 to 11.53%/d. Best Specific Growth Rate, Feed Efficiency and Productive Protein Value were obtained with diet containing 55% of protein. The highest body protein content was found with larvae fed by diet with 55% of protein ($P<0.05$). Feed Efficiency and Productive Protein Value of fish fed on 45, 50 and 60% dietary protein were not significantly different ($P>0.05$). Second degree polynomial regression and broken line models were used to analyze the relationships between dietary crude protein and SGR. Based on the results of this study, protein requirements of *P. obscura* larvae ranged from 45 and 55.5% of the diet.

Keywords: *Parachanna obscura* larvae, protein requirement, growth, feed utilization.

Introduction

Parachanna obscura is the most common species among African Snakeheads (Bonou and Teugels, 1985). Characteristics of this fish that make it a desirable species in aquaculture are its high market value, rapid growth, tasty flesh, few bones, tolerance of high stocking density, utilization of atmospheric oxygen for respiration in water with low dissolved oxygen and high ammonia levels (Gras, 1961; O' Bryen and Lee, 2007). Few data are available on farming on this fish. Domestication and intensive cultured of *P. obscura* should start to provide further guidance for fish farming and diversification of fish species used so far in farming in the world. In intensive larvae and fry culture, several factors influence survival rate, welfare, growth and production including dietary protein level (NRC, 1993), feeding level (Kpoguè and Fiogbé, 2012) and stocking density (Schram *et al*., 2006).

Protein is an essential component of fish diet, needed for growth, reproduction and survival of fish (Wilson and Halver, 1986). In fish food, protein provides the essential and non essential amino acids to synthesize body protein and in part provides energy for maintenance (NRC, 1993; Kaushik and Médale, 1994). When protein levels are inadequate in the diet of fish, a reduction of growth is observed. Dietary protein content is the most important factor affecting fish growth and feed cost (Lovell, 1989). Protein is the most expensive component in supplementary fish feed (Fagbenro *et al*., 1992). Feed constitutes 70% of total investment in intensive aquaculture (Pillay, 1990). Any reduction in dietary protein level without affecting fish growth can substantially reduce the cost of fish feed (Fiogbé, 1996; Kim *et al*., 2003; Jamabo and Alfred-Ockiya, 2008). For many fish species, there is an optimum requirement of dietary protein to supply adequate amino acids for maximizing growth (Siddiqui and Khan, 2009). If too much protein is supplied in the diet, only part of it is used to make new protein for growth, and the remainder will be converted into energy, which results in increased feed cost and increased ammonia nitrogen excretion. Therefore, from both economical and environmental perspective, it is important that inclusion of the dietary protein should be optimized (Siddiqui and Khan, 2009; Akpinar *et al*., 2011). Stocking density

and feeding level in *P. obscura* larvae has been investigated (Kpoguè and Fiogbé, 2012) but no study has yet been conducted in it protein requirement. Most snakehead culture relies on capture of wild fry, which are then trained to accept formulated feed (Diana *et al*., 1985) and the present study was undertaken in order to estimate the dietary protein requirement for maximum growth, survival, feed conversion and protein retention for *P. obscura* larvae collected in a swamp and reared in a re – circulating water system.

Material and Methods

Diets formulation and preparation

Five semi–purified experimental diets were formulated to be isoenergetic with different protein levels (35, 45, 50, 55 and 60 g/ 100 g of diet) (Table 1). These protein contents were chosen based on the results of the protein requirements of other snakeheads species such as *Channa striatus* (Mohanty and Samantary, 1996; Samantary and Mohanty, 1997; Aliyu – Paiko *et al*., 2010) and *C. punctatus* (Zehra and Khan, 2011). The various ingredients were ground with hammer mill, weighed, mixed, and pelleted using manual pelleter (meat mincer). The pelleted feed was re-ground into powder after sun drying at temperature of 28–35°C for about 2 days and passed through 0.8 mm and 1.2 mm mesh sieves.

Experimental Fish, Rearing Conditions and Feeding Trial

Larvae of *P. obscura* (initial weight = 0.04±0.02 g) were collected from a swamp "Dra" in Takon (Southeast Benin). Water temperature, pH and dissolved oxygen in the swamp were 27.7°C, 6.1 and 2.1 mg/L respectively. Once collected larvae were immediately shipped to the experimental station to the Research Unit in Wet Land of the Department of Zoology of the Faculty of Sciences and Techniques (University of Abomey - Calavi, Benin) and put in circular tank. Before the beginning of the experiment, larvae were fed with only live food for four days, mainly zooplankton (*Brachionus* spp) collected in a pond with planktonic net and newly hatched *Artemia* nauplii (EG grade, INVE, Dendermonde, Belgium). The food substitution was progressive and the larvae were reared up to 0.12 g. Indeed, based on results of Qin *et al*. (1997), snakehead larvae can successfully convert to formulated feed when it initial body weight is approximately 0.1 g. A mixture of the different experimental diets was used as feed during the food substitution phase. The experiment was conducted during 28 days in a re-circulated system composed of 15 tanks containing 20 L of water each. Acclimated larvae (0.12±0.01 g) were stocked at a density 50 fish/ tank. All tanks were linked to a 225 L re-circulated system, which received water from the experimental tanks. Water was re-circulated through mechanical and biological filter system before being pumped into

Table 1. Formulation and proximate composition of experimental diets

Ingredients (%)	Dietary protein				
	35%	45%	50%	55%	60%
Casein [a]	20.10	25.85	28.7	31.59	34.46
Cod meal [b]	14.07	18.09	20.09	22.11	24.12
Yeast [c]	6.03	7.75	8.61	9.48	10.34
Cod liver oil [d]	5.5	4.5	3.75	3.5	3.0
Soya oil [e]	5.5	4.5	3.75	3.5	3.0
Dextrin [a]	24.8	20	20	18	16.08
Glucose [a]	15	10.3	6.1	2.82	0
Premix (vit – min) [d]	8	8	8	8	8
Carboxymethylcellulose [a]	1	1	1	1	1
Proximate analyses					
Crude protein (%)	34.35	44.09	49.33	55.39	59.10
Crude lipid (%)	12.65	11.43	10.95	10.89	10.35
Ash (%)	11.11	11.58	13.84	11.86	12.57
Moisture (%)	8.95	8.86	8.84	9.06	8.96
NFE [f]	32.94	24.04	17.04	12.8	9.02
Gross energy (MJ/100 g)	2.01	2.02	2.02	2.03	2.04
Protein /Energy (g/MJ)	17.42	22.26	24.78	27.09	29.41

[a] SIGMA product; [b] Protibel (yeast Saccharomyces) Bel industries, 4 rue d'Anjou Paris 8ème, France;
[c] Rieber & Son, N. 5002 Bergen, Norway ;
[d] Drugstore , premix (vitamin – mineral) contains (‰):Vitamin A 4 000 000 U.I; Vitamin D 800 000 U.I; Vitamin E 40 000U.I; Vitamin K_3 1600 mg; Vitamin B_1 4 000 mg, Vitamin B_2 3 000 mg; Vitamin B_6 3 800 mg; Vitamin B_{12} 3 mg; Vitamin C 60 000 mg; Biotin 100 mg; Inositol 10 000 mg Pantothenic acid 8 000 mg; Nicotinic acid 18 000 mg; Folic acid 800 mg; Cholin chloride 120 000 mg; Colbat carbonate 150 mg; Ferrous sulphate 8 000 mg; Potassium iodide 400 mg; Manganese oxide 6 000 mg; Cuivre 800 mg; Sodium selenite 40 mcg; Lysine 10 000 mg ; Methionin 10 000 mg; Zinc sulphate 8 000 mg;
[e] Songhaï center (Republic of Benin);
[f] Nitrogen free extract, calculated as 100 – (protein + lipid + ash + moisture).

each tank at a flow rate of 0.5 L/min. All tanks were covered with nets throughout the experiment in order to prevent fish from jumping out. During the experiment, fish were hand fed daily, every hour from 08:00 AM to 08:00 PM, to apparent satiation. Each experimental diet was assigned three tanks (triplicate).

Each tank was daily finely siphoned in order to find uneaten food and likewise remove faeces and dead fish. After siphoning, the water volume (approximately 25%) was adjusted in each tank and dead fish were counted.

Water quality parameters such as temperature, dissolved oxygen and pH were daily measured. The mean values of these parameters were respectively 27.9 ± 0.18°C, 6.53 ± 0.02 mg/L and 6.74 ± 0.13.

At the beginning of each experiment, 30 larvae were weighed individually. All fish were counted and weighed every 4 days before being released into their corresponding tank and food ration adjusted accordingly. No feed was offered to the fish on the day the measurements were taken.

At the end of the experiment, all fish were counted and fish body weight per tank as well as individual weight was taken.

Chemical and Data Analysis

Fish samples were analyzed by standard methods for dry matter (oven drying) at 105° C for 24 h, crude protein (N- Kjeldahl x 6.25) and ash (oven incineration at 550° C). Total lipids were extracted according to Bligh and Dyer (1959).

The Specific Growth Rate (SGR), the Feed Efficiency (FE), the survival rate (SR), the Protein Efficiency Ratio (PER) and the Protein Productive Value (PPV) were calculated. SGR was calculated on the basis of the initial and final body weight, according to the duration of the experiment (number of days = d) as followed : SGR (%/d) = 100 x [ln(final body weight) – ln(initial body weight)]/d. FE was calculated on the basis of the total food distributed (FD, g), the Initial and Final Biomasses [IB and FB, respectively (g)], and Biomass of Dead fish (DB, g) as followed: (FB+DB-IB)/FD. Survival rate (%) = 100 x FN/IN (IN, FN = Initial and Final Number of fish respectively). PER = (final biomass – initial biomass)/(total feed intake x dietary protein). PPV = (final protein in fish – initial protein in fish)/(total feed intake per fish x dietary protein).

The mean values of final weight, SGR, FE, PPV, PER, survival rate and body composition were compared between treatments by one way analysis of variance (ANOVA 1) after verifying the homogeneity of variance using "Hartley' s test" for each treatment. Significant differences between treatments means ($P<0.05$) were determined using a Fisher's test (Saville, 1990). Results are given as means ± standard deviation.

Two mathematical (dose – response) models were used to assess the effect of dietary protein level on specific growth rate of *P. obscura* larvae.

The general equation of the broken line model (Robbins *et al*., 1979) is $y = L+U(R-X_{LR})$ where L is the ordinate and R, the abscissa of the breakpoint. R is taken as the estimated requirement (dietary protein that guarantees the maximum specific growth rate). X_{LR} means X less than R and U is the slope of the line for X_{LR}. By definition, $R-X_{LR}$ is zero when $X > R$.

The model of Brett and Grove (1979) was applied to the second order polynomial regression between dietary protein and specific growth rate. This model allows determination of the maximum dietary protein corresponding to the maximum specific growth rate.

Results

Survival rate in all treatments were not significantly affected by the dietary protein level ($P>0.05$) and varied from 68 to 78.67% (Table 2). Specific Growth Rate and final body weight were significantly influenced by dietary protein level ($P<0.05$) (Table 2). Final body weight of larvae fed 55% dietary protein is the highest. The Specific Growth Rate (SGR) and Feed Efficiency (FE) improved significantly as dietary protein level increased from 35 to 55% of the diet ($P<0.05$). The lowest FE was obtained with 35% dietary protein. FE of fish fed 45, 50 and 60% dietary protein were not significantly different ($P>0.05$). Inclusion of dietary protein above 55% of the diet did not produce any improvement in the SGR and the FE.

Table 2. Growth performances and nutrient utilization of *P. obscura* larvae fed diets containing different levels of protein

	Dietary protein				
	35%	45%	50%	55%	60%
Initial body weight (g)	0.12±0.01	0.13±0.01	0.12±0.01	0.12±0.01	0.14±0.01
Final body weight (g)	0.77±0.05^{a}	0.96±0.04^{a}	1.17±0.08^{b}	1.39±0.13^{c}	1.23±0.06^{b}
Survival rate (%)	78.67±8.33	74±9.17	84.67±2.31	68±6.93	76.67±9.45
SGR (%/d)	8.86±0.57^{a}	9.65±0.32^{a}	10.71±0.52^{b}	11.53±0.22^{c}	10.25±0.30^{b}
FE	0.72±0.05^{a}	0.91±0.02^{b}	1.03±0.06^{b}	1.18±0.08^{c}	1.15±0.08^{bc}
PER	2.09±0.14	2.07±0.05	2.08±0.12	2.17±0.16	1.94±0.04
PPV	0.09±0.01^{a}	0.18±0.01^{b}	0.32±0.03^{bc}	0.40±0.01^{d}	0.30±0.03^{bc}

Means on the same line followed by different superscripts are significantly different ($P<0.05$).

Relationships between dietary protein in diet and SGR have been used to estimate the optimum and maximum dietary protein requirements for *P. obscura* larvae. When a second degree polynomial regression analysis (Brett and Grove, 1979) was used to interpolate the data (Figure 1), maximum protein requirements is 55.5% of the diet. According to the model of the broken line model (Figure 2), maximum protein requirement for *P. obscura* larvae is approximately 55.5% of the diet.

The increase of the dietary protein level in the diet affected significantly the Protein Productive Value (PPV) ($P<0.05$). PPV increased with the dietary protein level up to 55% and decreased later on. The lowest PPV was found with the diet with 35% of protein (Table 2). PPV obtained with diets containing 45, 50 and 60% of protein are not significantly different ($P>0.05$). The highest Protein Efficiency Ratio (PER) was obtained for the larvae fed the diet with 55% of protein but was not significantly different ($P>0.05$) from that of larvae fed the others diets (Table 2).

The result of body composition analysis is presented in Table 3. Body protein content increased significantly up to the diet with 55% of protein and decreased later on. The lowest crude protein was found with dietary protein 35%. Body lipid increased significantly with the increase of dietary protein ($P<0.05$) up to 45%. There were no significant differences between body fat for fish fed diet with protein from 45 to 60% ($P>0.05$). Moisture and ash content were not significantly affected by dietary protein level ($P>0.05$).

Discussion

It is well known that protein is the most important and expensive item of the feed that should be supplied in adequate amounts to support good growth with minimal cost (Wee and Tacon, 1982; Zehra and Khan, 2011). According to the present results, survival rate of *P. obscura* larvae was not

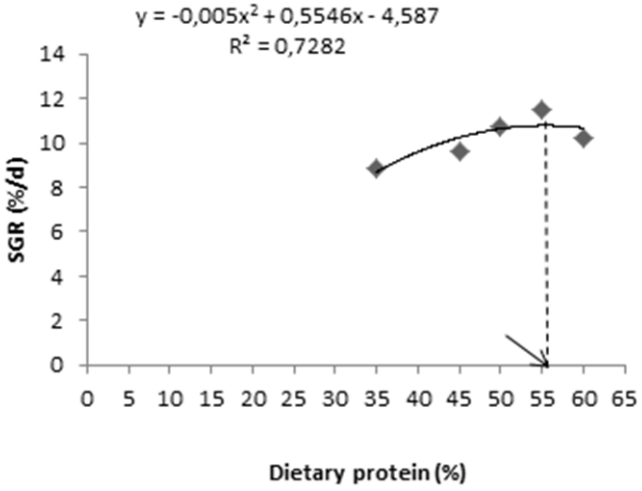

Figure 1. Determination of maximum dietary protein requirement of *P. obscura* larvae according to the Brett model

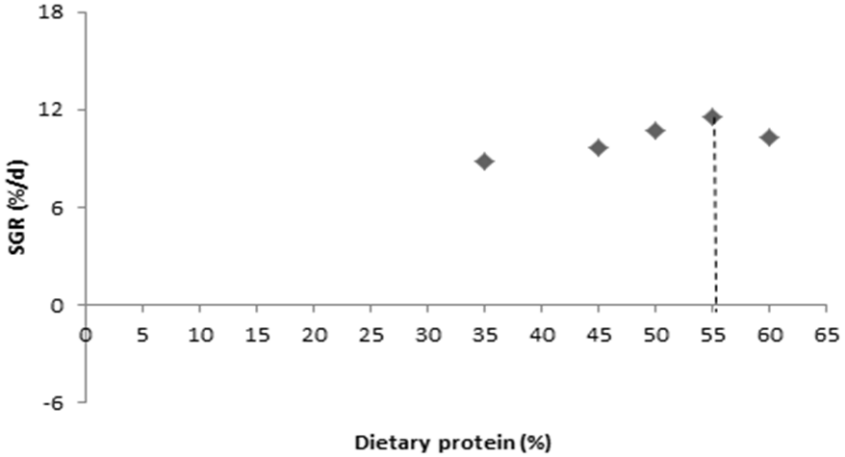

Figure 2. Determination of maximum dietary protein requirement of *P. obscura* larvae according to the broken line model

Table 3. Body composition data for *P. obscura* larvae fed with diets containing different levels of protein

Parameters	Crude protein	Crude lipid	Ash	Moisture
Initial fish	50.10±0.08	10.64±0.05	22.42±0.35	82.6±0.14
35%	51.49±0.10[a]	12.32±0.04[a]	20.34±0.01	78.1±0.02
45%	53.70±0.05[b]	18.11±0.32[b]	20.77±0.08	75.4±0.31
50%	58.27±0.71[c]	17.00±0.34[b]	21.07±0.28	79.5±0.21
55%	61.62±0.04[d]	15.22±0.17[b]	20.17±0.37	77.5±0.13
60%	59.03±0.58[c]	15.99±0.51[b]	18.21±0.19	78.6±0.45

Means on the same line followed by different superscripts are significantly different (P<0.05).

affected by the dietary protein level (P>0.05) but growth of larvae fed different dietary protein varied significantly. Growth (final body weight and SGR) increased with increasing dietary protein from 35 to 55% and decreased thereafter with further increases in dietary protein level. Similar trend of growth depression at lack or surplus levels of protein intake in the diets has also been observed in various fish species as *Carassius auratus* (Fiogbe and Kestemont, 1995); *Oreochromis niloticus* (Kaushik *et al*., 1995); *Perca fluviatilis* (Fiogbe, 1996); hybrid catfish (*Heterobranchus bidorsalis* x *Clarias anguillaris*) (Diyaware *et al*., 2009); *Heteropneustes fossilis* (Siddiqui and Khan, 2009); *Heterotis niloticus* (Monentcham *et al*., 2010); *Clarias gariepinus* (Farhat and Khan, 2011). When protein levels are inadequate in the diet of fish, a reduction of growth is observed. This observation could be attributed to the reduction in the available energy for growth due to inadequate non-protein energy necessary to deaminate and excrete excess absorbed amino acids (Houlihan, 1991; Kim *et al*., 2002). According to those authors, protein in fish is a main component constituent of tissue and organs. They are precursors of other nitrogen compounds (enzymes, hormones, slurry, neurotransmitters, cofactors, etc) and constitute an important energy source. Fish digest protein to obtain free amino acids, which are absorbed from intestinal tract and used by various tissues to synthesize new protein. Thus, a consistent intake of protein is required, since it is continually used by the fish to build new proteins. Inadequate protein levels in the diets results in a reduction of growth and loss of weight. However, when an excess of protein is supplied in the diet, only part of it is used for protein synthesis (growth) and the remaining is transformed into energy. The lowest growth obtained with the diet with 35% of protein can be due to the fact that most of the protein was used for maintenance making it unavailable for growth.

Relation between fish – diet – feeding has an important effect in the determination of nutrient requirement. FE, PER and PPV were used as indices of food and protein utilization in this study. Increase in dietary protein improved those parameters from 35 to 55% dietary protein and inclusion of dietary protein at 60% didn't exhibited best food and protein utilization by *P. obscura* larvae. FE and PPV of fish fed 45, 50 and 60% dietary protein were not significantly different (P>0.05). Thus, like the SGR, best FE, PER and PPV were obtained with 55% dietary protein. Several works show that the food and protein utilization decrease beyond maximal level of dietary protein and the requirements for maximal growth are always higher than the requirements for least cost (optimal) production (Yang *et al*., 2003; Kim and Lee, 2009; Siddiqui and Khan, 2009; Hossain *et al*., 2012). Based on the dose – response models applied between dietary protein and SGR, the maximum dietary protein requirement for *P. obscura* was found to be 55.5%. Our results show that at both dietary protein levels, 45 to 55.5% appeared to promote the most efficient food and protein utilization for *P. obscura* larvae.

This result is near to protein requirements recommended to other carnivorous fish larvae: 48-53% (*Salmo trutta*, Arzel *et al*., 1995); 45-53% (*Carassius auratus*, Fiogbe and Kestemont, 1995); 48.5-49.4 (*P. fluviatis*, Fiogbé, 1996); 42-56% (*Clarias gariepinus* and *Heterobranchus longifilis*, Kerdchuen, 1992); 50-55% (hybrid catfish *H. bidorsalis* x *C. anguillaris*, Diyaware *et al*., 2009). *P. obscura* larvae protein requirements obtained in this study are higher than the range determined for larvae of omnivorous species such as *O. niloticus* (28-35%, El Sayed and Teshima, 1992; 28-30%, De Silva *et al*., 1989; 30-36%, Shiau, 2002) and *Cyprinus carpio* (35-42%, Tacon, 1987). This variation in protein requirement among fish species may be attributed to the differences between phylogenetically distinct families or species (Akiyama *et al*., 1997).

The best SGR obtained in this study (11.53%/d) with dietary protein 55% is near to 12.24%/d obtained by Kpoguè and Fiogbé (2012) in *P. obscura* larvae feed with commercial food (Coppens with 56% of protein).

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