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Influence of dietary protein levels on growth, feed utilization and carcass composition of snakehead, *Parachanna obscura* (Günther, 1861) fingerlings

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Five isoenergetic semi-purified diets were formulated to evaluate the effects of dietary crude protein levels on growth and feed utilization of snakehead, *Parachanna obscura* (4.08 ± 0.07 g). Experimental diets were formulated to contain graded levels of crude protein (CP; 30, 40, 45, 50 and 60 g/100 g of diet). Fish feed on the tested diet in triplicate for 45 days. Seventy fingerlings were stocked per a 225 L cement tank. Growth performances and nutrient utilization parameters of fingerlings fed different diets varied significantly (P < 0.05) and the highest growth performance and nutrient utilization were obtained with fish fed on a 50% CP diet. The relationship between the dietary CP and specific growth rate (SGR) indicated that protein requirements of *P. obscura* fingerlings ranged from 42.5 to 53.5% of diet.

Key words: Parachanna obscura fingerlings, protein requirements, growth performances, nutrient utilization.

INTRODUCTION

Despite its enormous natural potentialities, sub-Saharan Africa region provides only 0.16% of world aquaculture production. Then, most African countries rely on imports of fish to meet local demand for fish products. In order to reduce imports and to better meet the needs of African fish consumers, fish production in this region should grow by 267% from 2006 to 2020 (FAO, 2006). The development of aquaculture through the farming of endemic species of African inland waters is necessary. Thus, some neglected fish species like snakehead, *Parachanna obscura*, (Günther, 1861) which reveal interesting aquacultural potential have been identified (Kpogue et al., 2013a). *P. obscura*, the most widespread African Channidae (Bonou and Teugels, 1985), is unfortunately an endangered species (Lalèyè et al., 1997; Babatunde and Olojede, 2010). It is being cultured in extensive farming system in some African countries like Ivory Cost and Nigeria (Lazard and Legendre, 1994; Bassey and Ajah, 2010). Intensive breeding of *P. obscura* is urgently required to kick-start its sustainable aquaculture. Indeed, this option can help to preserve, as well as strengthen the natural stocks of *P. obscura* and produce continually its juveniles for sale to aquatic farming.

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Table 1. Formulation and proximate composition of experimental diets.

Ingredient (%)	Dietary protein				
	30%	**40%**	**45%**	**50%**	**60%**
Casein[a]	10.80	21.60	25.86	32.40	43.10
Cod meal[b]	14.00	14.00	14	14.00	14.00
Yeast[c]	14.00	14.00	11.84	14.00	14.00
Cod liver oil[d]	9.80	7.80	6.00	6.00	6.00
Soya oil[e]	6.40	5.20	4.00	4.00	4.00
Dextrin[a]	20.00	20.00	17.00	10.00	2.90
Glucose[a]	14.00	6.40	10.30	8.60	5.00
Premix (vit – min)[d]	10	10	10	10	10
Carboxymethylcellulose[a]	1	1	1	1	1
Proximate analyses					
Crude protein (%)	27.35	39.01	44.09	49.33	59.06
Crude lipid (%)	13.15	11.93	11.43	11.43	11.43
Ash (%)	11.11	11.28	11.58	13.84	12.57
Moisture (%)	8.96	8.81	8.86	8.84	8.96
NFE [f]	39.43	28.97	24.04	16.56	7.98
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] Rieber & Son, N. 5002 Bergen, Norway; [c] Protibel (yeast Saccharomyces) Bel industries, 4 rue d'Anjou Paris 8[ème], France; [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).

It is well known that in intensive fingerlings culture, several factors like dietary protein level (NRC, 1993) and feeding level (El-Sayed, 2002; Giri et al., 2002) influence fish growth and survival. Indeed, in fish feeding, 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 level is inadequate in the diet of fish, a reduction of growth is observed. Protein is the most expensive component in supplementary fish feed (Fagbenro et al., 1992). As 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 (Fiogbe et al., 1996; Jamabo and Alfred-Ockiya, 2008). 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., 2012). Therefore, in this study, semi-purified diets were used in order to investigate the influence of dietary protein levels on growth, feed utilization and carcass composition of snakehead, *P. obscura* fingerlings and to estimate their protein requirement.

MATERIALS AND METHODS

Composition and preparation of the diets

Five isoenergetic experimental diets were formulated with semi-purified ingredients to contain graded levels of protein (30, 40, 45, 50 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 *Channa punctatus* (Zehra and Khan, 2011). The various ingredients were ground with hammer mill, weighed and mixed. Feed was prepared by mixing the dry ingredients with the addition of oil and water until a desirable paste-like consistency was reached. The resulting paste was transformed into pellets with 2 mm of diameter with the aid of food blender (MFM- 302- Denwa). After sun-drying at a temperature of 28 to 35°C for about 3 days, the pellets were manually broken.

Experimental fish, rearing conditions and feeding trial

Fish were collected from a swamp "Dra" in Takon (South - Est - Benin). Once collected, fish were transported to the experimental Station to the Research Unit of Wet Land, Department of Zoology, Faculty of Sciences and Technology, University of Abomey, Calavi, and put in circular tank for 2 weeks. During this period, fry were trained to accept progressively the formulated diet. A mixture of the

Table 2. Growth performances, survival rate and nutrient utilization of *P. obscura* fingerlings fed diets containing different levels of protein for 45 days.

Dietary protein	30%	40%	45%	50%	60%
Initial body weight (g)	4.04 ± 0.04	4.00 ± 0.04	4.06 ± 0.04	4.09 ± 0.02	4.19 ± 0.04
Final body weight (g)	6.00 ± 0.11^{d}	7.70 ± 0.09^{c}	8.79 ± 0.41^{b}	10.25 ± 0.14^{a}	9.16 ± 0.13^{b}
Survival rate (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
SGR (%/day)	1.08 ± 0.05^{d}	1.82 ± 0.02^{c}	2.13 ± 0.11^{b}	2.55 ± 0.04^{a}	2.17 ± 0.05^{b}
K	0.67 ± 0.12^{b}	0.76 ± 0.19^{b}	1.06 ± 0.34^{a}	0.98 ± 0.08^{b}	0.96 ± 0.24^{b}
FE	0.56 ± 0.03^{d}	0.75 ± 0.07^{c}	0.90 ± 0.08^{b}	1.17 ± 0.03^{a}	0.96 ± 0.06^{b}
PER	0.03 ± 0.00^{c}	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}	0.04 ± 0.01^{a}	0.03 ± 0.00^{c}
PPV(%)	3.68 ± 0.12^{d}	4.58 ± 0.07^{c}	5.44 ± 0.09^{b}	7.00 ± 0.21^{a}	5.20 ± 0.35^{b}
Sk	-0.64 ± 0.06^{bc}	0.27 ± 0.46^{ab}	-0.04 ± 0.31^{bc}	-0.15 ± 0.20^{bc}	0.19 ± 0.45^{b}

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

different experimental diets (20% of each) was used as feed during this phase. After this conditioning period, 70 fingerlings (4.08 ± 0.07 g) were stocked per a 225-L tank for 45 days and each diet was assigned by three tanks. Water in all tanks was renewed continuously (1 L/min). To prevent fish from jumping out, tanks were covered at 50% with a perforated wooden plank. During the experiment, fish were hand fed daily every 2 h from 08:00 am to 08:00 pm up to apparent satiation.

Water quality parameters such as temperature, pH, dissolved oxygen and nitrite were daily measured in each tank throughout the experimental period. These parameters were 27.8 ± 0.2°C, 6.1 ± 0.1, 6.28 ± 0.14 mg/L, and 0.01±0.00 mg/L, respectively.

At the beginning and the end of the experiment, all fish were counted and weighed per tank and 30 fingerlings were sampled for individual weight and total length. All fish were counted and weighed every 7 days for growth sampling during the experimental period and no feed was given to the fish on the day of sampling.

Chemical and calculations

Fish samples were analyzed by standard methods for dry matter (oven drying) at 105°C for 24 h, crude protein (CP) (N- Kjeldahl × 6.25) and ash (oven incineration at 550°C for 12 h). Total lipids were extracted according to Bligh and Dyer (1959).

After the feeding trial, fish were collected, counted, and weighed and the different parameters were calculated as follows:

Specific growth rate (SGR; %/d) = 100 × [Ln (final body weight) - Ln (initial body weight)] / days of the experiment

Feed efficiency (FE) = (FB+DB-IB) / FD

Where IB and FB are the initial and final biomasses and DB (g) is biomass of dead fish, and FD (g) is the total feed distributed.

Fish survival rate (%) = 100 × FN/IN; (IN, FN = Initial and Final Number of fish respectively).

Condition factor (K %) = 100 × final body weight /L^3; where L is fish length in cm;

Protein efficiency ratio (PER) = (final biomass - initial biomass) / (total feed intake × dietary protein);

Protein productive value (PPV) = 100 × (final protein in fish - initial protein in fish) / (total feed intake per fish × dietary protein).

Statistical analysis

The obtained data were statistically analyzed by one way analysis of variance (ANOVA) after verifying the homogeneity of variance using Hartley's test. Significant differences between means were determined using a Fisher's test at P < 0.05 (Saville, 1990). Results are given as means ± standard deviation. The coefficient of Skewness (Sk) was used to measure the degree of symmetry of body length distribution at different treatments (Sheskin, 2004). Relationships between diets and growth, and nutrient utilization performances were examined by Pearson's product - moment correlation.

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

(1) 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 SGR). 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.

(2) The model of Brett and Grove (1979) was applied to the second order polynomial regression between dietary protein and SGR. This model allows determination of:

(a) The maximum dietary protein (corresponding to the maximum SGR and calculated by taking the first derivative of the second order polynomial equation),

(b) The optimum dietary protein [obtained graphically and corresponding to the best feed efficiency (FE)].

RESULTS

Survival rate, growth performances and nutrient utilization parameters

Survival rate, growth performances and nutrient utilization parameters of *P. obscura* fingerlings during the feeding trial are shown in Table 2. In all the treatments, survival rates were 100% and were not significantly affected by the dietary protein levels (P > 0.05). Growth parameters namely the final body weight, SGR, and K factor were significantly influenced (P < 0.05) by the dietary protein levels. For these parameters, the value recorded with fish fed the diet with 30% of protein was the lowest, while the highest ones were obtained at 50% CP diet. The final

Table 3. Whole-body composition (%; on dry matter basis) for *P. obscura* fingerlings fed with diets containing different levels of protein for 45 days.

Parameter	Crude protein	Crude lipid	Ash	Moisture
Initial fish	40.46 ± 1.14	11.01 ± 0.20	17.60 ± 0.54	76.30 ± 1.22
30%	42.38 ± 5.02^{c}	13.31 ± 0.46^{e}	15.02 ± 0.33^{a}	78.60 ± 0.82^{bc}
40%	45.34 ± 0.27^{b}	16.50 ± 0.12^{a}	14.20 ± 0.50^{bc}	77.80 ± 0.44^{c}
45%	47.03 ± 0.27^{b}	15.70 ± 0.01^{b}	15.81 ± 0.37^{a}	76.80 ± 0.06^{d}
50%	50.15 ± 2.52^{a}	14.10 ± 0.26^{d}	13.91 ± 0.35^{cd}	79.21 ± 0.44^{ab}
60%	48.48 ± 2.74^{ab}	14.81 ± 0.37^{c}	13.75 ± 0.24^{d}	80.01 ± 0.04^{a}

Values with different letters within the same column are significantly different (P < 0.05).

Table 4. Linear regression characteristics describing relationships between dietary protein level and growth and nutrition utilization parameters.

Parameter	SGR (%/day)	K	FE	PER	PPV
Range	1.08 - 2.55	0.67 - 1.06	0.56 - 1.17	0.03 - 0.04	3.68 - 7.00
R^2	0.65*	0.55	0.62	0.02	0.40
P	0.09	0.14	0.11	0.8	0.24

Range = minimum – maximum. Correlation marked with (*) is significant at 95%.

body weight and the SGR improved significantly as dietary protein level increased from 30 to 50% and inclusion of dietary protein above 50% of diet did not improve the fish growth. As for K factor, it was significantly the highest with 45% CP (P < 0.05). Parameters of nutrient utilization (FE, protein efficiency rate (PER), and PPV) were significantly affected by the levels of dietary protein (Table 2; P < 0.05). FE, PER and PPV increased progressively with graded dietary protein level and was found to be the best for fish fed 50% CP diet (P < 0.05). Moreover, a significant fall in FE, PER, and PPV was obtained in fish fed 60% CP diet. No significant difference (P > 0.05) was recorded for the Sk in all treatments (Table 2).

Body composition

The result of whole-body composition analysis is presented in Table 3. CP, crude lipid, ash and moisture were significantly affected by dietary protein level (P < 0.05). CP increased with the dietary protein level up to 50% and decreased later on. The lowest CP was found in fish fed 30% dietary protein. There was no significant difference (P > 0.05) between body CP for fish fed diets with 50 and 60% protein. The dietary treatment which produced the highest growth performance (50% dietary protein) induced one of the lowest body lipid and ash contents. Initial body composition of fish contained less moisture than final body composition of fish, regardless of experimental diets.

Estimation of protein requirement

Table 4 showed that SGR is the parameter mostly correlated with the levels of dietary protein. Significant linear relationship was observed for SGR (R^2 = 0.65). No evidence was found for FE, K, PER and PPV. Thus, relationships between dietary protein in diet and SGR were used to estimate the dietary protein requirements for *P. obscura* fingerlings. Based on the second-order polynomial regression analysis (Brett and Grove, 1979) of SGR against dietary protein levels, optimum and maximum protein requirement for better growth of *P. obscura* fingerlings were 42.5 and 53.5, respectively (Figure 1). According to the model of the broken line model (Figure 2), the maximum protein requirement for *P. obscura* fingerling is 50.5%.

DISCUSSION

The obtained results showed that fish growth was improved significantly with the increase in dietary protein up to 50% after which fish growth was retarded. Similar observation was reported in various other cultivated fingerlings as *Oreochromis niloticus* (Kaushik et al., 1995), *Perca fluviatilis* (Fiogbe et al., 1996), *Heterotis niloticus* (Monentcham et al., 2010), *Clarias gariepinus* (Farhat and Khan, 2011), *Horabagrus brachysoma* (Giri et al., 2011), and *Cyprinus carpio* (Moreau, 2001; Ahmed et al., 2012). The trend of growth depression at surplus levels of protein intake in the diets could be attributed to

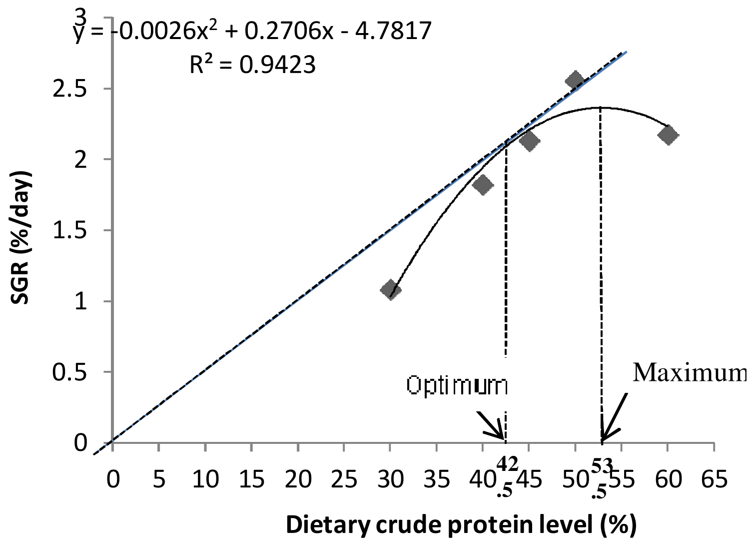

Figure 1. Second degree relationship between specific growth rate and dietary protein levels.

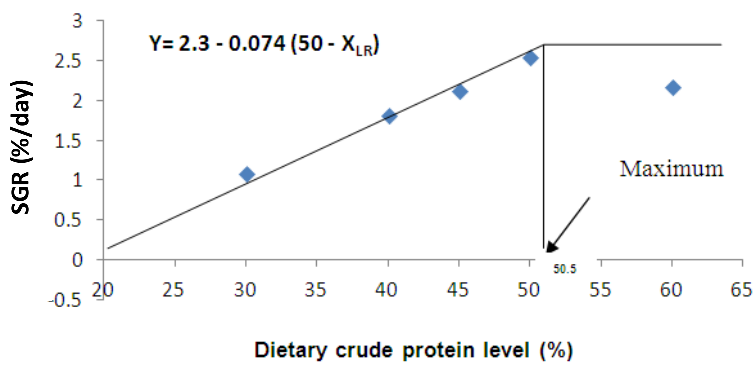

Figure 2. Relationship between specific growth rate and dietary protein levels according to the broken line model.

the reduction in the available energy for growth due to inadequate non-protein energy necessary to desaminate and excrete absorbed amino-acid (Houlihan, 1991; Kim et al., 2002; Köprücü and Özdemir, 2005). 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 low growth obtained in this study with 60% CP diet can be due to the fact that most of the protein was used for maintenance making it excess and unavailable for fish growth and the excess protein may be broken down to amino acids which were deaminated to become carbohydrates.

The K factor is significantly the best with 45% CP. This result showed that diet with 45% CP could be ideal for best growth of *P. obscura* fingerlings. The Sk coefficient ranged between -0.06 ± 0.06 and 0.29 ± 0.46. These results showed heterogeneity between fish size in all the treatments. Regardless of dietary CP level, dominance hierarchy was present in all tanks. Dominant fish being territorial and having a privileged access to the feed according to the values of the Sk coefficient. This hierarchy induced size heterogeneity into each group. Those observations tallied with Fiogbe et al. (1996) in *P. fluviatilis* fingerlings.

FE, PER and PPV are usually used as indices of feed and protein utilization in aquaculture. In this study, increase in dietary protein from 30 to 50% CP improved these parameters and the inclusion of dietary protein over 50% did not exhibited better feed and protein utilization in *P. obscura* fingerlings. In this regard, Yang et al. (2003), Kim and Lee (2009), Siddiqui and Khan (2009), Adewolu and Adoti (2010), Sotolu (2010), Ergün et al. (2010) and Ahmad et al. (2012) obtained the same trend and they have shown that the feed and protein utilization parameters decrease beyond the maximal level of dietary protein. However, protein requirement for maximal growth is always higher than the requirement for least cost (optimal) production.

The results of whole-body composition showed that CP increased significantly (P < 0.05) with increasing the dietary protein level up to 50% and decreased later on. However, crude lipid, ash and moisture did not show any coherent trends. Similar trends were obtained with Aliyu-Paiko et al. (2010) in *C. striatus* fingerlings.

According to the two mathematical models (dose - response) used to assess the effect of dietary protein level on SGR, protein requirements of *P. obscura* fingerlings ranged from 42.5 to 53.5% of diet. This result is similar to those recommended to other snakehead species fingerlings such as *C. striatus* and *C. punctatus*. For *C. striatus* fingerlings, protein requirements are 43% (Boonyaratpalin, 1980, 1981), 50% (Wee and Tacon, 1982; Wee, 1986), 40 to 45% (Samantary and Mohanty, 1997), 45% (Aliyu-Paiko et al., 2010) and 55% (Kumar et al., 2010). *C. punctatus* protein requirements are ranged between 43.8 and 44.4% (Zehra and Khan, 2011). *P. obscura* fingerlings dietary protein requirements are near to those determined for other carnivorous species fingerlings like *Carassius auratus* 42.5% (Bandyopadhyay et al., 2005), *P. fluviatilis* 43.6% (Fiogbe et al., 1996), hybrid *Hetero clarias* 50%, (Diyaware et al., 2009), *Pangasius hypophthalmus* 45% (Liu et al., 2011), *Chrysophrys major* 55% (Yone, 1976), *Dicentrarchus labrax* 50 to 53% (Alliot et al., 1974; Métailler et al., 1981), *Heteropneustes fossilis* 40 to 43% (Siddiqui and Khan, 2009), *C. gariepinus* 43 to 46% (Farhat and Khan, 2011) and *Chrysischthys nigrodigitatus* 40% (Adewolu and Benfey, 2009). Protein requirements between fish species is complicated by difference in species, size and age of fish, diet formulation, stocking density, protein quality, hygiene and experimental conditions between studies (NRC, 1993). However, the dietary protein requirements of *P. obscura* fingerlings as estimated in the present study, were slightly lower than those determined by Kpogue et al. (2013b) for the same species larvae (45.5 and 55.5%).

Despite its short duration (6 weeks), the results of this trial had shown clearly the effects of dietary protein levels on growth, feed utilization and carcass composition of endangered snakehead (*P. obscura*) fingerlings. This study confirmed that *P. obscura* is a hardy and rustic species. Growth performances and nutrient utilization of fingerlings fed different diets varied significantly (P < 0.05). SGR is the parameter mostly correlated with dietary protein level and two mathematical models (second order polynomial regression and broken line models) were used to analyze the relationships between the dietary CP and the SGR. According to our results, dietary CP requirements for *P. obscura* varied from 42.5 to 53.5% in formulated diets and the optimum level is 50.3%.

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