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Optimization of dietary protein to energy ratio (P/E ratio) for Sutchi catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) fingerlings

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Abstract
A 60 days feeding trial was conducted to investigate the optimum dietary protein to energy ratio (P/E ratio) in Sutchi catfish *Pangasianodon hypophthalmus*. The same aged uniform size fingerlings of *P. hypophthalmus* were randomly distributed into groups of 40 fish (averaging 2.55 ± 0.05g in weight) per 70-L fiber glass tank in triplicates following completely randomized design. Six experimental diets were formulated to contain two levels of crude protein (25 and 30%) and three lipid levels (5, 10 and 15%) formed 25/5, 25/10, 25/15, 30/5, 30/10 and 30/15% P/E ratio contained 15.50, 17.36, 19.38, 16.52, 18.38 and 20.35 kJ g^{-1} gross energy. Feeding rate and frequency were 10-8% of their body weight 3-2 times daily. The highest (P<0.05) weight gain was observed for fish consuming that diet which contained 30/10% P/E ratio. The optimum dietary P/E ratio found for *P. hypophthalmus* fingerlings is 16.33 mg protein kJ^{-1} of GE, for a diet containing crude protein 30%, crude lipid 10% and gross energy 18.38 kJ g^{-1}. Significantly indifferent (P>0.05) values of protein utilization were found in-between the both (higher and lower) protein diets. Higher lipid deposition (P<0.05) in whole body and liver was observed with increasing dietary lipid level at each protein diet and as higher (P<0.05) for the lower protein diets. Significantly (P<0.05) lower liver glycogen level was found with increasing dietary lipid at each protein level. No significant (P>0.05) difference was found in digestive enzyme activities (amylase, protease and lipase) in fish fed with different experimental diets. Hence, it may be concluded that Sutchi catfish *P. hypophthalmus* performed best the diet containing 30%, 10%, 18.38 kJ g^{-1} and 16.33 mg protein kJ g^{-1} GE protein, lipid, gross energy, and P/E ratio, respectively.

Keywords: Sutchi catfish (*Pangasianodon hypophthalmus*), protein to energy ratio, protein utilization, liver lipid, liver glycogen, digestive enzymes

1. Introduction

Sutchi catfish, *P. hypophthalmus* commonly known as Thai catfish, was introduced in 1989 as an aquaculture species from Thailand [1]. Stripped catfish (family Pangasiidae, order Siluriformes) is one of the most important native catfish in Thailand, Malaysia, Indonesia and China [1]. Nowadays, Sutchi catfish has become the most popular and commercial cultured species because of its omnivorous feeding habits, fast growth, lucrative size, good taste and high market demand [2].

The aquaculture industry in Bangladesh is faced with the challenges of inadequate supply, and the high cost of quality fish feeds. Protein is the most expensive component in fish feeds and plays a vital role in the growth of fish [3]. About diet composition, minimizing protein levels while optimizing energy balance is especially important because these factors can influence feed intake and nutrient utilization and retention in fish. Dietary protein to energy ratio (P/E ratio) has great importance for the formulation of well-balanced and low-cost diets. If the dietary P/E ratio is unbalanced so that non-protein energy is inadequate, dietary protein may be catabolized and used as an energy source to satisfy maintenance before growth [3-4]. Providing properly balanced ratios of protein to non-protein energy in diets can spare dietary protein from energy metabolism and also increase its utilization for fish growth [5-9]. On the other hand, excessive non-protein energy can reduce feed intake, produce fatty fish and inhibits the utilization of other nutrients [10]. Not only the assessment of dietary protein and energy are necessary but also understanding the relationship between these two requirements is important. The balance between dietary P and E is needed to maintain high growth rate and good food conversion (FCR) efficiency, improve protein utilization, minimize excessive accumulation of

lipid and glycogen in somatic tissue and liver, decrease undesirable nitrogen waste output and improve the quality of fish farm effluents [11-12].
Commercial culture and production of these fish have currently been expanded dramatically, but the benefit is decreasing gradually due to high operating costs contributed mainly by feed. Lack of current information on their nutrition and optimum P/E ratio are significant constraints in the development of pangas culture. Keeping these above points into considerations, a feeding trial was carried out in the laboratory, and the objective of the present study was to evaluate dietary protein and energy interactions and their influence on growth, feed and protein utilization and body composition leading to optimization of P/E ratio for *P. hypophthalmus* fingerlings.

2. Materials and Methods

2.1 Experimental Animals

P. hypophthalmus (pangas) fingerlings were procured from Authentic Fish Hatchery, Mymensingh and acclimatized for two weeks in the Wet Laboratory, Freshwater station of Bangladesh Fisheries Research Institute, Bangladesh.

2.2 Experimental design and procedure

The feeding trial was carried out a series of cylindrical fiberglass tanks (70-L each) for 60 days at Freshwater Station, Bangladesh Fisheries Research Institute. The same aged uniform size fingerlings of *P. hypophthalmus* (Pangas) distributed into groups of 40 fish (averaging 2.55 ± 0.05g) per 70-L fiberglass tank and three replicate tanks used for each test diet following completely randomized design (CRD). Artificial aeration used to maintain the dissolved oxygen level. The fishes were weighed at the start and end of the experiment by weekly. Weekly samplings of fish were done to adjust the daily feed ration for the following week. Water quality parameters such as temperature, pH, dissolved oxygen, ammonia-N, nitrite-N, and nitrate-N were monitored through weekly sampling.

2.3 Experimental feeds and feeding

Six experimental diets were formulated to contain two levels of protein (25 and 30%), each with three levels of lipid (5, 10 and 15%), in order to produce a range of protein to energy ratios shown in Table 1. Fish meal and mustard oil cake were used as protein source. A lipid source was soybean oil. Starch and wheat flour were used as sources of carbohydrate. Alpha cellulose was used as filler and carboxymethyl cellulose will be used as a binder at a rate of 2%. Vitamin and mineral premix were added at a rate of 0.20%. The bite-sized (1.0-2.0 mm) pellet feeds were made with the help of hand pellet machine. The fishes were offered the experimental and control diets at the rate of 10-8% of their body weight and sub-divided into 3 equal feeds at 9.00, 13.30 and 18.00 h. Feeding rate was adjusted based on weekly sampling weights of fish.
At the onset of the experiment, 10 fishes were killed for analysis of initial carcass proximate composition. At the termination of the experiment, 10 fishes were taken from each replication for determination of whole body composition, organ indices, liver lipid, liver glycogen, digestive enzyme activities and growth performance.

2.4 Analytical methods

The water sample were analyzed for parameters like temperature, dissolved oxygen, pH, free carbon dioxide, total hardness, ammonia-N, nitrite-N and nitrate-N levels following 'APHA' procedures [13]. Experimental diet and fish carcass samples were analyzed for proximate composition by employing 'AOAC' procedures [14]. Gross energy of the experimental diet was determined using an Automatic Oxygen Bomb Calorimeter (Gallenkamp & Co Ltd., England). All the samples were analyzed in triplicate.
Specific growth rate (SGR), % weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU), viscerosomatic index (VSI) and hepatosomatic index (HSI) was calculated as follows:
Net gain (g) = Final body weight (g) – Initial body weight (g)
SGR (% / day) = [(Ln. Final body weight (g) – Ln. Initial body weight (g)) / days × 100]
%Weight gain = [(Final body weight (g) – Initial body weight (g)/Initial body weight (g)] × 100
FCR = Feed fed (g dry weight)/ Live weight gain (g)
PER = Live weight gain (g) / Crude protein fed (g dry weight)
ANPU = (Final carcass protein – Initial carcass protein) / Total dry protein consumed × 100
VSI = Weight of viscera (g) / Total body weight (g) × 100
HSI = Weight of liver (g) / Total body weight (g) × 100

Collection of the intestine with its content from fish and enzyme extraction for digestive enzyme assays was carried out as described by Al-Owafeir [15]. Amylase activity was assayed according to the procedure of Worthington [16]. Protease activity was determined by the casein digestion method as described by Walter [17] with some modifications [18]. Lipase activity was determined using the method of Bier [19] with some modifications [20]. Liver lipid was extracted following the method of Folch *et al*. [21] and liver glycogen was determined by a method modified by Seifter *et al*. [22] and Hassid and Abraham [23].

2.5 Analysis of data

The statistical analysis was carried out by using statistical software package SPSS (21.0 for Windows). One-way ANOVA and Duncan's multiple range tests were used to determine the significant differences between the means. Comparisons were made at the 5% probability level [24].

3. Results

Proximate compositions of different experimental diets are shown in Table 1.Optimum water quality parameters were provided to the experimental animals throughout the experimental period shown in Table 2. Growth increment and feed utilization of fish were influenced by the levels of dietary protein and energy as lipid shown in Table 3. Maximum growth rate could be achieved at P/E ratio of 16.33 mg protein kJ^{-1} GE that significantly higher ($P<0.05$) growth rates were attained fish fed at higher protein diets. However, the highest dietary energy level resulted in reduced growth performance. The highest growth performances and feed utilization were found by fish fed 30% dietary protein, 10% dietary lipid and 18.38 kJ^{-1} GE with a P/E ratio of 16.33 mg protein kJ^{-1} GE. Fish fed diet 5 (protein 30%, lipid 10% and P/E ratio 16.33) and diet 6 (protein 30%, lipid 15% and P/E ratio 14.75) had the similar performances in terms of weight gain,%weight gain, SGR, FCR, PER and ANPU. While the performances of the fishes fed with diet 1 were significantly ($P<0.05$) lower than other groups. Protein utilization efficiencies (PER and ANPU) were not significantly ($P>0.05$) different among the experimental diets (Table 3).

The carcass composition (% wet weight basis) of the experimental fishes at the end of the experimental period is presented in Table 4. The final carcass composition of *P. hypophthalmus* fingerlings of different experimental groups differs significantly ($P<0.05$) among the groups fed with different protein energy ratio with highest values observed in diet 5 and diet 6. But there was no significant difference ($P>0.05$) found between diet 3 and diet 6. The carcass crude protein content of the fishes fed with different experimental diets ranged between 16.13% and 17.79% ($P>0.05$) while carcass crude lipid content of the fishes ranged between 6.35% and 8.52% ($P<0.05$). There was an overall trend towards the higher whole body lipid content and lower body moisture with the increase in dietary energy level at each protein level, sometimes significantly (Table 3).

Table 1: Formulation and proximate composition of the experimental diets (% dry weight) for *Pangasianodon hypophthalmus* for experimental period of 60 days

Diets number						
Diet No.: (Protein / Lipid), (%)	**1 (25/5)**	**2 (25/10)**	**3 (25/15)**	**4 (30/5)**	**5 (30/10)**	**6 (30/15)**
Ingredients:						
Fishmeal	32.00	32.00	32.00	40.70	40.70	40.70
Mustard Oil Cake	15.00	15.00	15.00	15.00	15.00	15.00
Rich bran (auto)	10.00	10.00	10.00	8.00	8.00	8.00
Starch	38.80	34.10	29.10	32.10	27.40	22.40
Soybean Oil	00	4.70	9.70	00	4.70	9.70
Alpha cellulose	1.50	1.50	1.50	1.50	1.50	1.50
Binder (Carboxymethyl cellulose)	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin and Minerals Premix*	0.20	0.20	0.20	0.20	0.20	0.20
Proximate composition:						
Crude Protein	25.05	25.05	25.05	30.02	30.02	30.02
Crude Fat	5.14	10.04	15.04	5.20	10.03	15.03
Ash	10.60	10.60	10.60	12.79	12.79	12.79
Fibre	3.56	3.56	3.56	3.45	3.45	3.45
NFE	43.50	43.50	43.50	42.65	42.65	42.65
GE (kJ g^{-1})	15.50	17.36	19.38	16.52	18.38	20.35
P / GE ratio	16.16	14.43	12.96	18.17	16.33	14.75

*Vit-Min premix Composition of vitamin mineral premix (quantity/kg) Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000mg; Vitamin E, 750mg; Vitamin K, 1,000mg; Vitamin B6, 1,000mg; Vitamin B12, 6mg; Calcium Pantothenate, 2500mg; Nicotinamide, 10g; Choline Chloride, 150g; Mn, 27,000mg; I, 1,000mg; Fe, 7,500mg; Zn, 5,000mg; Cu, 2,000 mg; Co, 450mg, L-Lysine, 10g; DL-Methionine, 10g; Selenium, 125mg.

NFE = Nitrogen free extractives, calculated as 100 – (% protein +% Lipid +% Ash +% Fibre)

GE = Gross energy content in kJ g^{-1}

P / GE ratio = Protein to energy ratio in mg protein/ kJ^{-1} GE

Table 2: Water quality parameters of experimental groups for *Pangasianodon hypophthalmus* during the experimental period of 60 days

Parameters	1 (25/5)	2 (25/10)	3 (25/15)	4 (30/5)	5 (30/10)	6 (30/15)
Temperature (°C)	25.2-32.0	25.6-32.5	25.1-32.8	25.7-32.6	26.2-32.9	25.3-32.4
pH	7.6-8.3	7.6-8.0	7.5-8.1	7.7-8.4	7.6-8.2	7.5-8.0
Dissolved O_2 (mg L^{-1})	6.3-7.0	6.5-7.1	6.2-7.3	6.4-7.2	6.3-7.2	6.4-7.1
Free CO_2 (mg L^{-1})	1.1-1.4	1.2-1.4	1.0-1.3	1.1-1.5	1.2-1.5	1.1-1.5
Hardness (mg L^{-1})	233-250	227-255	235-252	230-250	239-258	225-254
Ammonia-N (mg L^{-1})	0.03-0.07	0.04-0.09	0.02-0.07	0.03-0.08	0.04-0.07	0.03-0.09
Nitrite-N (mg L^{-1})	0.001-0.004	0.001-0.003	0.002-0.004	0.001-0.004	0.002-0.003	0.002-0.004
Nitrate-N (mg L^{-1})	0.02-0.05	0.03-0.05	0.02-0.06	0.03-0.06	0.02-0.05	0.02-0.06

Table 3: Growth performances and nutrient utilization of *Pangasianodon hypophthalmus* fed with different protein energy ratio for experimental period of 60 days

Diet no.: (Protein / Lipid), (%)	1 (25/5)	2 (25/10)	3 (25/15)	4 (30/5)	5 (30/10)	6 (30/15)
Initial body wt. (g)	$2.58^{a}\pm0.04$	$2.55^{a}\pm0.07$	$2.58^{a}\pm0.04$	$2.55^{a}\pm0.07$	$2.53^{a}\pm0.04$	$2.55^{a}\pm0.07$
Final body wt. (g)	$14.70^{d}\pm0.71$	$16.15^{c}\pm0.35$	$17.10^{bc}\pm0.99$	$18.80^{b}\pm0.99$	$19.83^{a}\pm0.32$	$19.00^{a}\pm0.42$
Weight gain (g)	$12.13^{d}\pm0.74$	$13.60^{c}\pm0.28$	$14.53^{bc}\pm0.95$	$16.25^{b}\pm0.92$	$17.30^{a}\pm0.35$	$16.43^{a}\pm0.46$
Weight gain (%)	$471.12^{d}\pm35.31$	$533.39^{c}\pm31.70$	$563.88^{bc}\pm29.33$	$637.00^{b}\pm18.38$	$685.32^{a}\pm23.60$	$638.05^{a}\pm26.61$
SGR (% day)	$2.91^{d}\pm0.11$	$3.08^{c}\pm0.01$	$3.16^{bc}\pm0.08$	$3.33^{b}\pm0.04$	$3.44^{a}\pm0.05$	$3.33^{a}\pm0.06$
FCR	$1.76^{a}\pm0.02$	$1.69^{a}\pm0.01$	$1.59^{b}\pm0.05$	$1.50^{b}\pm0.05$	$1.36^{c}\pm0.03$	$1.45^{bc}\pm0.04$
PER	$2.28^{a}\pm0.05$	$2.32^{a}\pm0.08$	$2.30^{a}\pm0.05$	$2.35^{a}\pm0.07$	$2.55^{a}\pm0.08$	$2.40^{a}\pm0.10$
ANPU (%)	$39.27^{a}\pm0.25$	$39.55^{a}\pm0.38$	$39.10^{a}\pm0.35$	$42.40^{a}\pm0.50$	$43.85^{a}\pm0.33$	$43.47^{a}\pm0.28$

Mean values are represented as Mean±standard error obtained from three replicates. ^{abcd}Mean values having different superscripts in a row differ significantly ($P<0.05$). SGR, Specific growth rate; FCR, Feed conversion ratio; FCE, Feed conversion efficiency, PER, Protein efficiency ratio, ANPU, Apparent net protein utilization

Table 4: Final carcass composition (% wet weight basis) of *Pangasianodon hypophthalmus* at the end of the experimental period of 60 days

Treatments	Initial	1 (25/5)	2 (25/10)	3 (25/15)	4 (30/5)	5 (30/10)	6 (30/15)
Moisture	74.08±0.34	73.98^{a}±0.45	73.60^{b}±0.43	71.53^{d}±0.62	73.90^{a}±0.21	72.73^{c}±0.28	71.85^{de}±1.16
Crude Protein	15.58±0.41	16.65±1.26	16.78±0.69	16.13±0.55	17.11±0.40	17.79±0.52	17.33±0.44
Crude Lipid	6.29±0.12	6.35^{d}±0.11	6.56^{cd}±0.14	8.43^{ab}±0.04	6.61^{c}±0.11	8.18^{b}±0.09	8.52^{a}±0.08
Ash	2.51±0.13	2.55±0.19	2.47±0.24	2.58±0.15	2.71±0.17	2.63±0.09	2.61±0.13

Mean values are represented as Mean±standard error obtained from three replicates. ^{abcde}Mean values having different superscripts in a row differ significantly ($P<0.05$)

Liver lipid level (Table 5) increased with an increase in dietary lipid at each protein level and liver lipid levels were higher ($P<0.05$) at the lower protein diet. Liver glycogen content (Table 5) was highest ($P<0.05$) in fish fed diet 1 and lowest ($P<0.05$) in fish fed diet 6. There was an overall trend of decreasing liver glycogen content with increasing dietary energy level at each protein level. Higher protein diets resulted in decreased liver glycogen content. Viscerosomatic indices (VSI) and hepatosomatic index (HSI) did not vary significantly ($P<0.05$) among the test groups (Table 5). No significant difference ($P>0.05$) was found in intestinal digestive enzyme activities (amylase, protease and lipase) in different experimental groups (Table 6).

4. Discussion

The results of the study confirmed that the level of dietary protein and energy influenced growth performance and nutrient utilization in *P. hypophthalmus* fingerlings. From the growth performance data, the best growth was achieved at 30% dietary protein, 10% dietary lipid, 18.38 kJ g^{-1} GE and P/E ratio of around 16.33 mg protein kJ^{-1} GE (diet 5). P/E ratio of 20.5 (20.5 mg protein kJ^{-1} GE), 20.81 (20.81 mg protein kJ^{-1} GE) and 20.55 (20.55 mg protein kJ^{-1} GE) was suggested for optimum growth in asian catfish, *Clarias gariepinus* [25-26], channel catfish, *Ictalurus punctatus* [27], and walking catfish, *Clarias batrachus* [28], respectively. The variations of dietary P/E ratio depends on the type of fish, feeding habits, rearing conditions, environmental parameters and diet composition including the digestibility of feed ingredients [29]. However, increased dietary lipid level above 10% at the high protein level did not further improve growth performance in terms of%weight gain or SGR. Authors have reported that with an increase in dietary energy levels beyond the optimum requirement level decreases the body weight gain in flounder (*Paralichthys olivaceous*) [30]. Similar findings also reported by different authors in *C. gariepinus* [25-26].

Table 5: Liver lipid, liver glycogen (% wet weight basis), viscerosomatic index (VSI) and hepatosomatic index (HSI) of *Pangasianodon hypophthalmus* at the end of the experimental period of 60 days

Treatments	1 (25/5)	2 (25/10)	3 (25/15)	4 (30/5)	5 (30/10)	6 (30/15)
Liver lipid	1.13^{e}±0.03	1.22^{de}±0.04	1.59^{b}±0.06	1.28^{d}±0.05	1.42^{c}±0.08	1.71^{a}±0.06
Liver glycogen	1.07^{a}±0.04	0.86^{ab}±0.05	0.85^{ab}±0.04	0.95^{ab}±0.03	0.78^{b}±0.03	0.75^{b}±0.03
VSI	0.84±0.11	0.82±0.14	0.85±0.09	0.73±0.12	0.77±0.15	0.79±0.10
HSI	0.23±0.02	0.22±0.03	0.24±0.03	0.19±0.04	0.21±0.03	0.20±0.03

Mean values are represented as Mean±standard error obtained from three replicates. ^{abcde}Mean values having different superscripts in a row differ significantly ($P<0.05$), VSI, Viscerosomatic index, HIS, Hepatosomatic index

Table 6: Digestive enzyme (amylase, protease and lipase) activities of *Pangasianodon hypophthalmus* at the end of the experimental period of 60 days

Treatments	1(25/5)	2 (25/10)	3 (25/15)	4 (30/5)	5 (30/10)	6 (30/15)
Amylase	2.76±0.41	2.86±0.34	2.91±0.23	2.82±0.26	2.95±0.37	3.05±0.29
Protease	0.32±0.09	0.34±0.06	0.36±0.07	0.37±0.05	0.39±0.08	0.41±0.10
Lipase	3.96±0.37	4.07±0.17	4.18±0.29	3.99±0.22	4.02±0.21	4.19±0.31

The maximum%weight gain and SGR observed in fish fed with diet 5 and diet 6 is attributed to proper balance between protein and energy ratio. Lowest growth (%weight gain and SGR) was recorded for the lowest dietary energy (as lipid) both dietary protein diets (25% and 30%). At lowest dietary energy levels dietary protein may be used for energy production, as has been demonstrated in other *Clarias* species [25, 31] and other fishes [32-33]. The fixed ration level (8% of the body weight) used might have prevented the fish from consuming more feed to compensate for energy supply from low energy diets. As a result, fish presumably catabolized dietary protein to meet some of their requirements for energy rather than using it for growth increment. This could be attributed to lower feed intake by fish. Similarly, high energy content of the diet resulted in low protein intake [34] or to the hindrance of digestion and absorption of other nutrients by the high energy content in the diet [35].

The final carcass proximate composition analysis indicated that whole-body lipid increased with increasing dietary energy (as lipid) level at each protein level. These observations seem in general agreement with results reported earlier for African catfish, *C. gariepinus* [25, 26, 29]. A similar finding was also reported in common carp (*Cyprinus carpio*) [36].

An increase in dietary lipid at each protein level also resulted in greater accumulation of liver lipid as with whole-body lipid with the lower protein diets having the higher liver lipid deposition. The positive correlation noted between dietary energy and liver lipid content compares favourably with other results obtained for *C. gariepinus* [26, 37]. Liver glycogen content gradually decreased with increasing dietary lipid at each protein level. There was a tendency for greater deposition of glycogen in fish fed the lower protein diets. The observation seems in agreement with results reported for Sunshine bass [26, 38]. Higher carbohydrate levels possibly result in increasing liver glycogen accumulation in fish fed lower dietary protein diets (Ali & Jauncey, 2005). It has been reported for several fish species that liver glycogen content generally increased with increasing dietary carbohydrate [27, 39]. No changes in the activity of digestive enzymes (amylase, protease and lipase) with dietary lipid or protein level were apparent. This is in agreement with earlier findings on *C. gariepinus* [26].

5. Conclusion

From the present study, it may be concluded that Sutchi catfish, *P. hypophthalmus* performed best the diet containing 30% dietary protein, 10% dietary lipid and 18.38 kJ^{-1} GE with a P/E ratio of 16.33 mg protein kJ^{-1} GE respectively. The findings of this research may be useful in developing a cost effective, eco-friendly and nutritionally balanced practical diet for the pond culture system of the species.

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