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Dietary protein and energy interactions- an approach to optimizing dietary protein to energy ratio in walking catfish, *Clarias batrachus*

M. Zulfikar Ali*, M. Enamul Hoq, M. Mominuzzaman Khan and S.U. Ahammed
Bangladesh Fisheries Research Institute
Freshwater Station, Mymensingh 2201, Bangladesh
*Corresponding author. Email zulfikar_bfri@yahoo.com

Abstract
An 8 weeks feeding trial was conducted in a static indoor rearing system to investigate protein to energy ratio (P/E ratio) in walking catfish *Clarias batrachus*. Six fishmeal based diets of two protein levels (25 and 35%), each with three lipid levels (5, 10 and 15%) resulted in P/E ratios ranging from 13.57 to 21.97 mg protein kJ^{-1} gross energy (GE) were fed to 50 fish in triplicate. Fish were fed 6% of their body weight three times per day adjusted fortnightly. Significantly higher ($p<0.05$) growth rates in terms of weight gain, % weight gain and specific growth rate (SGR) were evident in fish fed with higher protein diet. The highest growth rate was found by fish fed 35% protein, 17.06 kJ^{-} GE with a P/E ratio of 20.55 mg protein kJ^{-1} GE. Significantly better ($p<0.05$) feed conversion ratio (FCR) was also evident in fish fed with higher protein diet and best FCR was found by fish fed 35% protein, 10% lipid, 17.06 kJ^{-1} GE with a P/E ratio of 20.55 mg protein kJ^{-1} GE. Significantly indifferent ($p>0.05$) values of protein utilisation were found in between the both (higher and lower) protein diets. Higher lipid deposition ($p< 0.05$) in whole body was observed with increasing dietary lipid level at each protein diet and as higher ($p<0.05$) for the lower protein diets. The study reveals that *C. batrachus* performed best the diet containing 35%, 17.06 kJ g^{-1} and 20.55 mg protein kJ g^{-1} GE protein, gross energy and P/E ratio respectively.

Key wards: *Clarias batrachus,* Protein to energy ratio, Dietary protein and lipid levels

Introduction

Dietary protein to energy ratio (P/E ratio) in fish diets is of great importance and evidence of the use of dietary protein as an important energy source is widespread (Cowey 1980, Wilson 1989). If the dietary P/E ratio is unbalanced so that non-protein energy is inadequate, dietary protein may be catabolised and used as an energy source to satisfy maintenance before growth (Cowey and Sargent 1979, NRC 1993). Conversely, if excess dietary energy is provided, feed consumption and protein intake may be limited. The foregoing discussion does not consider other possible physiological effect like different dietary protein levels and varying protein to energy ratios may impose on protein metabolism. In general, dietary protein requirements seem to be ≥ 35% for *Clarias batrachus* and somewhat higher for *Clarias gariepinus* and hybrid *Clarias* catfish (Machiels and Henken 1985, Degani *et al.* 1989, Singh and Singh 1992, Jantrarotai *et al.* 1996, Ali and Jauncey 2005). Unfortunately, dietary energy requirements of these works were not uniformly expressed (GE= gross energy, DE= digestible energy or ME= metabolizable energy) resulting ununiform comparisons between studies.

The walking catfish, *Clarias batrachus* is a promising species for aquaculture by virtue of its omnivorous feeding habits, air-breathing characteristics and higher market price. They are hardy, can survive in adverse aquatic environment and cultured with high stocking densities. Information on nutrient requirements and appropriate P/E ratios in walking catfish, *Clarias batrachus*, fed with fishmeal based practical diets are restricted. Therefore, it is important to estimate the optimum P/E ratio in a fishmeal based practical diets. Hence, the objective of the present study was to evaluate dietary protein and energy interactions and their influence on growth, feed and protein utilisation and body composition leading to optimisation of P/E ratio for *Clarias batrachus*.

Materials and methods

Experimental diets

Six experimental diets were formulated with two levels of protein (25 and 35%), each with three levels of lipid (5, 10 and 15%), to produce a range of P/E ratios. Gross energy contents (GE) of diets were ranging from 15.94 to 18.46 kJg^{-1} and P/E ratios ranged from 13.57 to 21.97 mg protein kJ^{-1} GE. Diets were referred to by two numbers separated by a '/', the first number being the dietary protein level and the second the lipid level. Ingredients and proximate composition of test diets, which are more practical for farming this species, are shown in Table 1.

Table 1. Formulation and proximate composition of the experimental diets (% dry weight basis)

	Diets					
Diet number	1	2	3	4	5	6
(Protein / Lipid), (%)	(25/5)	(25/10)	(25/15)	(35/5)	(35/10)	(35/15)
Ingredients						
Fish meal[1]	34.00	34.00	34.00	51.30	51.30	51.30
Mustard oil cake[2]	9.00	9.00	9.00	9.00	9.00	9.00
Rich bran (auto)[3]	9.00	9.00	9.00	6.60	6.60	6.60
Starch[4]	43.85	38.85	33.85	29.10	24.10	19.10
Alpha cellulose[5]	1.50	1.50	1.50	1.50	1.50	1.50
Soybean oil[6]	0.15	5.15	10.15	00	5.00	10.00
Binder (Carboxymethyl cellulose)[7,8]	2.30	2.30	2.30	2.30	2.30	2.30
Vitamin & minerals premix[9]	0.20	0.20	0.20	0.20	0.20	0.20

Proximate composition						
Crude protein	25.05	25.20	24.96	35.27	35.10	35.20
Crude fat	4.95	10.20	14.88	5.10	10.06	14.90
Ash	11.22	11.22	11.22	15.54	15.54	15.54
Fibre	3.46	3.46	3.46	3.31	3.31	3.31
NFE	48.38	43.38	38.38	33.12	28.12	23.12
GE (kJ g^{-1})	16.23	17.34	18.46	15.94	17.06	18.17
P/ GE ratio	15.43	14.45	13.57	21.97	20.55	19.27

NFE= Nitrogen free extractives, calculated as 100 – (% protein + % Lipid + % Ash + % Fibre);
GE= Gross energy content; P/ GE ratio= Protein to energy ratio in mg protein kJ^{-1} of GE.
[1] Crude protein: 60.05; crude fat: 4.54; fibre: 0.65; ash: 26.87.
[2] Crude protein: 34.78; crude fat: 9.03; fibre: 8.25; ash: 9.16.
[3] Crude protein: 16.39; crude fat: 28.42; fibre: 11.10; ash: 14.05.
[4] Marck Ltd., India. [5] Sigma, UK. [6] Teer, City group, Bangladesh. [7] Carboxymethyl cellulose – Sodium salt, high viscosity. [8] BDH, Poole, UK. [9] Novartis (BD) Ltd.

The all required amount of ingredients along with vitamin and mineral premix were weighed as per formulae of experimental diets (Table 1) and mixed homogenously. During mixing, oil was gradually poured into the mixture to assure homogeneity. Adequate amount of water was added to moisten the mixture to get a definite dough texture and then the mixture was extruded through 1 mm diameter die of a pellet machine (Hobart mixture machine, Model A200). The resultant feeds were then sun dried. The experimental diets were separately packed in air-tight polyethylene bag and stored in a deep freeze for further use.

Experimental system and animals

The experiment was conducted in a static indoor rearing system with 18 cylindrical fibre glass tanks (80 cm diameter, 75 cm deep, 70-L each). Artificial aeration was used to maintain an adequate level of dissolve oxygen in each test tank. About sixty percent of the water in the system was replaced biweekly to avoid accumulation of waste products. Water quality parameters such as temperature (27.00-34.50^{0}C), pH (6.65–8.10), dissolved oxygen (5.50–7.50 mg/L) and ammonia (0.12–0.29 mg/L) remained within acceptable ranges for *Clarias batrachus* (Viveen *et al.* 1985, Hoffman *et al.* 1991). Eight hundred 10-week old (average weight 1.15±0.05 g) for *Clarias batrachus* fingerlings were collected locally. Before starting the experiment the fish were acclimatized to the experimental condition for two weeks.

Experimental procedure

Fish were randomly assigned into groups of 50 per 70-L cylindrical fibre glass tank. Each dietary treatment had three replications and the experiment was conducted for 8 weeks. The fish were individually weighed at the start and at the end of the experiment and bulk-weighed by tank fortnightly in between. Fortnightly bulk weights were used to adjust the daily feed ration for the following 2 weeks. The fish were offered the test diets three times daily at the rate of 6% of their body weight and sub-divided into three equal feeds at 9.30, 13.00 and 17.00 h. At the onset of the experiment, 15 fish were sacrificed for analysis of initial carcass composition. At the termination of the experiment, 4 fish were taken from each replication for determination of whole body composition.

Analytical methods and analysis of data

Proximate composition of diet ingredients, diets and whole body fish were analysed following AOAC (1990) methods. Nitrogen-free extract (NFE) was calculated by difference. Gross energy was calculated according to Jauncey (1998). All samples were analysed in triplicate. Specific growth rate (SGR), %weights gain, food conversion efficiency (FCR), protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) were calculated as follows:

SGR (%/day) = [(Ln. Final body weight – Ln. Initial body weight)/days xí 100]

% Weight gain = (Final body weight – Initial body weight/Initial body weight) x 100

FCR = Food 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 x 100

ANEU(%) = (Final carcass energy – Initial carcass energy)/Total dietary energy consumed x 100

The growth performance, feed utilization, and whole body composition data were analyzed using one way ANOVA. Paired mean comparisons among the treatments were made using Duncan's Multiple Range Tests (Duncan 1955). A significance level of $p<0.05$ was used. Standard deviation (±SD) was calculated to identify the range of means. Percentage data were arc-sine transformed (Zar 1984) prior to ANOVA and reversed afterward. Third order polynomial regression analysis (Zeitoun *et al.* 1976) was employed to determine regression of specific growth rate (SGR, % day) on protein to energy ratio (P/E ratio).

Results

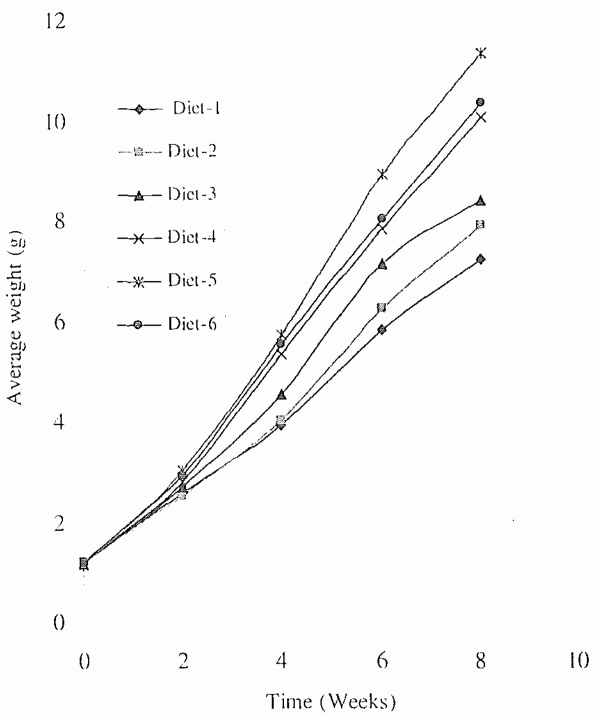
Growth performances in terms of final body weight, mean weight gain, specific growth rate (SGR, % day) and feed utilisation of fish fed the experimental diets were influenced by the levels of protein and energy as lipid (Table 2). The increase in average fortnightly fish weight is shown in Fig. 1. The relationship between SGR and P/E ratio at both protein levels depicted through a polynomial curve (Fig. 2) indicates that, with the form of energy provided, maximum growth rate could be achieved at P/E ratio of 20.55. Significantly higher ($p<0.05$) growth rates were attained fish fed at higher protein diets. However, the highest dietary energy level resulted in reduced performance. The highest growth performances and feed utilisation were found by fish fed 35% protein, 10% lipid and 17.06 kJ^{-1} GE with a P/E ratio of 20.55 mg protein kJ^{-1} GE. FCR values ranges between 1.25 to 1.66 with fish fed diet 5 (35% protein, 10% lipid and 17.06 kJ^{-1} GE with a P/E ratio of 20.55 mg protein kJ^{-1} GE) showing significantly ($p<0.05$) the lowest i.e. the best FCR. The fish fed the high protein with the highest lipid diet (6) exhibited a higher in FCR (Table 2). Protein utilisation efficiencies (PER and ANPU) did not significantly ($p>0.05$) different among the experimental diets. Fish fed diet 5 (protein 35%, lipid 10% and P/E ratio 20.55) had the highest ANPU value and diet 1 the lowest ($p>0.05$) (Table 2).

Table 2. Growth performance, feed and protein utilization of *Clarias batrachus* fed diets containing various P/E ratios for 8 weeks

	Diets					
Diet number (Protein / Lipid), (%)	1 (25/5)	2 (25/10)	3 (25/15)	4 (35/5)	5 (35/10)	6 (35/15)
Initial body wt. (g)	1.15^{a} ±0.07	1.18^{a} ±0.04	1.15^{a} ±0.07	1.15^{a} ±0.07	1.13^{a} ±0.04	1.18^{a} ±0.04
Final body wt. (g)	7.20^{d} ±0.42	7.88^{cd} ±0.25	8.40^{c} ±0.28	10.05^{b} ±0.35	11.33^{a} ±0.60	10.35^{b} ±0.21
Weight gain (g)	6.05^{d} ±0.35	6.70^{cd} ±0.21	7.30^{c} ±0.35	8.90^{b} ±0.28	10.20^{a} ±0.57	9.18^{b} ±0.18
Percent weight gain (%)	526.14^{d} ±2.61	565.20^{cd} ±17.96	636.93^{c} ±69.90	774.62^{b} ±23.04	906.33^{a} ±21.80	780.98^{b} ±8.46
Specific growth rate (SGR) (% day)	3.06^{d} ±0.01	3.18^{cd} ±0.01	3.33^{c} ±0.16	3.62^{b} ±0.05	3.85^{a} ±0.04	3.63^{b} ±0.01
Food conversion ratio (FCR)	1.66^{a} ±0.02	1.59^{a} ±0.01	1.48^{b} ±0.05	1.40^{b} ±0.05	1.25^{c} ±0.03	1.35^{bc} ±0.04
Protein efficiency ratio (PER)	2.38^{a} ±0.05	2.52^{a} ±0.08	2.40^{a} ±0.05	2.45^{a} ±0.07	2.65^{a} ±0.08	2.50^{a} ±0.10
Apparent net protein utilization (ANPU, %)	37.25^{a} ±0.25	37.55^{a} ±0.38	38.10^{a} ±0.35	40.60^{a} ±0.50	43.25^{a} ±0.33	41.27^{a} ±0.28

Note: Values are ± SD of three replications. Figures in the same row having different superscript are significantly different ($p < 0.05$).

There was a trend towards higher whole body lipid content and lower body moisture content with increasing dietary energy level at each protein level, sometimes significantly Whole body protein content did not significantly ($p>0.05$) different among the experimental diets (Table 3). Body lipid content was positively correlated ($Y= - 8.6 + 0.83X$; $r = 0.16$; $p<0.05$) with dietary energy content.



Diets 1, 2, 3, 4, 5 and 6 contained P / E ratio 15.43, 14.45, 13.57, 21.97, 20.55 and 19.27 mg protein per kJ^{-1} GE respectively.

$$Y = 54.88 - 8.98X + 0.51X^2 - 9.48E - 03X^3$$

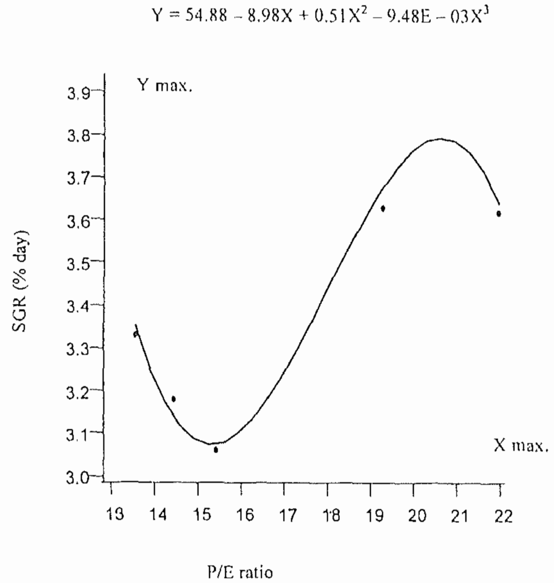


Fig. 1. The mean fortnightly growth response of *Clarias batrachus* maintained on the six experimental diets over 8 weeks.

Fig. 2. The third order polynomial regression of specific growth rate (SGR, % day) with dietary protein to energy ratio (P/E ratio) in *Clarias batrachus*

Discussion

The results of the present study demonstrated that the level of dietary protein and energy influenced growth performance in *Clarias batrachus*. From the growth performance data and the third order polynomial regression analysis it observed that the best growth was achieved at 35%protein, 10% lipid, 17.06 kJ g^{-1} GE and P/E ration of around 20.55 (diet 5). A P/E ratio of 20.55 (20.55-mg protein kJ^{-1} GE) is suggested for optimum growth, in agreement with the related species *Clarias gariepinus* (Ali and Januncey 2005, Machiels and Henken 1985). However, increased dietary lipid above 10% at the high protein level did not further improve fish growth in terms of percent weight gain or SGR. Similar trends have been obtained with previous studies in *C. gariepinus* (Machiels and Henken 1985, Ali and Januncey 2005).

Table 3. Whole body composition (% wet weight basis) *Clarias batrachus* at the start and end of the experiment

		Diets					
Diet number (Protein/Lipid, %) Parameters	Initial	1 (25/5)	2 (25/10)	3 (35/5)	4 (35/10)	5 (45/5)	6 (45/10)
Moisture	73.15	72.20^{b} 1.22	69.62^{ab} ±0.82	68.65^{a} ±0.62	72.53^{b} ±1.24	70.59^{ab} ±0.92	69.92^{ab} ±0.34
Crude protein	14.53	15.26^{a} ±1.12	15.75^{a} ±0.71	15.08^{a} ±0.42	16.76^{a} ±0.74	16.29^{a} ±0.54	16.16^{a} ±0.64
Crude lipid	6.56	6.15^{b} ±0.05	8.87^{c} ±0.58	9.62^{d} ±0.19	5.34^{a} ±0.46	7.05^{b} ±0.48	8.87^{bc} ±0.36
Ash	2.38	2.55^{a} ±0.26	2.43^{a} ±0.15	2.58^{a} ±0.19	2.52^{a} ±0.31	2.32^{a} ±0.12	2.54^{a} ±0.28

Note: Values are ± SD of three replications. Figures in the same row having different superscript are significantly different ($p<0.05$).

At both dietary protein diets, poorest growth (% weight gain, SGR) was recorded for the lowest energy (as lipid) diet. At low dietary energy levels protein may be used for energy, as has been demonstrated in other *Clarias* species (Machiels and Henken 1985, Jantrarotai *et al.* 1998) and other fishes (Hassan *et al.* 1995, Yamamoto *et al.* 2000). The fixed ration level (6% of 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 catabolised dietary protein to meet some of their requirements for energy rather than using it as growth.

In the present study changes in feed conversion ratio (FCR) was improved with the higher protein diets. At the lower protein level FCR improved with increased lipid/energy level in the diet. At the high protein level FCR was best for the intermediate (10% lipid) diet (5). Improved FCR, up to a certain level of dietary energy inclusion (through lipid), has also been reported by earlier workers (Hassan *et al.* 1995, Jantrarotai *et al.* 1998, Yamamoto *et al.* 2000). *C. batrachus* fed the high protein with the highest lipid diet (6) exhibited poor FCR. This could be attributed to lower feed intake by fish. The high energy content of the diet, resulted in low protein intake (Grove *et al.* 1978), or to the hindrance of digestion and absorption of other nutrients by the high energy content in the diet (Dupree *et al.* 1979). Whole body composition analysis indicated that whole body lipid increased and moisture decreased with increasing dietary energy (as lipid) level at each protein level. Whole body lipid levels were higher in fish fed the lower protein diets. These observations seem in general agreement with results reported earlier for African catfish, *Clarias gariepinus* (Machiels and Henken 1985, Henken *et al.* 1986, Ali and Jauncey 2005).

On the basis of growth performance, feed utilisation and whole body composition, it may be stated that the diet 5, containing 35% and 17.06 kJ/g protein and gross energy respectively, performed best. This diet presumably contained the most appropriate P/E ratio 20.55 (20.55 mg protein/ kJ of GE) in *Clarias btachus*. This study reveals that addition of dietary energy (lipid) at either protein level resulted in increases in growth and feed performance but that above 17.06 kJ g^{-1} GE at 35% protein performance was reduced. In conclusion, walking catfish *C. batrachus* performed best the diet containing 35%, 17.06 kJ g^{-1} and 20.55 mg protein kJ g^{-1} GE protein, gross energy and P/E ratio respectively.

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