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Effects of dietary protein levels on growth, feed utilization, protein retention efficiency and body composition of young *Heteropneustes fossilis* (Bloch)

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Abstract An 8-week growth trial was conducted to assess the effect of dietary protein on growth, feed utilization, protein retention efficiency, and body composition of young *Heteropneustes fossilis* (10.02 ± 0.09 g; 9.93 ± 0.07 cm). Isocaloric (4.15 kcal g^{-1}, GE) diets with varying levels of protein (25, 30, 35, 40, 45, and 50% of the diet) were fed near to satiation to triplicate groups of fish. Optimum dietary protein was determined by analyzing live weight gain (LWG%), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR%), and protein retention efficiency (PRE%) data. Maximum LWG% (167), best FCR (1.42), PER (1.75), SGR (1.76), and PRE (31.7%) were evident in fish fed 40% protein diet (Diet 4). Body protein data also supported the above level. However, second-degree polynomial regression analysis of the above data indicated that inclusion of dietary protein in the range of 40–43% is optimum for the growth of young *H. fossilis*.

Keywords Dietary protein levels · Growth · *Heteropneustes fossilis* · Protein retention efficiency

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Introduction

The intensification of fish culture has led to dependence on artificial feeds. Protein is the most expensive component in fish feeds and also the most important factor affecting growth performance of fish and feed cost (Lovell 1989; Luo et al. 2004). Reducing the feeding costs could be key factor for successful development of aquaculture. Fish have high dietary protein requirement (Deng et al. 2006). The significance of qualitative and quantitative feeds is well recognized (Jauncey 1982; Mohanty and Samantary 1996; Gunasekera et al. 2000; Yang et al. 2002; Giri et al. 2003; Deepak and Garg 2003; Yang et al. 2003; Sales et al. 2003; Kalla et al. 2004; Islam and Tanaka 2004; Luo et al. 2004; Cortes-Jacinto et al. 2005; Kim and Lee 2005; Tibbetts et al. 2005; Sa et al. 2006). Level of dietary protein is of fundamental importance, because it significantly influences growth, survival, and yield of fish as well as economics of a farming industry by determining the feed cost which is typically the largest operational cost. Increase in dietary protein has often been associated with higher growth rate in many species. However, there is a certain level beyond which further growth is not supported, and may even decrease (Mohanty and Samantary 1996; Shiau and Lan 1996; McGoogan and Gatlin 1999; Gunasekera et al. 2000; Kim and Lall 2001; Yang et al. 2002; Abbas et al. 2005; Debnath et al. 2007; Kvale et al. 2007). Considerable research effort has been

expended to determine the quantity and quality of dietary protein necessary to achieve optimum performance of fish.

Heteropneustes fossilis is an important tropical freshwater food fish (Mohamed and Ibrahim 2001) and its culture has gained attention because of its ability to efficiently utilize animal and plant origin feedstuffs and withstand adverse environmental conditions, in addition to its medicinal value and market potential for intensive culture (Pillay 1990; Jhingran 1991; Thakur 1991). Apart from these factors, it has exceptional tolerance to high ammonia and low oxygen for several months, inhabits derelict and stagnant, slow-flowing, water bodies, agricultural fields, or swamps and wetlands (Saha and Ratha 1998, 2007) which make it a successful candidate for aquaculture. Information on the basic nutritional requirements and feeding of *H. fossilis* is needed in view of emphasis on catfish culture in the country (Tripathi and Das 1976; Dehadrai and Thakur 1980). Protein requirements are generally higher for smaller fish. As fish grow larger, their protein requirements usually decrease. Information on some aspects of nutrient requirement of *H. fossilis* is available (Sing and Srivastava 1984; Akand et al. 1991; Anwar and Jafri 1992; Firdaus et al. 1994, 2002; Firdaus and Jafri 1996; Firdaus 1993; Jhingran 1991; Niamat and Jafri 1984; Mohamed 2001; Mohamed and Ibrahim 2001; Usmani and Jafri 2002; Usmani et al. 2003). Although information on effect of dietary protein level on growth, feed conversion, and body composition of Singhi *H. fossilis* fry (Akand et al. 1989) is available, no published data is available on this aspect of the young stage of *H. fossilis*. The present study was therefore conducted to optimize the dietary protein level for growth, protein retention efficiency, and body composition of young *H. fossilis*. The information will be useful in developing protein balanced diet for the culture of this fish species.

Materials and methods

Preparation of experimental diets

Six casein-gelatin based isocaloric (414.83 kcal/100 gross energy) diets containing graded levels of protein (25, 30, 35, 40, 45, and 50% crude protein) were formulated (Table 1). Diets were prepared taking into account the amount of protein contributed by casein and gelatin and made isocaloric by adjusting the dextrin. Calculated quantities of dry ingredients were thoroughly mixed and stirred in a volume of hot water (80°C) in a steel bowl attached to a Hobart electric mixer (Hobart, Troy, OH, USA). Gelatin powder was dissolved separately in a volume of water with constant heating and stirring and then transferred to the above mixture. Other dry ingredients and oil premix, except carboxymethyl cellulose, were added to the lukewarm bowl one by one with constant mixing at 50°C temperature. Carboxymethylcellulose was added last and the speed of the blender was gradually increased as the diet started to harden. The final diet, with the consistency of bread dough, was poured into a Teflon-coated pan and placed in a refrigerator to gel. The prepared diets were in the form of semi-moist cake (50% dry matter) from which cubes were cut and stored at −4°C in sealed polythene bags until used.

Feeding trial

Induced bred *H. fossilis* were procured from a local fish hatchery. These were transported to the wet laboratory, given a prophylactic dip in $KMnO_4$ solution (1:3,000), and stocked in circular aluminium plastic lining [Plastic Crafts, Mumbai, India; 4 × 3 × 3 (c. 1.2 × 0.9 × 0.9 m)] fish tanks (water volume 600 l) for 2 weeks. During this period, the fish were fed to satiation a mixture of soybean, mustard oil cake, rice bran, and wheat bran in the form of moist cake twice a day at 0700 and 1700 hours. These were then acclimatized for 1 week on casein-gelatin based (40% CP) H-440 diet (Halver 2002) near to satiation.

Young *H. fossilis* (10.02 ± 0.09 g; 9.93 ± 0.07 cm) were stocked randomly in triplicate groups in 70-l circular polyvinyl troughs (water volume 55 l) fitted with a continuous flow-through system at the rate of 10 fish per troughs for each dietary treatment levels. The fish were fed experimental diets to apparent satiation divided over two feeding schedule at 0700 and 1700 hours. No feed was offered to the fish on the day they were weighed. The feeding trials lasted for 8 weeks. Initial and weekly body weights were recorded on a top loading balance (Precisa 120A; PAG Oerlikon, Zurich, Switzerland). Troughs were siphoned off to remove fecal matter before

Table 1 Composition of experimental diets

Ingredients (g 100 g^{-1}, dry diet)	Diet (%)					
	1	2	3	4	5	6
Casein[a]	25	30	35	40	45	50
Gelatin[b]	6.25	7.50	8.75	10.0	11.25	12.5
Dextrin[c]	49.74	40.96	32.18	23.39	14.61	5.83
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0
Cod liver oil	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix[d]	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix[d,e]	3.0	3.0	3.0	3.0	3.0	3.0
α-Cellulose	–	2.53	5.07	7.6	10.14	12.67
Carboxymethyl cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Total	99.994	99.993	100	99.996	100	100
Calculated crude protein	25.0	30.0	35.0	40.0	45.0	50.0
Analyzed crude protein	24.93	29.19	34.45	39.65	44.15	49.03
Gross energy[f] (kcal/100 g, dry diet)	414.83	414.83	414.83	414.83	414.83	414.83
Estimated gross energy (kcal/100 g, dry diet)	411.0	399.21	402.0	415.1	412.67	414.0

[a] Crude protein (80%), Loba Chemie, India

[b] Crude protein (97%), Loba Chemie, India

[c] Loba Chemie, India

[d] Halver (2002); 1 g vitamin mix + 2 g α-cellulose

[e] 1 g vitamin mix + 2 g α-cellulose

[f] Calculated on the basis of fuel values 5.52, 4.83, 3.83, and 9.00 kcal/g for casein, gelatin, dextrin, and fat, respectively, as estimated on Gallenkamp ballistic bomb calorimeter

feeding daily. Any uneaten feed was siphoned off immediately, dried in a hot air oven and reweighed to measure the amount of feed consumed.

Water quality assessment

Water quality indices were monitored daily during the feeding trial. Water in each trough was sampled and analyzed for the average water temperature, dissolved oxygen, free carbon dioxide, pH, and total alkalinity, and were recorded following standard methods (APHA 1992). The average water temperature, dissolved oxygen, free carbon dioxide, pH, and total alkalinity based on daily measurements were 27.5–28.9°C, 67–7.1, 5.5–10.7, 7.5–7.8, and 65.7–80.5 mg l^{-1}, respectively.

Statistical analyses

All growth data were subjected to analysis of variance (Snedecor and Cochran 1968; Sokal and Rohlf 1981). Differences among treatment means were determined by Duncan's Multiple Range Test at a $P < 0.05$ level of significance (Duncan 1955). To predict more accurate responses to the dietary protein intake, the optimum level was estimated using second-degree polynomial regression analysis ($Y = aX^2 + bX + c$) as described by Zeitoun et al. (1976). Statistical analysis was done using Matlab (version 7.1) and SPSS (version 13.0).

Chemical analyses

Proximate composition of casein, gelatin, experimental diets, and initial and final carcass was analyzed using standard methods (AOAC 1995) for dry matter (oven drying at 105 ± 1°C for 22 h), crude protein (N-Kjeldahl X 6.25 using Kjeltec Tecator™; Technology 2300, Foss, Hoeganaes, Sweden), crude fat (solvent extraction with petroleum ether B.P. 40–60°C using Socs Plus; Pelican equipments, Chennai, India, for 2–3 h) and ash (oven incineration at 650°C for 4–6 h using muffle furnace; S.M. Scientific Instrument (p), Jindal Company, India). Gross energy

content was determined on a Gallenkamp ballistic bomb calorimeter-CBB 330 010L (Gallenkamp, Loughbrough, UK). Six subsamples of a pooled sample of 10 fish were analyzed for initial and final body composition. At the end of the experiment, five fish from each replicate of dietary treatments were pooled separately and three subsamples of each replicate ($n = 3 \times 3$) analyzed for final body composition.

Results

Growth parameters of young *H. fossilis* fed diets containing graded levels of protein are presented in Table 2. Live weight gain (LWG%), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR%), and protein retention efficiency (PRE%) were sensitive to the levels of protein in the diet and improved significantly ($P < 0.05$) as dietary protein level increased from 25 to 40% whereas at 45% dietary protein growth responses were almost constant or slightly reduced. However, a significant fall in growth and conversion efficiencies was noted at 50% protein of the diet. Maximum LWG (167%), best FCR (1.42), PER (1.75), SGR (1.76%), and PRE (31.7%) were obtained for the fish fed diet with 40% protein. Fish fed lower levels of protein in the diet exhibited significantly less ($P < 0.05$) growth and reduced feed utilization efficiency. Poorest FCR (3.52), PER (1.14), SGR (1.04) and PRE (14%) were observed for fish fed diet containing 25% protein (Diet 1).

At the end of the experiment, significant differences ($P < 0.05$) in body composition were noted among the groups (Table 3). Moisture content showed a continuous decrease with the increase in the levels of dietary protein. Maximum body protein was recorded for fish fed diet containing 40% protein (Diet 4). Fish fed diets containing 45% protein diet did not show any significant change ($P > 0.05$) in growth parameters and body protein content. However, significant falls in growth parameters and body protein were recorded in fish fed diet containing 50% protein (Diet 6). Similar trend was evident for protein retention efficiency (PRE%). However, a significant and continuous increase in body fat of the fish was noted with the increase in protein content of the diet from 25% (Diet 1) to 50% (Diet 6). Significant differences ($P > 0.05$) in ash content were evident in fish fed 25% (Diet 1) and 30% (Diet 2) protein diets whereas it remained non-significant ($P > 0.05$) for the groups receiving other diets (Diets 3–6).

On subjecting the LWG% data to second-degree polynomial regression analysis (Zeitoun et al. 1976), the optimum dietary protein level was found to be at 42.75% protein of the diet. The relationship was described by the following equations:

$$Y = -0.272X^2 + 23.256X - 338.177 (R^2 = 0.950)$$

The FCR of young *H. fossilis* fed 40 and 45% protein was significantly lower than those of the other dietary

Table 2 Growth and conversion efficiency of young *Heteropneustes fossilis*

	Dietary protein levels (% of the diet)					
	25	30	35	40	45	50
Average initial weight (g)[1]	10.03 ± 0.01[a]	10.08 ± 0.01[a]	10.04 ± 0.01[a]	10.05 ± 0.01[a]	10.05 ± 0.02[a]	10.14 ± 0.07[a]
Average final weight (g)[1]	17.95 ± 0.06[d]	20.85 ± 0.05[c]	23.65 ± 0.02[b]	26.87 ± 0.06[a]	26.18 ± 0.07[a]	23.92 ± 0.02[b]
Live weight gain[1,2]	79.03 ± 6.3[d]	106.9 ± 10.9[c]	135.5 ± 9[b]	167.4 ± 6[a]	160.4 ± 7[a]	140.6 ± 8[b]
Food conversion ratio[1,2,3]	3.52 ± 0.02[a]	2.55 ± 0.04[b]	1.89 ± 0.01[c]	1.42 ± 0.01[d]	1.43 ± 0.02[d]	1.50 ± 0.04[e]
Protein efficiency ratio[1,2,4]	1.14 ± 0.01[d]	1.30 ± 0.03[c]	1.51 ± 0.09[b]	1.75 ± 0.24[a]	1.55 ± 0.03[b]	1.34 ± 0.03[b]
Specific growth rate (%)[1,2,5]	1.04 ± 0.08[d]	1.29 ± 0.01[c]	1.52 ± 0.09[b]	1.76 ± 0.05[a]	1.71 ± 0.02[a]	1.53 ± 0.02[b]
Feed intake g dry diet	278.8 ± 0.12	274.6 ± 0.08	257.3 ± 0.04	238.8 ± 0.14	230.7 ± 0.12	206.7 ± 0.09

[1] Mean values of 3 replicates ± SEM. Mean values sharing the same superscripts in a row are not significantly different ($P > 0.05$)

[2] Live weight gain = Final body weight − Initial body weight/Initial body weight × 100

[3] FCR = Dry feed fed / Weight gain

[4] PER = Wet weight gain / Protein fed

[5] SGR% = 100 × (ln Final body weight − ln Initial body weight) / Number of days

Table 3 Body composition of young *H. fossilis*

	Initial	Dietary protein levels (% of the diet)					
		25	30	35	40	45	50
Moisture	76.98 ± 0.22	75.01 ± 0.11^{a}	74.55 ± 0.10^{b}	73.67 ± 0.15^{c}	72.87 ± 0.05^{d}	72.02 ± 0.30^{d}	71.58 ± 0.12^{e}
Protein	11.92 ± 0.22	12.11 ± 0.33^{d}	12.95 ± 0.24^{c}	14.69 ± 0.30^{b}	15.76 ± 0.15^{a}	15.42 ± 0.60^{a}	14.30 ± 0.60^{b}
Fat	5.54 ± 0.05	6.01 ± 0.10^{f}	6.99 ± 0.10^{e}	7.23 ± 0.03^{d}	7.98 ± 0.10^{c}	9.1 ± 0.15^{b}	10.91 ± 0.16^{a}
Ash	3.63 ± 0.05	4.23 ± 0.04^{a}	3.87 ± 0.40^{b}	3.49 ± 0.10^{c}	3.45 ± 0.03^{c}	3.50 ± 0. 40^{c}	3.48 ± 0.04^{c}
Protein retention Effiency %	–	14.0 ± 0.10^{e}	18.1 ± 0.14^{d}	25.2 ± 0.17^{b}	31.7 ± 0.12^{a}	27.20 ± 0.22^{b}	21 ± 0.20^{c}

Mean values of three replicates ± SEM ($n = 3 \times 3$). Mean values sharing the same superscripts in a row are not significantly different ($P > 0.05$)

protein levels. The FCR (Y) to dietary protein level (X) relationship was estimated by the following second-degree polynomial regression equation:

$$Y = 0.006X^2 - 0.502X + 12.541 (R^2 = 0.997)$$

Based on the above equation, the estimated FCR occurred at approximately 41.5% protein of the diet.

The PER of young *H. fossilis* fed 40% protein diet differed significantly from the other levels of dietary protein inclusion. The PER (Y) to dietary protein level (X) relationship was estimated by the second-degree polynomial regression equation:

$$Y = -0.0025X^2 + 0.198X - 2.319 (R^2 = 0.861)$$

Based on the above equation, the estimated PER occurred at a dietary protein level of approximately 40.0% of the diet.

Also, the SGR of young *H. fossilis* fed 40 and 45% protein diet were significantly higher than those of the other levels of dietary protein inclusion. The SGR (Y) to dietary protein level (X) relationship was estimated by the following second-degree polynomial regression equation:

$$Y = -0.00234X^2 + 0.198X - 2.486 (R^2 = 0.960)$$

Based on the above equation, the estimated SGR occurred at a dietary protein level of approximately 42.3% of the diet.

Similarly, the PRE% of young *H. fossilis* fed 40% protein diet differed significantly from the other levels of dietary protein inclusion. The PRE% (Y) to dietary protein level (X) relationship was estimated by the following second-degree polynomial regression equation:

$$Y = -0.070X^2 + 5.645X - 85.329 (R^2 = 0.882)$$

Based on the above equation, the estimated PRE% occurred at a dietary protein level of approximately 40.3% of the diet.

On the basis of the above polynomial equations, the maximum LWG%, best FCR, PER, SGR, and highest PRE% occurred at 42.75, 41.5, 40.0, 42.3, and 40.3% protein in the diet, respectively.

Discussion

In the present study, growth and conversion efficiencies increased with increasing dietary protein levels from 25 to 40%. Although growth of the fish levelled off when reared on 45% protein in the diet, the decline in growth was statistically insignificant ($P > 0.05$) and, hence, feeding fish with 45% protein in the diet would be uneconomical. Therefore, inclusion of 40% protein in the diet for young *H. fossilis* is appropriate. However, a significant fall in growth and conversion efficiencies was noted at 50% protein of the diet indicating that 40% protein diet (Diet 4) satisfied the requirement and is considered optimum for achieving maximum growth and excellent conversion efficiency. A similar trend has been observed in many other fish species irrespective of culture strategies (Jauncey 1982; Cho et al. 1985; Khan and Jafri 1991; Vergara et al. 1996; Bai et al. 1999; Ng et al. 2001; Kim et al. 2002; Kim and Lee 2005; Wang et al. 2006). The decline in growth performance at protein level above 40% can be attributed to the fact that the fish body cannot utilize

dietary protein once the optimum level has been reached (Phillips 1972). Excess protein in the diet could reduce the performance due to higher energy requirement for catabolism rather than for protein deposition. Once protein is catabolized, the nitrogen fraction of the amino acids are excreted by deamination which leads to the release of amino groups that cannot be recycled through metabolic processes. The amino groups, therefore, must be excreted which is done at the expense of energy resulting to the lesser utilization of energy for growth purposes and more towards excretion and deamination of the excess amino nitrogen resulting from the excess amount of dietary protein intake. Therefore, from a metabolic point of view, it could be said that dietary protein beyond 40% could not be used for body protein synthesis or tissue building in fish and was being oxidized to produce energy to deaminate and excrete the extra nitrogenous load from the body. However, the amount of NH_3 in the surrounding water of the fish fed high protein diet might have not affected the growth and health status of the fish, as the feeding trial was conducted in troughs fitted with water flow-through system at 1–1.5 l/min. The decrease in weight gain at protein levels above the optimum level may also be because of a reduction in available energy for growth and due to inadequate non-protein energy necessary to deaminate and excrete excess absorbed amino acids (Jauncey 1982; Cho et al. 1985; Vergara et al. 1996; Kim et al. 2002) Decrease in the protein utilization beyond requirement level of dietary protein is a well-documented phenomenon (Jobling and Wandshik 1983; Daniels and Robinson 1986; Tibbetts et al. 2000; Catacutan et al. 2001; Ng et al. 2001; Kim et al. 2002; Lee et al. 2002; Yang et al. 2002; Deepak and Garg 2003; Sales et al. 2003; Yang et al. 2003; Islam and Tanaka 2004; Kalla et al. 2004; Luo et al. 2004; Cho et al. 2005; Jacinto et al. 2005; Kim and Lee 2005; Tibbetts et al. 2005; Sa et al. 2006). In the present study, PER and protein retention efficiency increased with the increase in dietary protein content up to 40% and then decreased with further elevation of dietary protein level at 45 and 50% (Diets 5 and 6) which is also evident in other studies (Lee and Putnam 1973; Bromley 1980; Pongmaneerat and Watanabe 1991; Lee et al. 2002). Body protein was also found to increase with the increase in dietary protein up to 40% and declined thereafter. Body fat content increased with the increase in dietary protein levels which is in accordance with the findings of Khan et al. (1993). Higher body lipid content beyond the optimum protein level in the diet may be due to the fact that excess dietary protein gets deaminated and stored as body fat. In the present study, a negative correlation between moisture and fat content is evident. Feed intake was significantly different among the treatments during this study and ranged from 207 g at the highest dietary protein level to 279 g at the lowest dietary protein level. The variation in the feed intake may be due to the fact that fish eat to satisfy their energy requirement (NRC 1993) and they stop feeding once the dietary energy need is met. The low feed intake (207 g) at 50% protein diet may be as a result of this.

From the second-degree polynomial regression analysis of the growth and body composition data, the optimum dietary protein level for growth of young *H. fossilis* is found to be in the range of 40.0–42.75% of the diet. The value obtained during the present study is higher than the values reported (as percentages) for young grey mullet, *Mugil capito*, 24 (Papaparaskera-Papoutsoglou and Alexis 1986), Nile tilapia, *Oreochromis niloticus*, 25 (El-Saidy and Gaber 2005), Rohu, *Labeo rohita*, 25 (Khan et al. 2005), juvenile greenlip abalone, *Haliotis laevigata*, 27 (Coote et al. 2000), young tilapia, *Oreochromis mossambicus*, 28 (De Silva et al. 1989), *Notemigonus crysoleucas*, 29 and goldfish, *Carassius auratus*, 32 (Lochmann and Phillips 1994), walking catfish, *Clarias batrachus*, 30 (Chuapoehuk 1987). Nile tilapia, *O. niloticus*, 30 (Siddiqui et al. 1988), Shingi, *H. fossilis*, 27.73–35.43 (Akand et al. 1989), big head carp, *Aristichthys nobilis*, 30 (Santiago and Reyes 1991), juvenile silver perch, *Bidynus bidynus*, 31 (Yang et al. 2002), juvenile freshwater crayfish, *Cherax quadricarinatus*, (Cortes-Jacinto et al. 2003), juvenile silver perch, *Spinibarbus hollandi*, 32.7 (Yang et al. 2003), Catla, *Catla catla*, 30–35 (Seenappa and Devaraj 1995), South African abalone, *Haliotis midae*, 35.87 (Sales et al. 2003), and rohu, *L. rohita*, 35 (Satpathy et al. 2003), and is lower than the requirement reported for pike perch, *Sander lucioperca*, 43 (Nyina-wamwiza et al. 2005), African catfish, *Clarias gariepinus*, 43 (Ali and Jauncey 2005), bagrid catfish *Mystus nemurus*, 44 (Ng et al. 2001), grouper, *Epinephelus malabaricus*, 44 (Shiau and Lan 1996), Catla, *C. catla*, 47% (Singh and Bhanot 1988), Nile tilapia, *O. niloticus*, 45 (El-Sayed

and Teshima 1992), juvenile Florida pompano, *Trachinotus carolinus*, 45 (Lazo et al. 1998), juvenile spotted sand bass, *Paralabrax maculatofascinatus*, 45 (Alvarez-Gonzalez et al. 2001), Catla, *C. catla*, 47 (Singh and Bhanot 1988), American eel, *Anguilla rostrata*, 47 (Tibbetts et al. 2000), juvenile haddock, *Melanogrammus aeglefinus*, 49.9 (Kim et al. 2001), discus, *Symphysodon* spp., 44.9–50.1 (Chong et al. 2000), Mahseer, *Tor putitora*, 45–50 (Islam and Tanaka 2004), juvenile olive flounder, *Paralichthys olivaceus*, 46.4–51.2 (Kim et al. 2002), juvenile haddock, *M. aeglefinus*, 54.6 (Tibbetts et al. 2005), juvenile turbot, *Scophthalmus maximus*, 55 (Cho et al. 2005), *and Salmo trutta,* 57 (Arzel et al. 1995) and comparable to the requirement for African catfish, *C. gariepinus*, 40 (Degani et al. 1989), *C. batrachus*, 40 (Khan and Jafri 1990), Catla, *C. catla*, 40 (Khan and Jafri 1991), mangrove red snapper, *Lutjanus argentimaculatus*, 40 (Catacutan et al. 2001), juvenile masu salmon, *Oncorhynchus masuo*, 40 (Lee and Kim 2001), Mahseer, *T. putitora* , 40 (Hossain et al. 2002), juvenile blackspot seabream, *Pagellus bogaraveo*, 40 (Silva et al. 2006), cuneate drum, *Nibea miichthioides*, 40 (Wang et al. 2006), *Chanos chanos*, 40 (Jana et al. 2006), Persian sturgeon, *Acipenser persicus*, 40 (Mohseni et al. 2007), Mexican silverside, *Menidia estor*, 40.9 (Martinez-Palacios et al. 2007), juvenile sunshine bass, *Morone chrysops* × *M. saxatilis*, 41 (Webster et al. 1995) and Malaysian catfish, *M. nemurus*, 42 (Khan et al. 1993). The differences in protein requirements among these fish species may be due to different dietary formulations, fish sizes, and different methodologies applied (Akiyama et al. 1997; Luo et al. 2004; Tibbetts et al. 2005; Sa et al. 2006). Some of these discrepancies may be attributed to differences in experimental design, e.g., feeding level and frequency, in the experimental conditions, e.g., water quality, water flow rate, biomass rate, reference protein used, or energy density of the diet (Luzzana et al. 1998). Digestibility and energy content bring about variable effects (Simmons et al. 1999; De Silva et al. 2000). Variations may also be attributed to differences between phylogenetically distinct families or species (Akiyama et al. 1997).

The study indicates that the dietary protein levels influences the growth, conversion efficiency, and body composition of the fish, and that the inclusion of dietary protein in the range of 40–43% of the diet is optimum for the growth and efficient feed utilization of protein for growth of young *H. fossilis*. Data generated during the present study would be useful in developing protein balanced diets for the intensive culture of the young *H. fossilis*.

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