Indian J. Fish., 60(2) : 115-121, 2013

Substitution of dietary fish meal protein by a mixture of protein concentrate and meat and bone meal in the diet of climbing perch, *Anabas testudineus* (Bloch 1792)

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ABSTRACT

The effect of substitution of fish meal protein by a mixture (1:1) of protein concentrate and meat and bone meal (PCMBM) in the diet of climbing perch, *Anabas testudineus* fry was evaluated. Five experimental diets were formulated to substitute 0, 25, 50, 75 and 100% fish meal protein with PCMBM and fed to 0.89± 0.02 g fry stocked in 15 aquaria (46 × 41 × 41 cm) each with three replicates. Fish were fed to their apparent satiation three times daily for 8 weeks. Weight gain of fish fed with diet 3 (replacing 50% dietary fish meal) was significantly ($p < 0.05$) higher than fish fed with other diets. However, there was no significant difference ($p>0.05$) between the weight gain of fish fed with diets 4 and 1 (control). Fish fed with diet 3 had significantly higher specific growth rate and apparent net protein utilisation. Although there was no significant difference between the carcass protein content of fish fed with different diets, the carcass moisture and lipid contents differed significantly ($p>0.05$). The results demonstrated that up to 75% of fish meal protein can be replaced by a mixture (1:1) of protein concentrate, and meat and bone meal (PCMBM) without adverse effects on growth and feed utilisation.

Keywords: Climbing perch, Fish meal, Meat and bone meal, Protein concentrate

Introduction

The climbing perch, *Anabas testudineus* (Family : Anabantidae), locally known as 'koi' in Bangladesh, is a tropical fish, having wide range of geographical distribution and inhabits both fresh and brackishwaters. This species is also found in India, Pakistan, Burma, Sri Lanka, Thailand, China, Hong Kong, Philippines and Malaysia (Jayaram, 1981). Koi commonly grows up to 25 cm in length, and females are larger than the males. Mature males are dark colored and have longer knife edged anal fins than females. *A. testudineus* is a microplankton feeder during its larval and fry stage, but soon becomes insectivorous and voracious feeder (Singh and Samuel, 1981).

The recent rapid increase in aquaculture output in Bangladesh has been partly based on species diversification, one of which is the introduction of Thai strain of climbing perch, locally known as Thai koi (*A. testudineus*) in 2003 from Thailand. The popularity of Thai koi is because of its faster growth compared to native one. It has high demand and market price compared to other species, and can tolerate wide range of environmental conditions, and can be cultured with other catfishes like *Clarias batrachus*, *Heteropneustes fossilis* and *Mystus cavasius*. As the intensive culture of Thai koi is gaining popularity in Bangladesh, the farmers are facing problems due to the high price of commercial pelleted feeds. Because of the high price of feed, the profit margins of the farmers are decreased. Therefore, efforts are needed to develop a suitable low-cost diet for profitable Thai koi farming.

Feed cost generally constitutes the highest single operational cost of semi-intensive or intensive fish farming operation (Shang and Costa-Pierce, 1983). Throughout the world, fish meal is used as major protein source in most of the diets for finfish and crustacean species. Fish meal is one of the best quality protein source which is expensive. In the context of developing countries like Bangladesh, use of fish meal in fish feed is not feasible for poor farmers, because of its unavailability and high cost (Hasan, 1986). Use of different protein sources in various combinations is more effective than that of a single source in the substitution of fish meal in fish feed by preventing high inclusion level of any single anti-nutritional factor in the diet (Hossain and Jauncey, 1990). Protein concentrates (PC), meat and bone meal (MBM), and poultry byproducts meals (PBM), which are cheaper and commercially available in the market, can be considered as the alternative to fish meal for aquaculture feeds. Usage of combination of various animal or plant protein ingredients in the place of fish meal in fish diet has been demonstrated successfully (Millamena, 2002). Recently, Guo *et al*. (2007) also demonstrated that combination of rendered animal protein ingredients can replace most of the fish meal in practical diets for cuneate drum (*Nibea miichthiodes*).

In view of the above, the present study was designed to evaluate the effect of replacing dietary fish meal protein by a 1:1 mixture of protein concentrate (PC) & meat and bone meal (MBM) on the growth of Thai koi and to recommend the maximum level of inclusion of a mixture of protein concentrate & meat and bone meal (PCMBM) to prepare a cost effective diet for Thai koi, *A. testudineus*.

Materials and methods

The experiment was conducted in a recirculatory system in the wet laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh for a period of 8 weeks during August to September 2006.

Experimental system

A recirculatory system consisting of 20 glass aquaria (each of size 46 × 41 × 41 cm) with an effective water volume of about 65 l was used. Altogether 15 aquaria were used for this study. All the aquaria were kept on a rack made of iron angles to facilitate better observation and accessibility. Water from a deep tube well supply was used and the flow rate in each aquarium was maintained at about 1.5 l min^{-1}. Water was circulated through a common biological filter system under gravity before flowing into a sump tank so that all the replicate aquaria shared similar water. The circulating biofiltration system consisted of five biofilter tanks (each having 150 l capacity) containing plastic cones and gravels stones to remove particulate matter and provide substrate for nitrifying bacteria. At the mouth of the first filter tank, a sponge filter was placed to collect the solid wastes (feces) from water. Water replenishment was done with tap water to fill up the loss due to evaporation. The water temperature in the system was maintained at 28 ± 1°C using a Thermostat (Model SiF10A) heater. An adequate level of oxygen in each aquarium was maintained through aeration using an air pump (Johnson Pump, MDR-series (Johnson pumpen GmbH, Lohne, Germany). Natural photoperiod of 12 h light and 12 h dark (12L: 12D) was maintained throughout the experimental period.

Experimental fish and acclimation

Fry of Thai koi, *A. testudineus* were collected from Freshwater Station, Bangladesh Fisheries Research Institute (BFRI), Mymensingh. The collected fish were given a prophylactic treatment with salt (3% NaCl) solution for 10 min. During treatment, sufficient oxygen supply was maintained through artificial aeration. Before starting the experiment, fish were acclimatised to the experimental condition for one week and fed a commercial nursery shrimp feed (42% protein).

Feed formulation and preparation

Since quality fish meal was not available in the market, fish meal was prepared locally by grinding and mixing (1:1) dried *chela* (*Chela cachius*) and *kachki* (*Corica soborna*) collected from a local market in Mymensingh. Protein concentrate (protein concentrate 65%, Gold brand, manufactured by P. Raven and G. Walkee, Western Farmers Association, Seattle, Washington, USA), meat & bone meal (Australian origin) and vitamin-mineral premix (y SK+F, Eskayef Bangladesh Ltd.) were also collected from same market. The protein concentrate (PC) and meat & bone meal (MBM) was mixed in equal proportion (1:1) to obtain a new protein source termed as PCMBM. All the ingredients were finely ground and sieved to pass through a 0.5 mm mesh and were analysed for proximate composition (Table 1).

Five iso-nitrogenous diets were formulated to contain 40% protein and to be as iso-energetic as possible. Diet 1 contained fish meal as a sole source of protein and considered as the control. Diets 2 to 5 were formulated to replace 25, 50, 75 and 100% of fish meal protein by PCMBM respectively. The formulation of experimental diets is shown in Table 2. α-cellulose was used to adjust the protein and energy levels in different diets.

All the dietary ingredients were weighed (Table 2), mixed thoroughly, made into dough, and finally pelletised (2 mm) using a kitchen-type pellet machine (Mum 5580, Type UM60 ST2-M; Bosch, Leipzig, Germany). The pellets

Table 1. Proximate composition of the protein sources used (% dry matter basis)

Ingredients	Dry matter	Protein	Lipid	Ash	Crude fibre	NFE††
Fish meal*	88.84	61.12	15.46	21.38	0.52	1.52
PCMBM†	89.31	58.15	11.10	26.66	2.34	1.75

*Prepared locally by grinding and mixing (1:1) of dried *chela* (*Chela cachius*) and *kachki* (*Corica soborna*) collected from Mymensingh market.
†Prepared by mixing protein concentrate, and meat & bone meal in equal proportion (1:1).
††Nitrogen free extract calculated as 100 % (protein + lipid + ash + crude fibre)

Table 2. Ingredient composition (%) of experimental diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal†	65.44	49.08	32.27	16.36	-
PCMBM††	-	17.20	34.39	51.59	68.87
Soybean oil	-	0.50	2.20	1.75	2.50
Starch (soluble)	24.56	24.22	23.14	22.30	20.63
Binder‡	3.00	3.00	3.00	3.00	3.00
Vitamin-mineral premix‡‡	2.00	2.00	2.00	2.00	2.00
α cellulose	5.00	4.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00

† Fish meal manufactured by grinding of dry *chela* and *kachki* (1:1)

†† PCMBM prepared by mixing of protein concentrate and meat and bone meal (50:50)

‡ Carboxymethyl cellulose (high viscosity) from BDH England.

‡‡ Composition of vitamin-mineral premix (per kg mix) manufactured by SK+F, Eskayef Bangladesh Limited: vitamin A - 1000000 IU; vitamin D_3 - 200000 IU; vitamin E - 15000 mg; vitamin K_3 - 400 mg ; Thiamin (B_1) - 1500 mg; Riboflavin (B_2)- 2000 mg; Pyridoxin (B_6) - 1000 mg; vitamin (B12) - 3 mg; Calcium-d-Pantothenate - 4000 mg; Ascorbic acid - 15000 mg; Nicotinic acid - 10000 mg; Folic acid - 300 mg; Biotin - 50 mg; Cobalt (Co) - 10 mg; Copper(Cu) - 300 mg; Iron (Fe) - 5000 mg; Iodine (I) - 100 mg; Manganese (Mn) - 2000 mg; Zinc - 3000 mg; Selenium (Se) - 10 mg; Choline chloride (50%) - 300000 mg; Calcium carbonate - Q.S. to 1 kg .

‡‡‡ cellulose from BDH, England.

were then dried in an oven at 45 °C for 24 h. The pellets were broken into smaller particles and sieved to different size categories (0.5 to 1.0 mm) to be suitable for the fry. The diets were separately packed in air-tight polyethylene bags and stored in a freezer until use.

Experimental design and feeding rates

The experiment was conducted in a completely randomised design. Five experimental diets were randomly assigned to 15 aquaria each with three replicates. Fifteen numbers of uniform sized fry of *A. testudineus* with initial weight of 0.89 ± 0.02 g were randomly stocked in each aquarium. Fish were fed with experimental diets up to their apparent satiation. Feeding was done in such a way that left over's were minimum. The fish were fed to their apparent satiation three times daily at 9.00, 13.00 and 17.00 hrs.

Biweekly sampling of fish was done to observe the growth. Prior to weighing, fish were caught with a fine mesh scoop net and excess water was then removed from fish body by gently blotting on a soft tissue paper. Weight of fish in each sampling was measured by bulk weighing using a digital electronic balance (Model CT1200-S; OHAUS, Princeton, NJ, USA). During sampling, the fish were handled very carefully. Any mortality of fish during the study period was recorded. Water quality parameters (water temperature, dissolved oxygen, pH and total ammonia) were monitored weekly throughout the experimental period. Water temperature and pH were measured by a Hach pH meter and dissolved oxygen (DO) by Hach DO meter (models Sension 1 and 6; Hach, Dusseldorf, Germany) and total ammonia by a Hach kit (model DR2010) following the standard method. At the beginning of the experiment 25 fish from the stock was randomly sacrificed for proximate analysis and this was considered as initial carcass composition. At the end of the experiment, 5 fish from each replicate were sacrificed and used for final carcass composition analysis.

Calculation and analytical methods

The proximate analyses of feed ingredients and experimental diets and fish carcasses were carried out in triplicate according to AOAC (1990). The gross energy contents of the diets were calculated according to Jauncey and Ross (1982). Weight gain (g), specific growth rate (SGR % per day), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilisation (ANPU %) and survival (%) of the fish were calculated according to Castell and Tiews (1980). MSTAT package program and one way analysis of variance (ANOVA) were used to determine the effect of different diets on the growth of fish. This was followed by Duncan's New Multiple Range Test (Duncan, 1955) to identify the level of significance of variance ($p<0.05$) among the treatment means. Standard deviations (± SD) of treatment means were also calculated.

Results

There were slight variations in proximate composition of different experimental diets (Table 3). The protein content varied between 40.38 and 41.19%. The variations in lipid and ash contents among different experimental diets were also minimal. The lipid content of various diets ranged between 10.12 and 10.87%, the ash content ranged between 13.15 and 14.56%, crude fibre ranged between 4.18 and 4.46% and gross energy content varied between 18.45 and 18.80 KJ g^{-1}

Table 3. Proximate composition of experimental diets (% dry matter basis)

Components	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Dry matter	91.01	90.02	92.83	92.15	92.52
Protein	41.19	40.38	40.48	40.79	41.02
Lipid	10.70	10.84	10.87	10.28	10.12
Ash	13.15	13.66	13.76	14.17	14.56
Crude fibre	4.46	4.21	4.18	4.23	4.28
NFE[†]	30.50	30.91	30.71	30.53	30.02
Gross energy (KJ g^{-1})[‡]	18.80	18.73	18.73	18.54	18.45

[†]Nitrogen free extract calculated as 100-% (protein + lipid + ash + crude fibre)
[‡]According to Jauncey & Ross (1982)

The acceptability of different experimental diets was judged by a subjective behavioural assessment of the feeding responses. All fish were acclimatised to the experimental diets within 1-2 days of the start of feeding. The acceptability of all the diets was more or less similar. Fish were observed to feed actively and consumed all the feed within a few minutes of administration.

All the water quality parameters monitored during the experimental period were well within the acceptable range for fish culture. The ranges were as follows: temperature 27 – 29 °C, pH 6.9-8.1, dissolved oxygen (DO) 6.5-8.4 mg l^{-1} and total ammonia 0.10-0.21 mg l^{-1}.

The final weight of fish fed with diet 3 was significantly higher (p<0.05) than those fed with other diets but there was no significant difference (p>0.05) among the final weight of experimental fish fed with diets 1, 2 and 4 (Table 4). Significantly (p<0.05) lowest growth was observed in fish fed with diet 5. The SGR of fish ranged between 3.8 and 4.5. Diet 3 resulted in the highest SGR while diet 5 produced the lowest SGR value (Table 4). There was no significant differences between the SGR values in diets 2 and 4 but these values were higher than those in diet 1 and 5. The mean FCR values of different experimental diets ranged between 1.0 and 1.3 (Table 4). Diet 3 resulted in the lowest FCR but it was not significantly (p>0.05) different from the FCR values obtained with diet 1 and 4. Again there were no significant differences between the FCR values in diet 1, 2, 4 and 5. The PER values ranged between 2.2 and 2.9 with diet 5 producing significantly (p<0.05) lowest value. However, the PER values in other diets did not vary significantly among themselves. The ANPU values in different diets were fairly high, and ranged between 36.8 and 45.4%. The ANPU value in diet 3 was the highest (p<0.05) while it was the lowest in diet 5. There were no significant differences among the ANPU values in diets 1, 2 and 4, and the values were significantly higher (p<0.05) than that of diet 5.

The protein and lipid content of the final carcass composition increased markedly compared to that of the initial carcass composition (Table 5). The significantly (p<0.05) highest carcass moisture content was observed in fish fed with diet 5 and it was lowest in fish fed with diet 3. However, there was no significant (p>0.05) difference between the carcass moisture content of fish fed with diets

Table 4. Growth performance and feed utilisation of *A. testudineus* fed experimental diet for 8 weeks

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Mean initialweight (g)	0.91± 0.07^{a}	0.88±0.02^{a}	0.89±0.10^{a}	0.89±0.01^{a}	0.89 ±0.01^{a}
Mean final weight (g)	9.91± 0.11^{b}	9.65±0.12^{b}	11.32±0.19^{a}	9.73±0.18^{b}	7.56±0.16^{c}
Weight gain (g)	9.03±0.10^{b}	8.76±0.10^{b}	10.43±0.19^{a}	9.17±0.41^{b}	6.67±0.16^{c}
% weight gain	1022.33±15.31^{b}	989.00±14.18^{b}	1171.66±24.03^{a}	985.66±4.05^{b}	744.00±24.88^{c}
SGR (% per day)[†]	4.37± 0.09^{b}	4.26±0.02^{c}	4.54 ±0.03^{c}	4.25±0.03^{c}	3.80±0.05^{d}
FCR[††]	1.14±0.02^{ab}	1.29±0.19^{a}	1.06±0.03^{b}	1.17±0.04^{ab}	1.33±0.03^{a}
PER[‡]	2.86±0.05^{a}	2.89±0.06^{a}	2.98±0.06^{a}	2.86±0.07^{a}	2.22±0.04^{b}
ANPU (%) [‡‡]	42.47±0.59^{b}	43.12±0.40^{b}	45.42 ±0.48^{a}	42.46±0.47^{b}	36.84±0.62^{c}

*Values are mean ± standard deviation
**Values in the same row with same superscripts are not significantly different (p>0.05)
[†]Specific growth rate (SGR%/day) = 100 (ln final body weight – ln initial body weight)/total number of experimental days x100
[††]Feed conversion ratio (FCR) = total dry feed fed (g)/total live weight gain (g)
[‡]Protein efficiency ratio (PER) = live weight gain (g)/ dry protein fed (g)
[‡‡]Apparent net protein utilisation (ANPU%) = (final fish body protein-initial body protein) / total protein fed x 100

Table 5. Carcass composition of the fish sample at the start and end of the experiment (% fresh matter basis)

Components	Initial (all fish)	Final				
		Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moisture	77.59	68.92 ± 0.31^{b}	68.72 ±0.30^{b}	67.93 ±0.22^{c}	68.83 ±0.25^{b}	69.59 ±0.22^{a}
Protein	14.68	17.56 ± 0.18^{a}	17.58 ± 0.21^{a}	17.97 ±0.19^{a}	16.40 ±1.74^{a}	17.05 ±0.24^{a}
Lipid	2.49	5.78 ± 0.16^{a}	5.80 ± 0.17^{a}	6.09 ± 0.17^{a}	5.66 ± 0.16a	5.07 ± 0.28^{b}
Ash	4.95	7.67 ± 0.16^{b}	7.73 ± 0.11^{ab}	7.80 ± 0.14^{ab}	7.87 ± 0.18^{ab}	8.00 ± 0.20^{a}

*Values are mean ± standard deviation

†Values in the same row with same superscripts are not significantly different (p>0.05)

1, 2 and 4. There was no significant difference (p>0.05) between the carcass protein content of fish fed with different diets, which ranged between 17.05% and 17.97%. Fish fed with diet 5 resulted in the lowest (p>0.05) carcass lipid. However, there was no significant difference between the carcass lipids of fish fed with other diets (Table 5). The carcass ash contents progressively increased with the inclusion of PCMBM in the diets.

Discussion

Fishery products, either in the form of low value trash fish or rendered as fish meal, are presently the major sources of protein in growout culture of most fish species and contribute up to 70% by weight of their diet (Tacon, 1995). As the demand for fish meal for aquaculture increases, while their availability decreases, the cost is expected to rise. In the long run many developing countries may not be able to depend on fish meal as a protein source for aqua feeds. Therefore, dependable supply of cost-effective alternative sources of protein must be provided for aquaculture to be profitable. Meat and bone meal (MBM) are generally considered inferior animal protein and less preferred for inclusion in commercial diets as main protein source. Replacement of fish meal with MBM generally compromised the growth performance of the fish (Shimeno *et al*., 1993; Kikuchi *et al*., 1997; Kureshy *et al*., 2000).

The present study, however, has demonstrated that up to 75% replacement of fish meal protein with PCMBM (protein concentrate and meat & bone meal, 1:1) allowed growth rate similar to those exhibited by the control group (fish meal-based diet). Fish in Diet 3 receiving 50% fish meal and 50% PCMBM protein even resulted in significantly higher growth than those in the control group. The fish readily accepted the diets at all levels of fish meal replacement by PCMBM as was shown by the better FCR values of 1.0 to 1.3. This conforms to other studies where diets with inclusion levels of meat and bone meal ranging from 30 to 70% as substitute for fish meal have been readily accepted by both omnivorous and carnivorous fish species such as tilapia, rainbow trout, yellowtail and grouper (Davies *et al*., 1989; Shimeno *et al*., 1993; Watanabe *et al*., 1993; Millamena, 2002).

Fish fed with diets replacing up to 75% dietary fish meal protein by PCMBM grew well, showing comparable or higher specific growth rates (SGR) than that with fish meal based diet (diet 1). In this study, growth, FCR and PER values either improved or were not significantly affected by 0 - 75% replacement of fish meal with PCMBM. The higher PER values indicated better utilisation of protein from different diets. Fish meal has been completely replaced in practical diets for tilapia (El-Sayed, 1998) but complete replacement of fish meal in this study has been less successful. However, based on the results in the present study, combination of animal protein sources, *i.e.,* protein concentrate + meat and bone meal (1:1) can be used to replace up to 75% dietary fish meal.

In the present study, the ANPU values were fairly high and ranged between 36.8 and 45.4%. These values are higher than those reported for sea bream, *Sparus aurata* (Davies *et al*., 1993) but similar to those for tilapia, *Oreochromis niloticus* (Hossain *et al*., 2002). The higher ANPU values in the present study indicate better assimilation of protein by fish from different diets.

Most individual animal protein sources, such as meat and bone meal, feather meal, blood meal, and poultry by-products meal have been able to replace less than 50% of fish meal in diets of salmonids (Lee *et al*., 2001). High levels of meat & bone meal in fish diets showed different results in different fish species. When fish meal is replaced with combination of ingredients, the interpretation of results is difficult because many interactions between nutrients may be involved in nutrient metabolism. The limitations in use of alternative protein sources may be due to three factors: i) lower feeding rate with replacement of fish meal by alternative proteins (Robaina *et al*., 1997); ii) imbalances of essential amino acids in alternative protein sources (Ai and Xie, 2005); iii) lower digestibility with incorporation of alternative protein (Bureau *et al*., 1999).

Some previous studies showed that feeding rate decreased with increasing dietary meat & bone meal because of reduced palatability, which accounted for growth reduction (Robaina *et al.,* 1997; Xue and Cui, 2001). In the present study, satiation feeding was employed and the feeding rate was not different among dietary treatments and to some degree increased with the increase of dietary PCMBM, suggesting that PCMBM could be readily accepted by koi and hence feeding rate did not account for the growth reduction.

One of the possible reasons for the reduced growth of *A. testudineus* at total replacement with PCMBM may be the deficiencies in essential nutrients such as essential amino acids. Most of the terrestrial animal byproducts meals including poultry byproducts meals, hydrolysed feather meal, blood meal, and meat & bone meal have high protein and favourable essential amino acid profiles (NRC, 1983). But these feed sources may be deficient in one or more of the EAAs, especially lysine, isoleucine and methionine (Tacon and Jackson, 1985). However, if these ingredients are used in a proper combination in the diet, the quality of this diet is likely to improve (Davies *et al.*, 1989). Though the amino acid composition of the diets in the present study were not analysed, the combination of fish meal and PCMBM in diet 3 might have had been balanced in terms of essential amino acids and resulted in the best growth performance of fish.

Other possible explanation for the lowest performance of *A. testudineus* at the highest level of fish meal substitution by PCMBM may be the resulting effect on digestibility (Millamena, 2002). Although the digestibilities of diets were not determined in this study, it is well documented that high ash content in meat & bone meal could reduce protein digestibility (Alexis, 1997; Robaina *et al.*, 1997; Kurshey *et al.*, 2000). Robaina *et al.* (1997) suggested that more than 12.5% ash content in diets would lead to lower digestibility of protein. Thus, increased dietary ash content (Table 3) with increasing dietary PCMBM probably accounts for reduced growth at highest PCMBM level diets.

The whole body moisture was inversely correlated with body lipids. Similar observation has been reported elsewhere (Hossain and Jauncey, 1990). Previous studies showed that there were no significant differences in body composition (protein, lipid, ash and moisture) among fish fed diets with graded levels of meat and bone meal (Robaina *et al.*, 1997). PCMBM in the present study significantly affected lipid, moisture and ash content although protein was not significantly different. Similar observation has been reported by Ai *et al*. (2006). In the present study, lipid content in fish, however, decreased with increasing dietary PCMBM, especially when replacing more than 50% fish meal protein. Since the diets had the same lipid, the decreased lipid deposition suggests lower utilisation of lipid with increasing PCMBM. This is likely because of higher saturated fatty acids at higher dietary PCMBM levels (though, fatty acid levels were not analysed in the present study).

Most of the studies so far done have evaluated fish meal replacements in fish feed from biological or nutritional view points. Little attention has been paid to economic analysis of alternative protein sources. Only few studies have been conducted in this subject and these have indicated that those unconventional protein sources were more economical than fish meal because of their local availability at low prices. Based on wholesale market price of 2006 (local Mymensingh town), the price of fish meal (although quality fish meal is not available in the market) is Tk. 40.00 per kg (1USD =Tk 68) and estimated cost of PCMBM is Tk. 28.00 per kg, (PC, Tk. 36.00 per kg and MBM, Tk. 20.00 per kg respectively). Therefore, it is expected that at least Tk. 5.00 per kg diet of *A. testudineus* could be saved, if 75% of the dietary fish meal protein (diet 4) is replaced by PCMBM. Use of PCMBM in fish diet as fish meal replacement could be more cost-effective in the future, if the price of fish meal continues to rise. Thus, the results of the present study indicated that PCMBM could be used as a main protein source to replace fish meal up to 75% of dietary protein without significant negative effect on growth and feed utilisation of *A. testudineus.* Thus, farmers can use a mixture of protein concentrate, and meat and bone meal (1:1) to substitute fish meal up to 75% level in the diet of *A. testudineus* to reduce the cost of production without compromising the growth.

Acknowledgements

The authors are grateful to the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh for logistic support in conducting the experiment. Use of trade name or commercial products is solely for purpose of providing specific information and does not imply endorsement by the authors.

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Date of Receipt : 11.08.2012
Date of Acceptance : 16.11.2012