International Journal of Aquatic Science
ISSN: 2008-8019
Vol 3, No 1, 2012

Effects of partial replacement of fishmeal in the diet by mulberry leaf meal on growth performance and digestive enzyme activities of Indian minor carp *Labeo bata*

Kausik Mondal*[1], Anilava Kaviraj[2] and Pratap Kumar Mukhopadhyay[3]

1) Department of Zoology, Tamralipta Mahavidyalaya, Tamluk – 721636, India
2) Department of Zoology, University of Kalyani, Kalyani – 741235, India
3) Central Institute of Freshwater Aquaculture, ICAR, Bhubaneswar – 751002, India

Abstract: Fermented mulberry leaf (*Morus indica*) meal (MLM) was used as the main protein supplement to partially replace fishmeal (FM), mustard oil cake (MOC) and rice bran (RB) in the formulation of four experimental diets for the Indian minor carp *Labeo bata*. The four diets contained 0, 65, 75 and 80% MLM thereby replacing respectively 0, 50, 75 and 80% of FM, 0, 64, 64 and 74 % of MOC and 0, 77, 90 and 90% of RB. The diet containing 65% MLM (replacing 50% of FM, 64% of MOC and 77% of RB) appeared to be best diet in terms of growth, nutrient deposition and digestive enzyme activities of the *L. bata* fingerlings. It was concluded that inclusion of MLM as feedstuff to replace fishmeal in the formulation of diet of *L. bata* was a viable option provided crude fibre content of the diet did not exceed 5.63% of the dry weight of the diet.

Key Words: Mulberry, Fishmeal, Diet, *Labeo bata*, Enzyme, Growth

Introduction

Replacement of fishmeal by cost effective protein source is a priority research in aquaculture throughout the world because of growing scarcity of quality fishmeal supply and escalation of its cost. The efficacy of various plant sources for partial or complete replacement of fishmeal in aquatic diets has been investigated by a number of workers (Tacon, 1993; Ray and Das, 1995; Mondal and Ray, 1999; Lee *et al*. 2006). Although plant proteins (PP) are cost effective, their use is limited by deficiencies in essential amino acids and minerals, and the presence of anti-nutritional factors (ANFs) and complex carbohydrates (National Research Council (NRC), 1993 and Vielma *et al*. 2003). Fermentation is a simple and cheap method to decrease the anti-nutritional factors and crude fibre contained in the plant by-products (Bairagi *et al*. 2002). We used this technique

(✉) kausik.mondal2007@gmail.com

to remove ANFs from mulberry leaves and use fermented mulberry leaf meal as feed stuff to replace fish meal in carp diet.

Mulberry leaves are rich in protein and mineral elements (Majumdar *et al*. 1967; Datta *et al*. 2002). Incorporation of mulberry leaves in the diet of poultry (Narayana and Setty, 1977) and rabbit (Bamikole *et al*. 2005) resulted in better egg production of poultry and growth of rabbit. However, unlike oilseeds, legumes and cereal grains which are traditionally used as protein or energy concentrate in fish feed formulation (Gatlin III 2007), this plant resource has not so far been experimented in fish diet to a great extent, despite an historical link between integrated mulberry cultivation - sericulture - pond aquaculture in dike-pond systems in China, dating back to the XIV century (Lo 1996). Recently we introduced fermented mulberry leaf meal as a protein source in the diet of the Asian air breathing catfish *Heteropneustes fossilis* and found the fish to accept the diet well (Mondal *et al*. 2011). But there is no record yet of introduction of mulberry leaves as a diet supplement for Indian carps, while several aquatic plants have been successfully utilized as protein supplement in the formulation of diet for these groups of fish (Nandeesha *et al*. 2001; Bairagi *et al*. 2002; Kalita *et al*. 2008). The objective of the present study was to evaluate if fermented mulberry leaf meal could be used as feedstuff to replace fishmeal in the formulation of diet for the Indian minor carp *Labeo bata*.

Materials and Methods

Formulation of experimental diets

One reference and three experimental diets were formulated using mustard oil cake (MOC), rice bran (RB), fishmeal (FM) and mulberry (*Morus indica*) leaf meal (MLM). The reference diet contained MOC, RB and FM along with vitamin and mineral mixture (Table 1), while the experimental diets contained FM and the fermented product of MOC, RB and MLM. For fermentation, the MLM, MOC and RB were mixed at proportion mentioned in table 1. The mixture was added to a solution of microbial suspension (10^8 cell·mL^{-1}) (*Lactobacillus* sp., *Rhodopseudomonas* sp., *Azotobacter* sp. and *Saccharomyces* sp.) (the microbial suspension (EM^{TM}) was a gift from M/S, Maple Orgtech Pvt. Ltd. Kolkata.), molasses and water (2.5 mL : 2.5 g : 100 mL) and was fermented anaerobically in an anaerobic fermentation chamber under ambient temperature (27–30 °C) for 40 to 44 days, depending on the proportion of MLM. Final fermented product was mixed with fishmeal, vitamin, and mineral mixture to formulate three experimental diets. The reference and the experimental diets were made isonitrogenous (crude protein level 30 % approximately) (Table 1). The diets were ground, blended and pelleted with 0.5%

carboxymethyl cellulose and 1% chromic acid (Cr_2O_3) as non absorbent reference substance and then the diets were sun dried for a few days before using in the trial.

Table 1: Formulation and proximate analyses of reference and experimental diets

	Content	Reference	Experimental		
		T1	T2	T3	T4
Ingredients [%]	Mustard Oil Cake*	39	14	14	10
	Rice Bran**	39	9	4	4
	Mulberry Leaf Meal***	--	65	75	80
	Fishmeal#	20	10	5	4
	Vitamin Premix†	0.5	0.5	0.5	0.5
	Mineral Premix‡	0.5	0.5	0.5	0.5
	Cr_2O_3◊	1	1	1	1
Proximate composition [% dry matter basis]§	Dry Matter	93.50	92.65	93.25	93.65
	Moisture	6.50	7.35	6.75	6.35
	Protein	30.94	30.65	30.16	30.10
	Lipid	11.63	12.50	14.63	14.55
	Crude Fiber	3.83	5.63	8.26	8.65
	Ash	11.40	22.80	24.60	25.20
	NFE	47.10	43.87	40.2	40.35
	Gross Energy ($kJ.g^{-1}$)	18.72	18.44	18.53	18.51
	P:E Ratio¶	16.53	16.62	16.28	16.26

Notes: *Dry Matter(DM)-87%, Crude Protein(CP)- 34.50%, Crude Lipid(CL)-6.50%, Ash 9.20%

**DM-95.35%, CP-13.40%, CL-4.80%, Ash-22.00%

***DM-89.60%, CP-28.60%, CL-4.14%, Ash-10.24%

#DM-93.20%, CP-76.80%, CL-6.20%, Ash-8.20%.

†Vitamin mixture (%): (Ambiplex; Brihans Lab, Pune): Vit B1: 7.14, Vit B2: 2.55, Vit B6: 1.02, VitB12: 0.012, Biotin: 0.025, Calcium Pantothenate: 2.55, Niacin: 76.50, Cholin Chloride (B4): 10.20 ;Vitamin C in the form of ascorbyl polyphosphate was added to vitamin mixture at 100mg/kg mixture

‡Mineral mixture (%): (Agrimin; Glaxo India Ltd, Mumbai): Copper: 3.12, Cobalt: 0.45, Magnesium: 21.14, Iron: 9.79, Iodine: 1.56, Zinc: 21.30, Calcium: 30.00, Phosphorous: 8.25

◊Used as non absorbent reference substance only in diets used in digestibility experiments

§Number of samples per each determination = 3

¶Protein to energy ratio in mg protein/kJ of total energy

Experiments

Two experimental systems were utilized: one in the in indoor glass aquaria (50 L) to evaluate voluntary diet intake and apparent protein digestibility (APD) of the diets and other in the outdoor cement tanks (400 L) to evaluate growth and biochemical composition of the fish. Deep tube-well water stored in an overhead tank was used in both the trials. Fingerlings of *L. bata* (mean initial length 8.26 ± 0.24 cm and mean initial weight 5.60 ± 0.20 g) were obtained from a local fish farm and were acclimatized to the laboratory conditions for seven days prior to start of the experiment. The fingerlings were fed to satiation (twice a day six days a week) with the reference diet (Table 1) during acclimatization. The acclimatized fingerlings were randomly distributed at the rate of 10 per aquarium for the digestibility trial and 40 per tank in the growth trial. The aquaria or tanks were laid out in a completely randomized block design (Gomez and Gomez, 1984) with three replicates for each of the seven diet treatments.

The fish, in the indoor trials, were fed a ration at 5% of their body weight. The ration was provided at 08:00 hours and the fish were allowed to eat for 6 h. Left over diets were collected after 6 h of feeding, oven-dried, weighed and stored at –20°C. The leaching rate was estimated by placing weighed diets in aquaria without fish for 6 h and then recollecting, drying and re-weighing the diets. The average leaching rate was used to calibrate the amount of uneaten diets. Faecal samples were collected from each aquarium by immediate pipetting method (Spyridakis *et al*. 1989) continuously at a 3 to 4 h interval for a period of 17 h after the removal of uneaten diets. To minimize nutrient leaching, only fresh and intact faeces were collected and dried to a constant weight at 60°C in an oven and weighed before preserving at –20°C. Apparent protein digestibility (APD) of the diet was calculated from the proportion of Chromium (Cr) and protein in the diet and faeces following the methods described by Ellestad *et al*. (2002). The digestibility trial was continued for 10 days. Water temperature in the aquaria ranged from 22–24°C and aeration was provided to maintain a dissolved oxygen level of approximately 8.5–8.9 $mg.L^{-1}$.

In the outdoor growth trial, the fish were fed the same ration (5% of the body weight) everyday, but in two equal instalments', one at 10.00 h and again at 16.00 h. The quantity of the diet given was readjusted every 15 days after weighing the fish. Samples of water were collected every week to determine selected parameters like dissolved oxygen, free carbon dioxide, total ammonia, alkalinity, hardness, and pH following the standard procedures (APHA, 1995). All fish from each outdoor tank were sampled at the end of 60 days; length and weight of the fish were recorded and five sampled fish from each tank were subjected to

biochemical analyses to determine moisture, crude protein, lipid and ash content of the fish. Determinations were made on pooled samples of fish from each tank thereby giving a total of three replicates for each diet. Rest of the sampled fish was used to determine increase in weight, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and apparent net protein utilization (ANPU) using standard methods (Castell and Tiews, 1980). Daily growth coefficients (DGC) were calculated as 100 X (($FBW^{0.3333}$ – $IBW^{0.3333}$)/duration), since this growth index is considered more appropriate for fish grown at constant temperature (Cowey, 1992). Thermal growth coefficient was determined following the methods of Cho (1992):

$$TGC = (FBW^{1/3} - IBW^{1/3}) \times 1000 / \sum(\text{temp.}(°C) \times \text{feeding days})$$

Analytical methods

Proximate composition analyses of the experimental diets, carcass and faecal samples were performed following the AOAC (1990) procedures as follows: moisture was determined by oven drying at 105°C for 24 h; crude protein (nitrogen × 6.25) was determined by Kjeldahl method, after acid hydrolysis; lipid was extracted by petroleum ether (boiling point 40–60°C) for 7 to 8 h in a Soxhlet apparatus followed by determination of lipid gravimetrically, crude fibre was determined as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH and ash was determined by combustion at 550°C, in a muffle furnace, till a constant weight. Nitrogen-free extract (NFE) was calculated by taking the sum of values for crude protein, crude lipid, crude fibre and moisture and subtracting this from 100 (Maynard *et al*. 1979). Gross energy was calculated on the basis of methodology of Brafield (1985). Tannin content in both fermented and raw mulberry leaf was determined using Folin–Denis reagent (Schanderi, 1970). Phytic acid content was determined according to Wheeler and Ferrel (1971). Chromium was determined in the diets and faecal samples by Atomic Absorption Spectrophotometer (AAS). The detailed methodology has been followed from Saha and Gilbreath (1991).

Statistical analysis of data

The nature of distribution of the observations of each response variable from both the trials was verified by Kolmogorov-Smirnov (K-S) and Shapiro-Wilks (S-W) tests to ensure a Gaussian distribution. Since all data were found normally distributed they were subjected to single factor ANOVA, without any further transformation, followed by least significant difference (LSD) test to compare mean between the treatments (Gomez and Gomez, 1984; Johnson and Wichern, 1992).

Results

Crude protein level of the experimental diets ranged from 30.10 to 30.94% (Table 1). Diets containing MLM (T2 to T4) showed higher total lipid content (12.50 to 14.63%) than the reference diet (T1, 11.63%). Crude fibre level of the experimental diets (3.83 to 8.65%) increased with the increase in concentration of MLM. Concentrations of the anti-nutritional factors, tannin and phytic acid, in the ingredient mixture ranged from 0.26 to 0.40% and 0.28 to 0.39% respectively. None of these anti-nutritional factors could be detected in the experimental diets prepared with fermented mixture containing mulberry leaves.

The survival rate of the *L. bata* fingerlings during the digestibility trial in the glass aquaria ranged from 94 to 96% and showed no significant variation between the dietary treatments. The diets containing MLM (T2-T4) showed apparent protein digestibility (APD) similar to reference diet (T1). Feed intake rate did not vary between the treatments.

The survival rate of the *L. bata* fingerlings during the growth trial in the outdoor cement tanks ranged from 92 to 94% and showed no significant variation between the dietary treatments. Data on growth performance and diet utilization of *L. bata* fingerlings in terms of diet intake rate, weight gain, SGR, TGC, DGC, FCR, PER and ANPU are presented in table 2. Growth (in terms of weight gains, SGR, DGC, TGC and FCR) significantly increased in T2 (65% MLM replacing 50% of FM, 64% of MOC and 77% of RB) as compared with the reference diet (T1). Further increase of MLM (75 to 80%) (T3 to T4) replacing more amounts of FM (75 to 80%), MOC (64-74%) and RB (90%) resulted in decrease of growth as compared with the reference diet. However, protein efficiency ratio (PER) was significantly higher in T3 (75% MLM) and ANPU was significantly higher in both T3 and T4 as compared with the reference diet (T1). The whole body composition of the experimental fish determined before and after the experiment is given in table 3. The deposition of crude protein (CP) in the whole body was significantly higher in fish fed T2 (18.71%) diets but CP decreased in fish fed T3 (15.08%) and T4 (14.92%) diets as compared with those fed reference diet (T1, 15.17%). The lipid content of the whole body was significantly higher in the experimental diets T3 (2.99%) as compared with the reference diet (T1, 2.72%). Ash content did not vary significantly between the diet groups. However, maximum value of ash were recorded in T2 (5.13%) followed by T4 (4.99%).

Table 2: Digestibility, growth performance and diet efficiency of L. bata fingerling fed experimental diets for 60 days. (Data are mean ± standard deviation (n = 5)).

Diets	Indoor trial		Outdoor trial						
	Diet Intake (g/100g BW/d)	APD* (%)	Increase in Weight (%)	FCR#	SGR† (%.d^{-1})	PER‡	ANPU§	DGC	TGC
T1	2.598a ± 0.16	88.99a ± 0.61	134.17a ± 9.80	1.50a ± 0.11	1.42a ± 0.07	1.84a ± 0.13	35.68a ± 1.13	0.352a ± 0.02	0.970a ± 0.06
T2	2.576a ± 0.04	89.29a ± 0.75	156.90b ± 8.03	1.27b ± 0.06	1.57b ± 0.05	3.40b ± 0.17	47.33b ± 1.44	0.397b ± 0.02	1.093b ± 0.04
T 3	2.592a ± 0.15	89.25a ± 0.36	142.74a ± 2.77	1.40a ± 0.03	1.48a ± 0.02	3.23b ± 0.06	38.62c ± 2.03	0.369a ± 0.01	1.017c ± 0.02
T4	2.606a ± 0.17	88.78a ± 0.11	121.07c ± 18.22	1.68c ± 0.25	1.32c ± 0.14	2.84c ± 0.43	39.44c ± 2.05	0.324c ± 0.04	0.893a ± 0.11

Means with dissimilar superscripts in the same row indicates significant difference (LSD) between the means at 5% level

Notes: *APD = 100 -100 X ((% Cr in diet / % Cr in faeces) X (% protein in faeces / % protein in diet))

#FCR = Dry weight of diet given / increase in weight of the fish

†SGR = {(ln final weight – ln initial weight)/days on trial} X 100

‡PER = Wet weight gain of fish / Protein consumed

§ANPU = (Net increase in carcass protein / Amount of protein consumed) X100

Table 3: Proximate composition of carcass (% wet weight) of the experimental fish (L. bata) at the start and end of the 60 days dieting trial. (Data are mean ± standard deviation (n = 5)).

Components	Initial	Final			
		T1	T2	T3	T4
Crude Protein	13.76 ±0.06	15.17 ± 0.41a	18.71±0.50b	15.08 ± 0.68a	14.92 ±0.67a
Crude Lipid	2.41 ± 0.06	2.72 ± 0.15a	2.58 ± 0.12b	2.99 ± 0.47c	2.64 ± 0.20a
Ash	4.31 ± 0.12	4.59 ± 0.22a	5.13 ± 0.22b	4.48 ± 0.15a	4.99 ± 0.12cb

The activities of the digestive enzymes in the intestine of *L. bata* are presented in fig. 1. The α-amylase activity significantly increased in all the experimental diets (T2 to T4) as compared with the reference diet (T1). Maximum activity (7.92 mg maltose liberated mg protein^{-1} h^{-1}) was found in fish fed T2 diet followed by T3 diet. Lipase activity was significantly higher (4.65 LU mg protein^{-1} min^{-1}) in fish fed T3 diet followed by T4 diet. T2 diet showed significantly

higher protease activity (10.383µg histidine liberated mg protein^{-1} h^{-1}) than all other experimental diets.

Water quality parameters recorded during the growth trial (temperature: 27.00-27.73ºC, pH: 6.99-7.56, dissolved oxygen: 7.16-7.86 mg.L^{-1}, free carbon dioxide: 3.75-4.65 mg.L^{-1}, total alkalinity: 202-217 mg.L^{-1} as $CaCO_3$, hardness: 160-168 mg.L^{-1} as $CaCO_3$ and total ammonia: 0.09-0.35 mg.L^{-1} were within the optimum range required for rearing carp fingerlings.

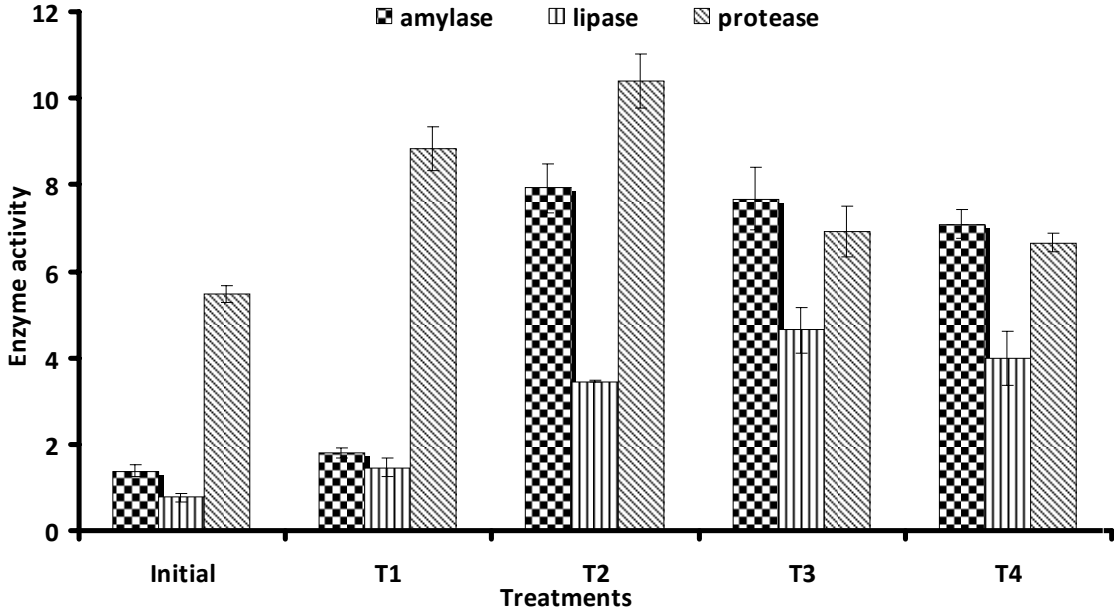

Figure 1. Enzyme assay of *L. bata* after 10 days acclimatization (6 hours after dieting) Amylase Activity = mg maltose lib (mg protein)$^{-1}$ h^{-1}; Protease Activity = µg histidine lib (mg protein)$^{-1}$ h^{-1}; Lipase Activity = LU (mg protein)$^{-1}$ min^{-1} (1LU = micromole free fatty acids lib min^{-1})

Discussion

Results of the present study indicate that mulberry leaf meal is a good source of protein and can be used as feedstuff in carp diet formulation if it is fermented with suitable microorganisms. Lactic acid bacteria (LAB) are the key microorganisms responsible for fermentation of vegetable materials, although several other microorganisms take part in different stages of the fermentation process. The suspension used in the present study contained *Lactobacillus* sp. along with the photosynthetic bacteria *Rhodopseudomonas* sp., nitrogen fixing bacteria *Azotobacter* sp. and

the yeast *Saccharomyces* sp. However, the LAB *Lactobacillus* sp. alone or similar strain isolated from fish gut are capable of fermenting plant materials for incorporation in fish diet (Mukhop-adhyaya and Ray 2005; Ramachandran *et al*. 2005). Termination of fermentation as indicated by decline of pH depends upon the substrate and quality of the innoculum. The present study indicates that diets supplemented by fermented mulberry leaf meal are accepted well by the fingerlings of *Labeo bata*.

Apparent protein digestibility of the experimental diets varied from 88.78-89.29% and significantly differed from each other, T2 diet showing highest digestibility (89.29%) and T4 diet showing the least (88.78%). The results indicate that protein digestibility decreases with increase in the level of MLM. Similar decrease in protein digestibility was found with increasing level of duckweed meal replacing FM, MOC and RB in the eight isonitrogenous and isocaloric diets formulated for *L. rohita* fingerlings (Bairagi *et al*. 2002). Although fermentation removed anti-nutritional factors like phytic acid and tannin completely from the diets containing MLM meal in the present investigation, crude fibre content persisted in these diets in considerable proportion and the level of crude fibre increased with the increase in proportion of MLM in the diets. Crude fibre content play a significant role in reducing the digestibility of the diets (Bairagi *et al*. 2002).

L. bata is a slow growing fish. Under natural conditions in rivers it attains a length of only 133 mm in one year (Chatterji *et al*. 1979). We observed that fingerlings of *L. bata* attained a 156% growth in 2 months culture on diets supplemented by 65% fermented MLM and 10% FM (T2). Typically, growth rate of fish increases with increase in the level of dietary protein till the optimum level is reached. Since the diets in the present study were isonitrogenous (crude protein level ≈ 30.00 %) the growth was unlikely to be affected by dietary protein level. But crude lipid level of the experimental diets increased with the increase of MLM level in spite of reduction in MOC which is also a source of lipid. This could extend a protein sparing effect on the growth of *L. bata* and explain a better growth in diet containing 65% fermented MLM replacing 50% of FM, 64% MOC and 77% RB (T2). Lipid as a non-protein energy source allows protein sparing by effectively reducing organic matter and nitrogen losses. Protein sparing effects of dietary lipids have been demonstrated for salmonids and sea bass (Cho and Kaushik, 1990; Arzel *et al*. 1994; Dias *et al*. 1998), common carp (Gilbey *et al*. 2001; Manjappa *et al*. 2002), grass carp (Du *et al*. 2005). So far there is no such report of protein sparing effect of lipid on any Indian minor carp. However, it was revealed from the present study that the protein sparing by lipid was effective as long as the crude fibre level remained low (5.63% at T2). When the crude

fibre level increased (in T3 and T4) it produced anti-nutritional effects. Protein sparing effects of some carbohydrates have also been found in the Indian cyprinidis except mrigal which tolerates less carbohydrate than other two Indian major carp species (Das *et al*. 1991). Protein sparing effect of carbohydrate in the T2 diet of the present study also cannot be ruled out because MLM is a rich source of carbohydrate, although RB, another source of carbohydrate was considerably reduced in this diet as compared to the reference diet.

Higher activities of the protease, lipase and amylase of the fish fed the T2 experimental diets as compared with the reference diet indicate that the protein, lipid and carbohydrates of MLM are easily digestible. However, protease activities reduced with the increase of MLM obviously due to increased fibre content.

L. bata is an herbivorous fish. But the present study shows that it can digest protein most efficiently. Proteolytic activity is less dependent on the nutritional habits (Hidalgo *et al*. 1999). Kuz'mina (1996) also found a high proteolytic potential in non carnivorous fish for utilizing animal and plant protein sources efficiently. The present study indicates that *L. bata* is also efficient in digesting protein from mulberry leaf meal.

It is concluded from the present study that MLM is a promising alternative of protein in the formulation of diet of the Indian major carp *L. bata*. Fifty percent replacement of fish meal is possible when 65% MLM is fermented along with MOC (14%) and RB (9%) and is used as ingredient in the formulated diet. Fermentation results in increase the quality of the experimental diets. However, a level higher than 65% of MLM reduces protein digestibility of the diet and growth of the fish.

Acknowledgements

This research was funded by University Grants Commission, New Delhi, India. The authors are thankful to Head, Department of Zoology, University of Kalyani and the Principal, Tamralipta Mahavidyalaya for providing neces-sary facilities for this research.

References

- ✓ AOAC (1990) In: Helrich, W. (Ed), Association of Official Analytical Chemists. Official Methods of Analyses. vol.I, 15th ed. Association of Official Analytical Chemists, Washington, DC.
- ✓ APHA (1995) Standard methods for the examination of water and wastewater, American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington, DC, U.S.A.
- ✓ Arzel J., Martinez Lopez F.X., Métailler R., Stéphan G., Viav M., Gandemer G. and Guillaume J. (1994) Effect of dietary lipid on growth performance and body composition of brown trout (*Salmo trutta*) reared in seawater. Aquaculture 123: 361-375.
- ✓ Bairagi A., Sarkar Ghosh K., Sen S.K. and Ray A.K. (2002) Duckweed (*Lemna polyrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu (*Labeo*

rohita Ham.) fingerlings after fermentation with a fish intestine bacterium. Biores. Technol. 85: 17-24.

- ✓ Bamikole M.A., Ikhatua M.I., Ikhatua U.J. and Evenwa I.V. (2005) Nutritive value of Mulberry (*Morus* spp.) leaves in the growing rabbits in Nigeria. Pakistan J. Nutr. 4: 231-236.
- ✓ Brafield A.E. (1985) Laboratory studies of energy budgets. P. Tytler and P. Calow, eds. Fish energetics: New perspectives. Croom Helm, London: 257-281.
- ✓ Castell J.D. and Tiews K. **(**1980) Report of the EIFAC, IUNS and ICES Working Group on the standarisation of methodology in fish nutrition research, EIFAC Technical Paper 36, Hamburg, Federal Republic of Germany, 21-23 March.
- ✓ Chatterji A., Siddiqui A.Q. and Khan A.A. (1979) Studies on the age and growth of the bata, *Labeo bata* (Ham.) (Cyprinidae, Teleostei) from the river Kali, India. Hydrobiologia 63: 99-192.
- ✓ Cho C.Y. (1992) Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. Aquaculture 100: 107–123.
- ✓ Cho C.Y. and Kaushik S.J. (1990) Nutritional energetic in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). World Rev. Nutr. Diet. 61: 132-172.
- ✓ Cowey C.B. (1992) Nutrition: estimating requirements of rainbow trout. Aquaculture 100: 177-189.
- ✓ Das K.M., Mohanty S.N. and Sarkar S. (1991) Optimum dietary protein to energy ratio for *Labeo rohita* fingerlings. Proceeding Fourth Workshop Asian Fish Nutrition, pub no. 5, S. S. De Silva (ed.), Asian Fisheries Society, 69-73.
- ✓ Datta R.K., Sarkar A., Rao P.R.M. and Singhvi N.R. (2002) Utilization of mulberry as animal fodder in India. In Sánchez, M.D. (ed.), Mulberry for animalproduction. FAO, Rome.
- ✓ Dias J., Alvarez M.J., Diez A., Arzel J., Corraze G., Bautista J.M. and Kaushik S.J. (1998) Regulation of hepatic lipogenesis by dietary protein/energy in juvenile European seabass (*Dicentrarchus labrax*). Aquaculture 161: 169–186.
- ✓ Du Z.Y., Liu Y.J., Tian L.X., Wang J.T., Wang Y. and Liang G.Y. (2005) Effect of dietary lipid level on growth, feed utilization and body composition by juvenile grass carp (*Ctenopharyngodon idella*). Aquac. Nutr. 11: 139–146.
- ✓ Ellestad L.E., Angel R. and Soares J.H.Jr. (2002) Intestinal phytase II: a comparison of activity and in vivo phytate hydrolysis in three teleost species with differing digestive strategies. Fish Physiol. Biochem. 26: 259-273.
- ✓ Gatlin III D. M., Barrows F. T., Brown P., Dabrowski K., Gaylord T.G., Hardy R.W., Herman E., Hu G., Krogdahl A., Nelson R., Overturf K., Rust M. , Sealey W., Skonberg D., Souza E. J., Stone D., Wilson R. and Wurtele E. (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38: 551-579.
- ✓ Gilbey J., Ali M. and Wootton R.J. (2001) Effect of rations of different lipid levels on the growth, food conversion efficiency and lipid contents of the common carp (*Cyprinus carpio* L.). Pakistan J. Zool. 33: 265–269.
- ✓ Gomez K.A. and Gomez A.A. (1984) Statistical Procedures for Agricultural Research. 2nd edn., John Wiley and Sons, New York.
- ✓ Hidalgo M.C., Urea E. and Sanz A. (1999) Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. Aquaculture 170: 267–283.
- ✓ Johnson R.A. and Wichern D.W. (1992) Applied Multivariate Statistical Analysis. Prentice Hall of India, New Delhi, India: 241-284.
- ✓ Kalita P, Mukhopadhyaya P. K. and Mukherjee A. K. (2008) Supplementation of four non-conventional aquatic weeds to the basal diet of *Catla catla* and *Cirrhinus mrigala* fingerlings: effect on growth, protein utilization and body composition of fish. Acta. Ichthyol. Piscat. 38: 21–27.
- ✓ Kuz'mina V.V. (1996) Influence of age on digestive enzyme activity in some freshwater teleosts. Aquaculture 148: 25–37.
- ✓ Lee K.J., Rinchard J., Dabrowski K., Babiak I., Ottobre J.S. and Christensen J.E. (2006) Long-term effects of

dietary cottonseed meal on growth and reproductive performance of rainbow trout: Three-year study. Anim. Feed Sci. Technol. 126: 93-106.

- ✓ Lo C.P. (1996) Environmental impact on the development of agricultural technology in China: the case of the dike-pond ('jitang') system of integrated agriculture-aquaculture in the Zhujiang Delta of China. Agric. Ecosyst. Environ 60: 183-195.
- ✓ Manjappa K., Keshavanath P. and Gangadhara B. (2002) Growth performance of common carp (*Cyprinus carpio*) fed varying lipid levels through low protein diet, with a note on carcass composition and digestive enzyme activity. Acta. Ichthyol. Piscat. 32: 145–155.
- ✓ Majumdar B.N., Momin S.A. and Kehar N.D. (1967) Studies on tree leaves as cattle fodder. I. Chemical composition as affected by the stage of growth. Indian J. Vet. Sci. 37: 217–223.
- ✓ Maynard L., Loosil J., Hintz H. and Warner R. (1979) In: Animal Nutrition, 7th edn (ed. By C.R. Zappa), McGrawHill, NewYork , USA: 13-14.
- ✓ Mondal T.K. and Ray A.K. (1999) The nutritive value of *Acacia auriculiformis* leaf meal in compounded diets for *Labeo rohita* fingerlings. The Fourth Indian Fisheries Forum Proceedings 1996, Kochi: 295–298.
- ✓ Mondal K., Kaviraj A. and Mukhopadhyay P.K. (2011) Introducing Mulberry Leaf Meal along with Fish Offal Meal in the Diet of Freshwater Catfish, *Heteropneustes fossilis*. Electron. J. Biol. 7: 54-59.
- ✓ Mukhopadhyay N. and Ray A.K. (2005) Effect of fermentation on apparent total and nutrient digestibility of linseed, *Linum usitatissimum,* meal in rohu, *Labeo rohita*, Fingerlings. Acta. Ichthyol. Piscat. 35: 73–78.
- ✓ Nandeesha M.C., Gangadhara B. Manissery J.K. and Venkataraman L.V. (2001) Growth performance of two Indian Major carp catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels *Spirulana platensis*. Biores. Technol. 80: 117-120.
- ✓ National Research Council (NRC) (1993) Nutrient Requirements of Fish. National Academy Press, Washington, DC: 124.
- ✓ Narayana H. and Setty S.V.S. (1977) Studies on the incorporation of mulberry leaves (*Morus indica*) in layers mash on health, production and egg quality. Indian J. Anim. Sci. 47: 212-215.
- ✓ Ramachandran S., Bairagi A. and Ray A.K. (2005) Improvement of nutritive value of grass pea (*Lathyrus sativus*) seed meal in the formulated diets for rohu*, Labeo rohita* (Hamilton) fingerlings after fermentation with a fish gut bacterium. Biores. Technol. 96: 1465–1472.
- ✓ Ray A.K. and Das I. (1995) Evaluation of dried aquatic weed, *Pistia stratiotes* meal as a feedstuff in pelleted feed for rohu, *Labeo rohita* fingerlings. J. Appl. Aquacult. 5: 35-44.
- ✓ Saha D.C. and Gilbreath R.L. (1991) Analytical recovery of chromium in diet and faeces determined by colorimetry abd atomic absorption spectrophotometry. J. Sci. Food Agric. 55: 433-446.
- ✓ Schanderi S.H. (1970) Method in Food Analysis. Academic Press, New York.
- ✓ Spyridakis P., Metallier R., Gabaudan J. and Riaza A. (1989) Studies on nutrient digestibility in European sea bass (*Dicentrarchus labrax*). Methodological aspects concerning faeces collection. Aquaculture 77: 61–70.
- ✓ Tacon A.G.J. (1993) Feed formulation and on-farm feed management. In M.B. New, A.G.J. Tacon and I. Csavas, eds. Farm-made aquafeeds, p. 61-74. Proceedings of the FAO/AADCP Regional Expert Consultation on Farm-Made Aquafeeds. Bangkok, FAO-RAPA/AADCP.
- ✓ Vielma J., Koskela J., Ruohonen K., Jokinen I. and Kettunen J. (2003) Optimal diet composition for European whitefish (*Coregonus lavaretus*): carbohydrate stress and immune parameter responses. Aquaculture 225: 3 -16.
- ✓ Wheeler E.L. and Ferrel R.E. (1971) A method for phytic acid determination in wheat and wheat fractions. Cereal Chem. 48: 312-320.