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Growth performance of Indian minor carp *Labeo bata* fed varying inclusions of fermented fish-offal and mulberry leaf meal based-diets

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Abstract

Fermented mixtures containing fish-offal meal (FOM) and mulberry leaf meal (MLM) were used as protein supplement to partially replace fish meal (FM) in the formulation of diets for the Indian minor carp *Labeo bata*. The diets included a reference diet (20 % FM), three diets of fermented mixture of FOM and MLM (T2–T4) replacing 50 to 75 % of FM. Formulation of diet with 30 % FOM, 24 % MLM and 5 % FM, thereby replacing 75 % of FM, appeared to be the best diet in terms of growth of the *L. bata* fingerlings. It was concluded that effectiveness of fermented FOM in replacing FM could be substantially increased by limited inclusion of MLM in the formulation of diet of minor carp.

Keywords: Fish-offal meal, Mulberry leaf meal, Recycling, Fermentation, Diet, Carp

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Introduction

Huge quantities of fish-offal consisting of viscera, heads and bones of fish are obtained from the retail fish markets and industrial processing units of fish in India. Approximately, 12-14 tones of fish offal are generated from the retail fish markets of Kolkata alone. Disposal of this huge quantity fish offal is a burden not only for Kolkata but also for many other major cities of the world. In Japan, production of fish-offal is estimated to be approximately two million tons per year, half of which is discarded as industrial waste (Hirai, 2001). The fish-offal, which is generated in fish markets of India, is a potential nutrient-laden raw material for formulation of diet for fish. Mondal *et al*. (2006) observed that carp fish-offal generated from Kolkata fish market contained high amount of protein (31.50 to 38.90 %) and lipid (40.60 to 43.80 %). These large quantities of offal result from the inefficient utilization of this limited natural resources and the disposal of the offal greatly affects the local environment. It is established that fermented fish-offal (mainly viscera of carps) can be effectively used as protein source to replace fish meal in the formulation of diet for the Indian major carp *Labeo rohita* (Mondal *et al*., 2007), *Heteropneustes fossilis* (Mondal *et al*., 2008) and *Labeo bata* (Mondal *et al*., 2011). Replacement of fish meal by cost effective protein source is a priority research in aquaculture in the world. 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 (NRC, 1993; Vielma *et al*., 2003). Fermentation is a simple and cheap method to decrease the antinutritional factors and crude fibre contained in the plant and animal by-products (Mukhopadhyay and Ray, 2005; Mondal *et al*., 2012). Fermented fish-offal alone could replace fifty percent of fish meal in the diet of above carps (Mondal *et al*., 2007), and catfish (Mondal *et al*., 2008). 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 *L.bata* resulted in better growth (Mondal *et al*., 2012). The present study was therefore undertaken to investigate if fermented mixture of fish-offal meal and mulberry leaf meal together could effectively replace fish meal in terms of amino acids and other biochemical composition of the diets and response of a carp fish fed such diets for growth and nutrient deposition.

Materials and Methods

Experimental diet formulation and preparation

Fish-offal meal (FOM), sun dried mulberry leaf meal (MLM), mustard oil cake (MOC) and rice bran (RB) were mixed and fermented to produce four experimental diets. For fermentation, the FOM, MOC, RB and MLM 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 30 to 38 days, depending on the proportion of FOM and MLM. Final fermented product was mixed with fish meal, vitamin, and mineral mixture to formulate diets, which contained not less than 30% crude protein (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 then the diets were sun dried for a few days before using in the trial.

Table 1: Formulation and proximate analyses of experimental diet. Diet preparation with fish offal meal (FOM) and *Morus* sp (mulberry leaf meal (MLM)).

Contents	T1 (Reference)	T2	T3	T4
Ingredients (%)				
Mustard oil cake[1]	40	24	24	24
Rice bran[2]	38	15	15	15
Fish-offal meal[3]	--	30	25	20
Mulberry leaf meal[4]	--	24	24	35
Fish meal[5]	20	5	10	4
Vitamin premix[6]	0.5	0.5	0.5	0.5
Mineral premix[7]	0.5	0.5	0.5	0.5
Cr_2O_3(g.kg diet^{-1})[8]	1	1	1	1
Proximate composition (%)[9]				
Dry matter	94.00	91.00	92.00	93.00
Protein	30.20	30.20	30.40	30.60
Lipid	11.63	19.12	17.20	14.10
Crude fiber	3.46	2.81	3.22	5.10
Ash	11.20	10.10	12.40	15.50
NFE	48.71	38.97	41.18	43.20
Gross energy (kJ.g^{-1})	18.83	19.99	19.71	18.92
P:E Ratio[10]	16.04	15.05	15.42	16.17

[1]Dry matter (DM)-87%, Crude Protein (CP)- 34.50%, Crude Lipid (CL)-6.50%, Ash-9.20%.
[2]DM-95.35%, CP-13.40%, CL-4.80%, Ash-22.00%.
[3]DM-82.00%, CP-35.20%, CL-42.20%, Ash-2.50%.
[4]DM-89.60%, CP-28.60%, CL-4.14%, Ash-10.24%.
[5]DM-93.20%, CP-76.80%, CL-6.20%, Ash-8.20%.
[6]Vitamin mixture (%): (Ambiplex; Brihans Lab, Pune): Vit B1: 7.14, Vit B2: 2.55, Vit B6: 1.02, Vit B12: 0.012, Biotin: 0.025, Calcium Pantothenate: 2.55, Niacin: 76.50, Choline chloride (B4):10.20 ;Vitamin C in the form of ascorbyl polyphosphate was added to vitamin mixture @ 100mg/kg mixture.
[7]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, Phosphorus: 8.25.
[8]Used as non absorbent reference substance only in diets used in digestibility experiments.
[9]Number of samples per each determination = 3
[10]Protein to energy ratio in mg protein/kJ of total energy.

Growth trials

Two experimental systems were utilized for the feeding trials: one in outdoor cement tanks (400 L) to evaluate growth and biochemical composition of the body, and the other in indoor glass aquaria (50 L) to evaluate voluntary diet intake and apparent protein digestibility (APD). Deep

tube-well water stored in an overhead tank was used in the feeding trial. Fingerlings of *L. bata* (mean initial length 8.12±0.16 cm and mean initial weight 5.20±0.12 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 (T1) 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 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 by siphoning from each aquarium 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. APD of the diet was calculated from the proportion of 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 9.2–9.6 $mg.L^{-1}$.

The fish were fed the same ration (5 % of body weight per day) this time in two equal installments, 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 e.g. 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 $100\times((FBW^{0.3333}– IBW^{0.3333})/duration)$, 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):

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

Analytical methods

Proximate composition analyses of the experimental diets, carcass and faecal samples were performed following the Association of Official Analytical Chemists, (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, moisture and ash and subtracting this from 100 (Maynard *et al*., 1979). Gross energy was calculated on the basis of methodology provided by 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. The detailed methodology has been followed from Saha and Gilbreath (1991).

For amino acid analysis defatted diet samples were dried. Dried and lyophilized samples were hydrolyzed with 2(N) HCl (Rosenberg, 2005) followed by analysis of the amino acids in HPTLC using TLC aluminum sheet silica gel [60 F_{254} 20×10 cm or 10×10 cm (Merck KGaA; 1.05554.0007)] as the stationary phase and the n-Butanol: Acetone: Acetic acid: Water [35:35:10:20] as the mobile phase (Wagner and Bladt, 1996). All the essential and non-essential amino acids were identified in samples against each amino acid used as standards. From the standard curve having best regression equation (R^2), the amount of amino acids content was determined.

Blood parameters analyses

Initial fish blood samples were collected before feeding trial by cutting of the caudal peduncle (Keene *et al*., 1998). Subsequent blood samples were collected on 1st, 20th, 40th and 60th day. The blood samples were collected in disodium salt of Ethylene Diamine Tetra-acetic acid (EDTA) bottles for analyses. The count of erythrocytes and leukocytes were enumerated in an improved Neubaeur haemocytometer, using Hayem and truck diluting fluids (Blaxhall and Daisley, 1973). The hemoglobin (Hb) was determined according to cyanomethemoglobin procedure (Blaxhall and Daisley 1973). Non-clotted blood (20 µL) was diluted with Drabkin solution (5 mL) and left standard 5 min. The absorbency of the mixture was read at 540 nm and the amount of Hb was calculated from a hemoglobin standard. Hb content was expressed in g per dL. The MCV was calculated according to Seiverd (1964). Hematocrit (packed cell volume, PCV) was determined by the method described

by Blaxhall and Diasley (1973) with commercially available heparinzed capillary tubes of 25 mm. The mean corpuscular volume (MCV) was calculated as quotient of hematocrit and number of red blood cells (RBC), MCV = (PCV / RBC) × 100. The value of MCV was expressed in femtolitre (fL).

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) using SPSS 10 program.

Results

Crude protein level in diets containing FOM, FM and MLM (T2 to T4) ranged from 30.20 to 30.60 %. Total lipid content significantly increased in diets containing mixture of 30% FOM and 24% MLM. Diets containing 30% FOM and 24% MLM (T2) showed higher total lipid content (19.12 %) than the other diets. Amino acids profile of FOM, MLM and the experimental diets are given in Table 2. Raw fish-offal meal contained 62.64 % total amino acid and 37.29% total essential amino acid, MLM contained 23.41 % total amino acid and 16.02% total essential amino acid. Percentage of total amino acid was highest in T4 diet (20% FOM and 35 % MLM and 4 % FM). Maximum proportion of essential amino acids was also found in T4 diet (11.70 %) and total amino acid 25.62 %. Concentrations of tannin and phytic acid (the anti-nutritional factors) in the ingredient mixture before fermentation were 0.07 and 0.08 %, in T2, 0.06 and 0.14 % in T3 and both 0.21 % in T4, respectively. None of these anti-nutritional factors could be detected in the fermented products.

The survival rate of the *L. bata* fingerlings during the digestibility trial ranged from 95 to 98 % and showed no significant variation between the dietary treatments. Mixture of 30% FOM and 24% MLM containing diets showing higher APD (95.71 %) than the other diets (Table 3), except that low FOM and high MLM containing diet (T4) exhibited APD that was comparable with the reference diet (T1).

The survival rate of the *L. bata* fingerlings during the growth trial ranged from 90 to 91% and showed no significant variation between the dietary treatments. Data on growth performance and diet utilization of *L. bata* fingerlings in terms of percentage of diet intake, weight gain, SGR, TGC, DGC, FCR, PER and ANPU are represented in Table 3. Growth (in terms of weight gains, SGR, DGC, and TGC) significantly increased in T2 (5 % FM, 30 % FOM and 24 % MLM) and T3 (10 % FM, 25 % FOM and 24 % MLM) diet, replacing 75 % and 50 % FM respectively, as compared with the reference diet (T1). However, ANPU and PER were significantly higher in T2 as compared with other diets.

Table 2: Amino acid content (% of total protein) amino acids present in dry samples of the raw materials and combined experimental diet.

Amino Acids	Samples					
	Fish-offal meal	Mulberry leaf meal	T1	T2	T3	T4
Essential amino acids						
Arg	4.47	1.72	0.37	0.32	0.68	0.51
His	2.28	1.64	0.96	1.69	1.74	1.68
Ile	6.48	1.84	0.94	1.84	0.78	0.89
Leu	4.83	2.68	1.86	0.96	1.78	0.88
Lys	6.95	1.88	1.46	1.86	0.66	0.92
Met	1.48	0.68	1.24	1.48	1.64	1.54
Phe	2.64	1.86	1.64	0.64	0.88	1.74
Thr	2.84	1.84	1.44	1.06	1.14	1.92
Trp	1.86	--	0.32	0.96	0.98	0.54
Val	3.46	1.88	1.64	1.22	1.00	1.08
Non-essential amino acids						
Ala	4.88	1.24	1.29	1.40	1.64	2.14
Asp	5.48	1.14	2.64	1.68	2.38	2.41
Glu	4.86	2.14	1.76	1.87	2.00	2.08
Gly	2.46	0.67	0.86	1.43	1.60	2.17
Pro	1.68	0.30	0.76	1.22	0.84	1.02
Ser	1.67	0.80	1.62	1.00	1.00	1.14
Cys	1.68	0.46	0.88	0.20	1.10	1.18
Tyr	2.64	0.64	1.24	0.46	1.24	1.78
TAA	62.64	23.41	22.92	21.29	23.08	25.62
TEAA	37.29	16.02	11.87	12.03	11.30	11.70

TAA—total amino acid, TEAA—total essential amino acids.

Table 3: Digestibility, growth performance and diet efficiency of *Labeo bata* fingerling fed experimental diets for 60 days.

	Indoor trial		Outdoor trial						
Diets	**Diet intake (g/100g BW/d)**	**APD[1] (%)**	**% increase in Weight**	**FCR[2]**	**SGR[3] (%.d^{-1})**	**PER[4]**	**ANPU[5]**	**DGC**	**TGC**
T1	2.498^{a}±0.16	89.00^{a}±0.61	128.11^{a}±9.80	1.86^{a}±0.11	1.24^{a}±0.07	1.62^{a}±0.13	34.26^{a}±1.13	0.352^{a}±0.02	0.970^{a}±0.06
T2	2.547^{a}±0.10	96.00^{b}±0.10	168.57^{b}±14.55	1.19^{b}±0.10	1.64^{b}±0.09	4.00^{b}±0.33	54.19^{b}±0.86	0.418^{b}±0.02	1.153^{b}±0.07
T3	2.575^{a}±0.04	93.30^{c}±0.64	153.88^{c} ±7.68	1.35^{c}±0.06	1.49^{c}±0.05	3.88^{c}±0.19	51.65^{c} ±1.71	0.402^{bc}±0.01	1.109^{bc}±0.04
T4	2.517^{a}±0.17	89.27^{a}±0.54	152.26^{cd}±11.54	1.32^{cd}±0.10	1.44^{cd}±0.08	3.73^{d}±0.28	48.95^{d}±1.69	0.388^{ad}±0.02	1.068^{cd}±0.06

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

[1]APD = 100 -100 × ((% Cr in diet / % Cr in faeces) × (% protein in faeces / % protein in diet))

[2]FCR = Dry weight of diet given / increase in weight of the fish

[3]SGR = {(ln final weight – ln initial weight)/days on trial} × 100

[4]PER = Wet weight gain of fish / Protein consumed.

[5]ANPU = (Net increase in carcass protein / Amount of protein consumed) × 100

The whole body composition of experimental fish determined before and after the experiment is given in Fig. 1. The deposition of crude protein in the whole body was significantly higher in fish fed T2 (18.19 %) and T3 (17.40 %) diets containing both FOM and MLM as compared with those fed reference diet (T1, 15.12 %). The lipid content of the whole body was significantly higher in the diet T2 (2.82 %) as compared with the reference diet (T1, 2.42 %). Ash content was significantly difference between the diet groups. Maximum value of ash was recorded in T2 (5.26 %).

The activities of the digestive enzymes in the intestine of *L. bata* are presented in Fig. 2. The α- amylase activity significantly increased in all the experimental diets (T2 to T4) as compared with the reference diet (T1). Maximum activity (5.711 mg maltose liberated mg protein^{-1} h^{-1}) was found in fish fed T4 diet (20 % FOM, 35 % MLM and 4%FM) followed by T2 diet (30 % FOM, 24 % MLM and 5% FM). Lipase activity was significantly higher in diets containing FOM (T2 to T4). Maximum activity (10.043 LU mg protein^{-1} min^{-1}) was recorded in fish fed T2 diet (30 % FOM, 24 % MLM and 5 % FM) followed by T3 (25 % FOM) and T4 (20 % FOM) diet. T1 diet (40 % MOC, 38% RB and 20 % FM) showed significantly higher protease activity (8.839 µg histidine liberated mg protein^{-1} h^{-1}) than all the experimental diets.

Fish blood parameters were determined before the start of the experiment (initial) and at day 1, 20, 40 and 60. The parameters have been recorded in Fig. 3. PCV irrespective of diet groups significantly increased from initial level to the end of experiment at 60th day (24.32% to 25.26%). Results of Hb also significantly increased from its initial value to the end of experiment in all diet groups (8.87% to 9.35%). RBC significantly increased from initial value of 2.50 (×10^6/ml) to 2.62 (×10^6/ml). White Blood Cells (WBC) did not change from initial value to the end of experiment while MCV showed marginal decrease from the initial value to the end of the experiment. At 60th day WBC did not show any significant variation between the diet groups while all other parameters were either significantly increase in experimental diets (T2-T3) or remained comparable to reference diet (T1).

Water quality parameters recorded during the growth trial (temperature: 27.50-27.83ºC, pH: 7.00-7.56, dissolved oxygen: 8.16-8.89 mg.L^{-1}, free carbon dioxide: 3.95-4.95mg.L^{-1}, total alkalinity: 180.00-190.00 mg.L^{-1}, total hardness: 190.00-195.00 mg.L^{-1} as $CaCO_3$ and total ammonia: 0.12-0.38 mg.L^{-1} were within the optimum range required for rearing carp fingerlings.

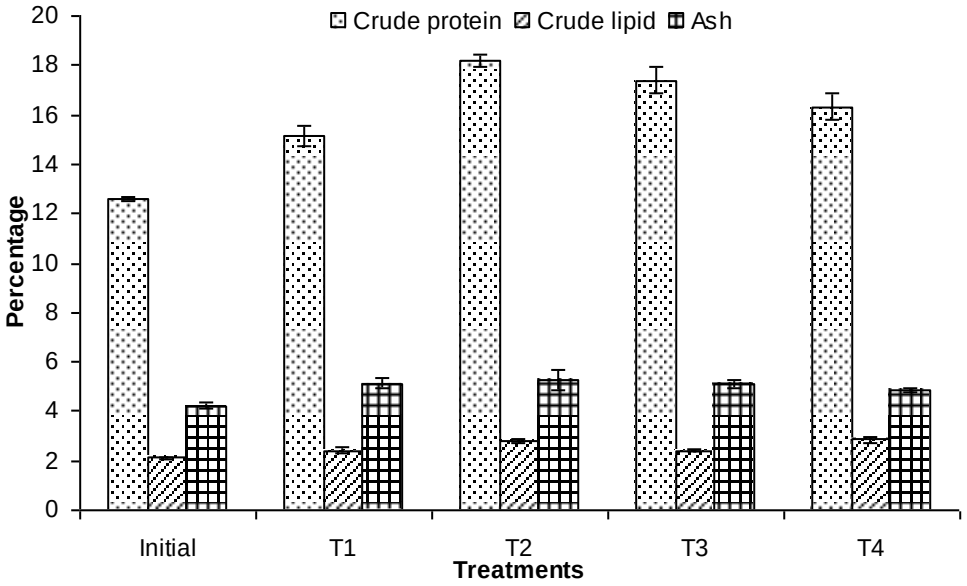

Figure 1: Proximate composition of carcass (% wet weight) of the experimental fish (*Labeo bata*) at the start and end of the 60 days dieting trial. Bars represent standard deviation of the mean.

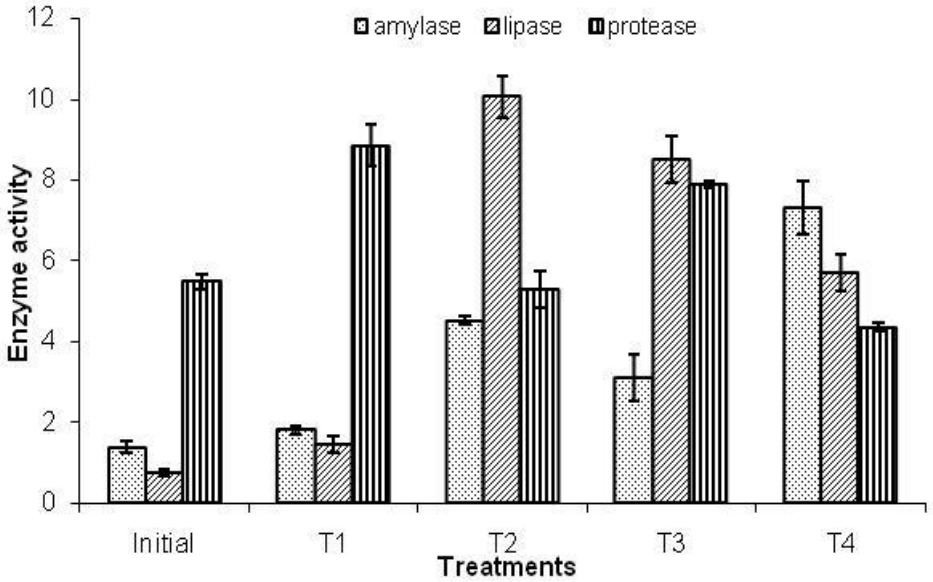

Figure 2: Enzyme assay of Bata after 10 days acclimatization (6 hours after dieting). Bars represent standard deviation of the mean.

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}) × 10^{3}/mL

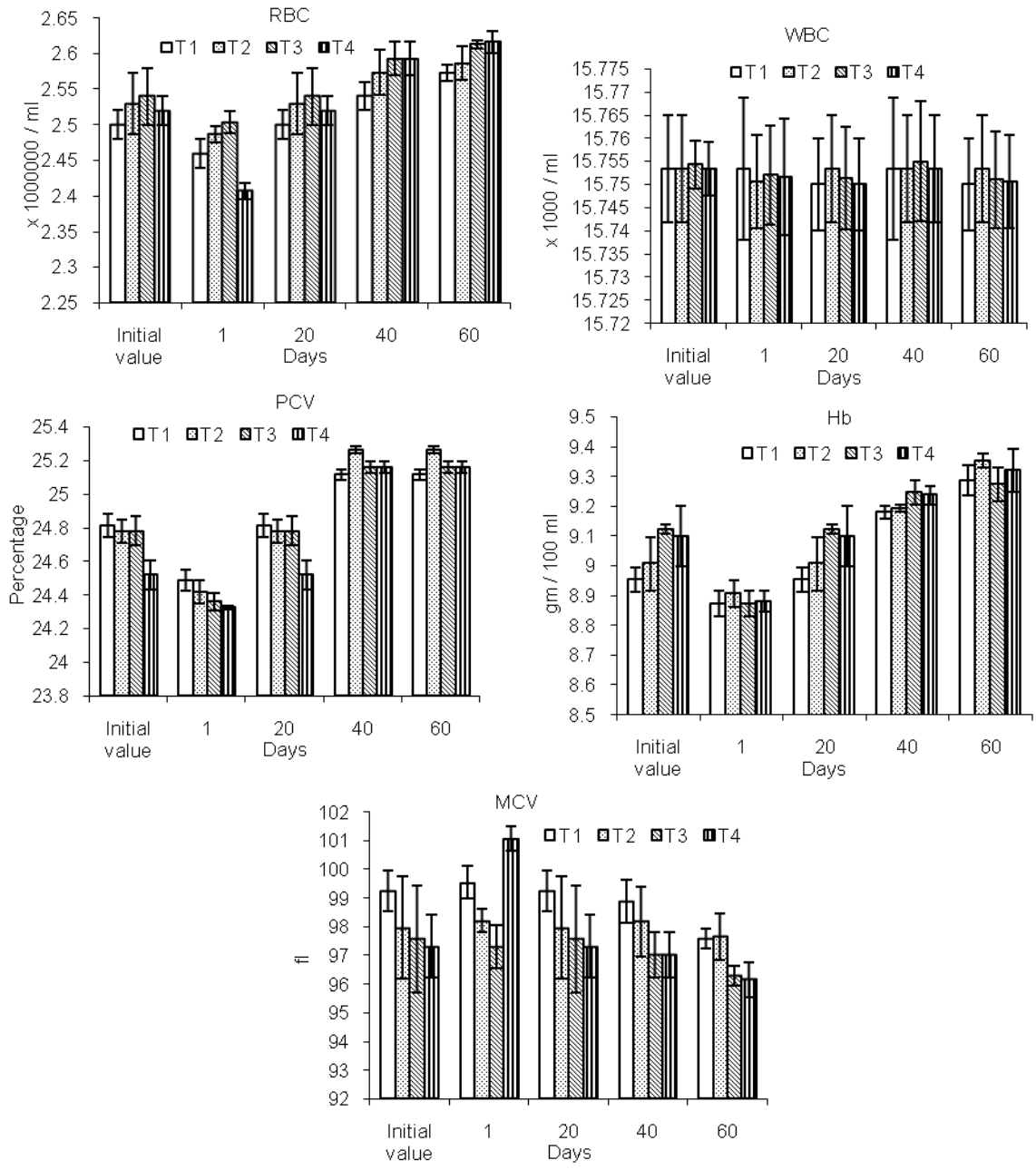

Figure 3: Hematological composition of *Labeo bata* fed fermented FOM (fish-offal meal) and MLM (mulberry leaf meal) based-diets for 60 days. Bars represent standard deviation of the mean.
RBC: Red Blood Cells, WBC: White Blood Cells, PCV: Packed Cell Volume, Hb: Hemoglobin, MCV: Mean Corpuscular Volume.

Discussion

Results indicate that diets supplemented by fermented mixture of FOM and MLM are accepted well by fingerlings of *L. bata*. The FOM and MLM containing diet can be utilized in carp diet formulation if it is fermented with suitable microorganisms. FOM could be used up to 30 % in combination with FM (5 %) and MLM (24 %) (T2 diet). Complete fermentation of the mixture of FOM and MLM (T2 to T4) required a little longer period (30 to 38 days) as compared to fermentation period

(12 to 22 days) required for FOM alone (Mondal *et al*., 2007).

The diet intake rate of the fingerlings of *L. bata* in the present investigation (2.498 to 2.575 g per 100 g BW per day) was higher than the rate observed by Mondal *et al*. (2011). Among the seven diets used in the present investigation protein digestibility varied from 89.00-96.00% and significantly differed from each other, T2 diet showing highest digestibility (96.00%). The present results indicate that protein digestibility decreases with increase in the level of MLM. Similar decrease in protein digestibility was found with increasing level of mulberry meal in the formulated diet of *L. bata* fingerlings (Mondal *et al*., 2012) when fish meal was replaced (75%) by fermented mulberry leaf meal. The results indicate that although fermentation removed phytic acid and tannin completely from the diets containing MLM meal, crude fibre content was still higher than the other diets and might play a role in reducing the digestibility of the diets (Mondal *et al*., 2012).

We observed fingerlings of *L. bata* grew better on diets supplemented by fermented FOM and MLM mixture (T2) as compared with the reference diet (T1). Typically, growth rate of fish increases with increase in the level of dietary protein till the optimum level is reached. Interestingly, in the present investigation the T2 diet (30 % FOM, 24 % MLM and 5 % FM) having lower level of dietary protein (30.20 %) and protein energy ratio (15.05 g protein.kj^{-1}) than the reference diets tested produced the best growth of the fish which is higher than the growth of *L. bata* (Mondal *et al*., 2011). Even other diets containing mixture of FOM and MLM (T3 and T4), showed better growth than the reference diet. This could be explained as protein sparing effect of the higher level of lipid in the diet containing mixed FOM and MLM. Diets containing FOM and MLM exhibited protein sparing effects in the diet of *L. rohita* (Kaviraj *et al*., 2012). 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; Dias *et al*., 1998), common carp (Manjappa *et al*., 2002), and grass carp (Du *et al*., 2005). So far there is no such report of protein sparing effect of lipid on any Indian minor carps.

Total amino acid level was maximum in T4 (20% FOM, 35 % MLM and 4 % FM) followed by T3 and T2. However, the best growth was obtained in T2 followed by T3 and T4. T2 diet contained higher amount of essential amino acid and all other experimental diets (T1 to T4). Bioavailability of the essential amino acids to fish is not uniform and there is marked difference in metabolism of a particular amino acid between species of fish (Conceição *et al*., 2003; Saavedra *et al*., 2007). From the better growth of *L. bata* fed diets containing mixture of FOM and MML it is assumed that such mixture renders higher amount of bioavailable amino acids in the formulated diet.

Higher lipase activities in the experimental diets as compared with reference diet is correlated with the level of lipid in the diets. Augmentation of the

lipase activity is necessary to facilitate digestion of excess lipid in the experimental diets to meet increased energy and fatty acid requirement for a rapid growth (Johnston *et al*., 2006; Mondal *et al*., 2012). Maximum lipase activity and best growth of *L. bata* was observed in T2 diet indicate that the fish is capable of digesting the excess lipid present in FOM and convert it in to the growth. There is a possibility that increased level of lipid in the diet containing FOM might influence sparing of protein. Lipid as a non-protein energy source allows protein sparing by effectively reducing organic matter and nitrogen losses, but high level of dietary lipid may lead to deposition of fat in the body of fish and depression of activities of lipogenic enzymes (Arnesen *et al*., 1993; Alvarez *et al*., 1998). Fish fingerlings are equipped with lipase enzyme necessary for digesting lipid which is the most important sources of energy and essential fatty acids for the fish for stress resistance, securing high membrane fluidity for rapid cellular divisions and growth (Watanabe, 1982; Murray *et al*., 2003). The capacity of fish fingerlings to digest dietary lipid is therefore of great importance for optimal nutrition in development (Bolasina *et al*., 2006, Kaviraj *et al*., 2012).

The experimental diets also showed higher amylolytic activity than the reference diet, which might be due to contribution of carbohydrate from the MLM. Proteolytic activity of the experimental diet was lower than the reference diet because of higher protein level in the later. Although *L. bata* is a herbivorous fish, it is capable of digesting protein diets and show high proteolytic activity. Proteolytic activity is less dependent on the nutritional habits (Hidalgo *et al*., 1999). Kuz'mina (1996) found a high proteolytic potential in non carnivorous fish for utilizing animal and plant protein sources efficiently. The present study indicates that *L. bata* having high proteolytic potential is better prepared to digest protein from fish meal or non conventional sources like fish-offal meal and mulberry leaf meal.

Hematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc. (Blaxhall, 1972; Duthie and Tort, 1985). In this study, WBC count of experimental diets remained the same as initial value. Other hematological parameters including PCV, Hb and RBC increased from the initial value. There was no reduction of these blood parameters in any experimental diets indicating absence of toxic action of the newly incorporated ingredients (Adeyemo, 2005; Osuigwe *et al*., 2005).

It is concluded from the present study that MLM is a promising alternative of protein in the formulation of diet of the Indian minor carp *L. bata*. Addition of limited amount of MLM (24%) in the fermentation of FOM (30%) produces a fermented mixture that can replace 75 % of FM in the formulation of diet, thereby substantially reducing the cost of the diet. Fermentation results in increase the quality of the experimental diets.

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References

Adeyemo, O.K., 2005. Haematological and histopathological effects of Cassava Mill Effluent in *Clarias gariepinus*. *African Journal of Biomedical Research*, 8, 179–183.

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.

Alvarez, M.J., López-Bote, C.J., Diez, A., Corraze, G., Arzel, J., Dias, J., Kaushik, S.J. and Bautista, J.M., 1998. Dietary fish oil and digestible protein modify susceptibility to lipid peroxidation in the muscle of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*). *British Journal of Nutrition*, 80, 281–289.

Arnesen, P., Krogdahl, A. and Kristiansen, I.O., 1993. Lipogenic enzyme activities in liver of Atlantic salmon (*Salmo salar* L.). *Comparative Biochemistry and Physiology-Part B: Biochemistry and Molecular Biology*, 105**,** 541–546.

Blaxhall, P.C., 1972. The haematological assessment of the health of freshwater fish. *Journal of Fish Biology*, 4, 593-605.

Blaxhall, P.C. and Daisely, K.W., 1973. Routine hematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771-781.

Bolasina, S., Perez, A. and Yamashita, Y., 2006. Digestive enzymes activity during ontogenetic development and effect of starvation in Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 252, 503–515.

Brafield, A.E., 1985. Laboratory studies of energy budgets. In P. Tytler and P. Calow, eds. Fish energetics: New perspectives. Croom Helm, London. pp. 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.

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.

Conceição, L.E.C., Grasdalen, H. and Rønnestad, I., 2003. Amino acid requirements of fish larvae and post-larvae: new tools and recent findings. *Aquaculture*, 227, 221–232.

Cho, C.Y. and Kaushik, S.J., 1990. Nutritional energetic in fish: energy and protein utilization in rainbow trout

(*Salmo gairdneri*). *World review of Nutritional Dietetics*, 61, 132-172.

Cowey, C.B., 1992. Nutrition: estimating requirements of rainbow trout. *Aquaculture*, 100, 177-189.

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 animal production. FAO, Rome. Available at: http://www.fao.org/docrep/005/X9895E/x9895e0h.htm#bm17.

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*). *Aquaculture Nutrition*, 11, 139–146.

Duthie, G.G. and Tort, L., 1985. Effect of dorsal aortic cannulation on the respiration and haematology of the Mediterranean dogfish, *Scyliorhinus canicula*. *Comparative Biochemistry and Physiology-Part A: Molecular and Integrative Physiology*, 81, 879-883.

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 Physiology and Biochemistry*, 26, 259-273.

Gomez, K.A. and Gomez, A.A., 1984. Statistical procedures for agricultural research. 2nd edn, John Wiley and Sons, New York.

Hirai, M., 2001. New advanced technology for fish waste. *Food and Development*, 35, 11–13.

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, pp.241-284.

Johnston, C.S., Corte, C. and Swan, P.D., 2006. Marginal vitamin C status is associated with reduced fat oxidation during submaximal exercise in young adults. *Nutrition and Metabolism*, 3, 35.

Kaviraj, A., Mondal, K., Mukhopadhyay, P.K. and Turchini, G.M., 2012. Impact of fermented mulberry leaf and fish offal in diet formulation of Indian major carp (*Labeo rohita*). *Proceedings of the Zoological Society*, 66(**1**), 64–73.

Keene, J.L., Noakes, D.L.G., Moccia, R.D. and Soto, C.G., 1998. The efficacy of clove oil as an anesthetic for rainbow trout, *Oncorhynchus mykiss*. *Aquaculture Research*, 29, 89-101.

Kuz'mina, V.V., 1996. Influence of age on digestive enzyme activity in some

freshwater teleosts. *Aquaculture*, 148, 25–37.

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 Journal of Veterinary Science*, 37, 217–223.

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 Ichthyologica et Piscatoria*, 32, 145-155.

Maynard, L., Loosil, J., Hintz, H. and Warner, R., 1979. In: Animal Nutrition, 7th edn (ed. By C. R. Zappa), McGrawHill, New York , USA, pp.13-14

Mondal, K., Kaviraj, A. and Mukhopadhyay, **P.K., 2006**. Fish waste in urban and suburan markets of Kolkata: problems and potentials. *Aquaculture Asia*, 11, 22-25.

Mondal, K., Kaviraj, A., Mukhopadhyay, P.K., Datta, M. and Sengupta, C., 2007. Evaluation of fermented fish offal in formulated diet of the Indian major carp, rohu, *Labeo rohita* (Hamilton). *Acta Ichthyologica et Piscatoria*, 37, 99–105.

Mondal, K., **Kaviraj, A. and Mukhopadhyay, P.K., 2008.** Evaluation of fermented fish-offal in the formulated diet of the freshwater catfish *Heteropneustes fossilis*. *Aquaculture Research***,** 39, 1443-1449.

Mondal, K., Kaviraj, A. and Mukhopadhyay, P.K., 2011. Partial replacement of fishmeal by fermented fish-offal meal in the formulation of diet for Indian minor carp *Labeo bata*. *Journal of Applied Aquaculture*, 23, 41-50.

Mondal, K., Kaviraj, A. and Mukhopadhyay, P.K., 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. International Journal of Aquatic Science*, 3, 72-83.

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 Ichthyologica et Piscatoria*, 35, 73–78.

Murray, H.M., Gallant, J.W., Perez-Casanova, J.C., Johnson, S.C. and Douglas, S.E., 2003. Ontogeny of lipase expression in winter flounder. *Journal of Fish Biology*, 62, 816–833.

National Research Council (NRC), 1993. Nutrient requirements of fish. National Academy Press, Washington, DC, 124P.

Osuigwe, D.I., Obiekezie, A.I. and Onuoha, G.C., 2005. Some haematological changes in hybrid catfish (*Heterobranchus longifilis* × *Clarias gariepinus*) fed different dietary levels of raw and boiled jackbean (*Canavalia ensiformis*) seed meal. *African Journal of Biotechnology*, 4, 1017-1021.

Rosenberg, I.M., 2005. Protein analysis and purification: Benchtop Techniques Springer. 520P.

Saha, D.C. and Gilbreath, R.L., 1991. Analytical recovery of chromium in diet and faeces determined by colorimetry abd atomic absorption spectrophotometry. *Journal of the Science of Food and Agriculture*, 55, 433-446.

Saavedra, M., Beltran, M., Pousão-Ferreira, P., Dinis M.T., Blasco, J. and Conceição, L.E.C., 2007. Evaluation of bioavailability of individual amino acids in *Diplodus puntazzo* larvae: Towards the ideal dietary amino acid profile. *Aquaculture*, 263, 192-198.

Schanderi, S.H,. 1970. Method in food analysis. Academic Press, New York. 709P.

Seiverd, C.E., 1964. Hematology for medical technologist's. Lea and febiger, Philadelphia, 946P.

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.

Watanabe, T., 1982. Lipid nutrition in fish. *Comparative Biochemistry and Physiology-Part B: Biochemistry and Molecular Biology*, 73, 3–15.

Wagner, H. and Bladt, S., 1996. [Indian reprint 2004]. Plant drug Analysis, 2nd ed. Springer Verlag Berlin.

Wheeler, E.L. and Ferrel, R.E., 1971. A Method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry*, 48, 312-320.