www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 11: 199-207 (2011) DOI: 10.4194/trjfas.2011.0204

Evaluation of Nutritive Value of Water Hyacinth (*Eichhornia crassipes*) Leaf Meal in Compound Diets for Rohu, *Labeo rohita* (Hamilton, 1822) Fingerlings after Fermentation with Two Bacterial Strains Isolated from Fish Gut

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Received 01 September 2009
Accepted 13 December 2010

Abstract

Nine isonitrogenous (30% crude protein approximately) and isocaloric (18.23 kJ g^{-1}) experimental diets (D1-D9) were formulated either with raw or treated (fermented with fish intestinal bacteria) *Eichhornia crassipes* leaf meal at 20%, 30% and 40% levels replacing other ingredients partially from a fish meal based reference diet (RD). Two specific strains of fish intestinal bacteria, *Bacillus subtilis* CY5 (isolated from *Cyprinus carpio*) and *B. megaterium* CI3 (isolated from *Ctenopharyngodon idella*) having extracellular cellulolytic and amylolytic activities, were used for fermentation of *Eichhornia* leaf meal for 15 days at 37°C. A commercial preparation of lactic acid bacteria (LAB), *Lactobacillus acidophilus* (Lactobacil) was used as feed supplement along with *Bacillus subtilis* CY5. Fermentation of leaf meal resulted in reduction of crude fibre, cellulose and hemicellulose contents and the anti-nutritional factors, tannin and phytic acid. However, free amino acids and fatty acids increased in the fermented leaf meal. The response of rohu fingerlings (4.0±0.14 g) fed the experimental diets for 80 days was compared with fish fed a RD. Both the inclusion level and type of *Eichhornia* leaf meal in diets significantly affected the growth performance of rohu. Fish fed diets containing 30% leaf meal fermented with CI3 strain performed better in terms of growth response, feed conversion ratio, protein efficiency ratio and apparent net protein utilization followed by diets containing 20% CI3 fermented leaf meal and 20% *Bacillus subtilis* + LAB fermented leaf meal in comparison with those with the RD. The apparent protein digestibility (APD) was better in fish fed diets containing fermented leaf meal. Highest deposition of protein in carcass was recorded in the group of fish fed 30% *Bacillus subtilis* + LAB fermented leaf meal diet whereas lipid deposition was highest in the fish fed the RD. It is concluded from the present study that *Eichhornia* leaf meal fermented with fish gut bacteria exhibiting extracellular enzyme activity can be recommended as a dietary ingredient in diets of *Labeo rohita* fingerlings up to 40% incorporation level replacing fish meal without any adverse effect on growth of the fish to produce cost effective formulated fish feed.

Keywords: *Eichhornia crassipes*, Fish gut bacteria, fermentation, diets, growth, *Labeo rohita* fingerlings.

Su Sümbülü (*Eichhornia crassipes*) Yaprak Ununun Balık Bağırsağından İzole Edilen İki Bakteri Türü ile Fermentasyonu Sonrası Yavru Rohu, *Labeo rohita* Diyetindeki Besinsel Kıymetinin Değerlendirilmesi

Özet

Dokuz adet izonitrojenik (yaklaşık olarak %30 ham protein) ve izokalorik (18.23 kJ g^{-1}) deneysel diyet hazırlanmıştır (D1-D9). Diyetler balık bağırsak bakterileri ile fermente edilmiş ve fermente edilmemiş %20, %30 ve %40 oranlarında su sümbülü (*Eichhornia crassipes)* yaprağı unu ile ikame edilerek formüle edilmiştir. Ayrıca balık unu içeren referans diyet (RD) hazırlanmıştır. Ekstra-hücresel selülolitik ve amilotik faaliyetler gösteren balık bağırsak bakterilerinin iki özel suşu, *Bacillus subtilis* CY5 (*Cyprinus carpio'*dan izole edilen) ve *B. megaterium* CI3 (*Ctenopharyngodon idella'*dan izole edilen) 37°C'de 15 gün boyunca *Eichhornia* 'nın yaprağının fermentasyonu için kullanılmıştır. Ticari hazırlanmış Laktik asit bakterisi (LAB) *Lactobacillus acidophilus* (Lactobacil) *Bacillus subtilis* CY5 ile birlikte yem katkısı olarak kullanılmıştır. Su sümbülü yaprağının fermantasyonu, ham selülozun, selüloz ve hemiselüloz içeriğine ve anti-beslenme faktörlerine, tanen ve fitik asite indirgenmesi ile sonuçlanmıştır. Ancak, serbest asitler ve yağ asitleri fermente su sümbülü yaprağında yükselmiştir. 80 gün boyunca deneysel diet ile beslenen Rohu fingerling'in (4.0±0.14 g) tepkisi, RD ile beslenen balık ile kıyaslanmıştır. Dietlerdeki *Eichhornia* yaprağının hem içerik seviyesi hem de tipi rohu'nun büyüme performansını önemli şekilde etkilemiştir. %30 C13 türü ile fermente edilmiş su sümbülü yaprağı içeren balık yem diyetleri; büyüme tepkisi, yem dönüşüm oranı, protein verimlilik oranı ve belirgin net protein kullanımı açısından daha iyi performans göstermiştir. Daha sonra RD ile kıyasla %20 CI3 ile fermente edilen su sümbülü yaprağı ve %20 *Bacillus subtilis* + LAB ile fermente edilen su sümbülü yaprağı içeren yemler gelmektedir. Görünen protein sindirimi (APD) fermente su sümbülü yaprağı içeren balık yemlerinde daha iyidir. %30 *Bacillus subtilis* + LAB ile fermente edilmiş yaprak yemi ile beslenen balık grubunda karkasta en yüksek protein depolanması kaydedilmiştir, oysa yağ depolanması en fazla RD ile beslenen balıklarda görülmüştür. Yürütülen projenin sonucuna göre, balık bağırsak bakterileri ile fermente edilmiş ekstraselüler enzim aktivitesi sergileyen *Eichhornia* 'nın yaprakları, etkin maliyetli formüle edilmiş balık yemi üretmek için, fingerling *Labeo rohita'*nın dietine balığın büyümesine herhangi ters bir etkisi olmayan %40 seviyesinde katılarak ikame balık yemi olarak kullanılması önerilmiştir.

Anahtar Kelimeler: *Eichhornia crassipes*, balık bağırsak bakterileri, fermantasyon, diet, büyüme, *Labeo rohita* fingerlings.

Introduction

Several studies in recent past to make supplementary feeding of fish cost-effective have been directed to substitute the high cost fish meal with less expensive protein sources. This aspect of feed development research is centered on the search for inexpensive, readily available and nutritious protein sources that can supply all the nutritional needs of the fish. One obvious approach involves the greater utilization of ingredients of plant origin. Aquatic and terrestrial macrophytes have been used as supplementary feeds in fish farming since early days of freshwater fish culture and still play an important role as fish feeds in extensive culture systems. The efficacy of the leaves of various terrestrial and aquatic macrophytes for partial replacement of fish meal in carp diets has been investigated by a number of workers (Ray and Das, 1992, 1994, 1995; Mondal and Ray, 1998, 1999; Bairagi *et al.*, 2002a, 2004). The aquatic weeds have been shown to contain substantial amount of protein and minerals (Ray and Das, 1994). These weeds, which otherwise remain unutilized, and often make the water body unsuitable for fish culture, may be converted into valuable fish flesh through their incorporation as an ingredient in carp diets. However, several factors are known to limit the higher incorporation of these ingredients in fish feeds, such as (i) low protein content (Devendra, 1985), (ii) amino acid imbalance (Tacon, 1987), (iii) presence of anti-nutritional factors (Tacon and Jackson, 1985), (iv) presence of crude fibre, cellulose, hemicellulose and lignin (De Silva and Anderson, 1995). Another important factor is that fish generally do not possess the enzyme cellulase or significant symbiotic gut flora capable of hydrolyzing cellulose present in macrophytes (Wee, 1991). Therefore, enhancement of nutrient value of plant ingredients by some processing means to increase the bioavailability of nutrients, reduction or removal of anti-nutritional factors and crude fibre and the inclusion of appropriate additives to correct known deficiencies could result in their higher level of incorporation in fish feeds. It is believed that the inclusion rate could be increased with proper processing and by the addition of exogenous enzymes to break down plant cell wall to liberate the nutritious cellular contents (Wee, 1991). Microbial fermentation and nutrient synthesis is typically important in organisms with a diet high in fibre i.e., a diet mainly composed of carbohydrates resistant to endogenous digestive enzymes (Annison, 1993). Fermentation is a simple and cheap process where there may be an increase in the nutrient level through microbial synthesis (Wee, 1991), apart from microbial degradation of apparently 'indigestible' plant cellulosic substances. The water hyacinth, *Eichhornia crassipes* is one of the most abundantly growing aquatic weeds in tropical and sub-tropical countries. It is considered as one of the world's noxious weeds due to its vigorous growth rate. It has been reported that during warm weather, the plant can multiply at a phenomenal rate of 15% surface area per day (Majid, 1986). This fast growing troublesome, indestructible plant is a good source of animal feed, organic fertilizer, biogas and fibre. The plant acts as a scavenger when it grows on polluted waste water. Water hyacinth, one of the rich sources of organic resources, has received attention for its control as well as utilization.

A number of studies have been conducted on the utilization of water hyacinth as feed for different fish species including Indian major carps (Edwards *et al.*, 1985; Hasan *et al*., 1990; Ray and Das, 1994). However, information on the use of processed leaf meals of aquatic weeds in aquafeeds are scanty (Bairagi *et al.*, 2002a; El-Sayed, 2003). In the present study, *Eichhornia* leaf meal was fermented by two cellulose-degrading fish gut bacteria, *Bacillus megaterium* isolated from grass carp, *Ctenopharyngodon idella* (strain CI3) and *Bacillus subtilis* (strain CY5) isolated from the gut of common carp, *Cyprinus carpio.* The bacterial strain CY5 was used in combination with the commercially available lactic acid bacteria (LAB; 'Lactobacil' Intercare Ltd., Mehsana, Gujarat, India), *Lactobacillus acidophilus*. The Indian major carp, *Labeo rohita* is primarily a herbivorous to omnivorous species and prefers to feed on plant materials (Jhingran, 1997). The present study aims to evaluate the possible utilization of fermented *Eichhornia* leaf meal as a partial substitute of fish meal in diets for the fingerlings of the Indian major carp, *Labeo rohita* (Hamilton).

Materials and Methods

Isolation of Cellulase-Producing Bacteria

Cellulolytic, amylolytic and proteolytic bacteria were isolated from the gastrointestinal tract of common carp, *Cyprinus carpio* and grass carp, *Ctenopharyngodon idella* following enrichment culture technique on sterilized agar media (Bairagi *et al.*, 2002b). The isolates were screened for quantitative production of extracellular amylase (Bernfeld, 1955), cellulase (Denison and Kohen, 1977) and protease (Walter, 1984). The bacterial strains were further characterized on the basis of morphological, physiological and biochemical tests. On the basis of the morphological, physiological and biochemical characteristics, and according to the description in the Bergey's Manual of Systematic Bacteriology (Williams *et al.*, 1986), the bacterial strains isolated from common carp and grass carp were identified as *Bacillus subtilis* CY5 and *B. megaterium* CI3, respectively.

Preparation of Bacterial Seed Culture and Inoculation of *Eichhornia* Leaf Meal

The selected bacteria were grown in shake

bottles in 4% tryptone soya broth (Hi-media, Mumbai, India) for seed culture at 37°C for 24 hours to obtain an average viable count of about 10^7 cells ml^{-1} broth in both the cases. Leaves of *Eichhornia crassipes* were sun-dried and finely ground and passed through a fine meshed sieve to ensure homogeneity. Weighed amount of ground leaf meal was moistened with 50% w v^{-1} liquid basal medium containing (g L^{-1}): KH_2PO_4, 4; $NaHPO_4$, 4; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.001; $FeSO_4.7H_2O$, 0.004 and autoclaved for sterilization. The sterilized leaf meal was inoculated with *Bacillus subtilis* and *B. megaterium* culture separately at the rate of 10^7 bacterial cells per gram of dried leaf and kept for 15 days at 37°C for fermentation. No further study was conducted to detect the bacteria after inoculation.

Experimental Diets

Nine isonitrogenous (approximately 30% crude protein) and isocaloric (18 kJ g^{-1} gross energy approximately) experimental diets (D1 to D9) were formulated incorporating either raw or fermented *Eichhornia* leaf meal at three different levels (20%, 30% and 40%) replacing other ingredients including fishmeal from the reference diet (RD) so that the crude protein level of the diets remain approximately 30%. Diets D1, D2 and D3 were prepared with raw *Eichhornia* leaf meal, D4, D5, D6 with *Bacillus megaterium* CI3 inoculated *Eichhornia* leaf meal and diets D7, D8 and D9 were formulated with *Eichhornia* leaf meal fermented with *Bacillus subtilis* CY5 along with a commercial preparation of *Lactobacillus acidophilus* (LAB; 'Lactobacil' Intercare Ltd., Mehsana, Gujarat, India) at the rate of 10^6 cells per gram to determine the synergistic effect of LAB, if any. To each of the formulated diet 1% chromic oxide was added as digestibility marker (Table 1). The diets were prepared in pelleted form using 0.5% carboxymethylcellulose as binder and the pellets were sun dried and stored in airtight containers in a refrigerator until used.

Experimental Design

The experiment was conducted in flow-through 90L circular fibre-glass tanks. Rohu (*Labeo rohita*) fingerlings obtained from a local fish seed dealer, were acclimatized to laboratory conditions for 15 days and fed with a mixture (1:1) of rice bran and mustard oil cake prior to the commencement of the feeding trial. Rohu fingerlings (mean weight 4.0±0.14 g) were randomly distributed at the rate of 15 fish per tank with three replicates for each treatment. Each experimental tank was supplied with unchlorinated water from a deep tube well with continuous aeration. All the fish were fed once daily at 10:30 hours at a fixed feeding rate of 3% body weight per day for 80 days. Each group of fish were weighed every 15^{th} day and the quantity of feed given was adjusted accordingly. Any left over feed was collected 6 h after each feeding by pipetting to determine the feed consumption. Since carboxymethylcellulose was used as binder during preparation of diets, the uneaten feeds were not dissolved in water. The uneaten feeds could be easily distinguished from faecal matter by their size which were carefully pipetted and weighed after oven drying. The digestibility experiment was

Table 1. Composition (% dry weight) and proximate analysis of experimental diets (on dry matter basis)

Ingredients	RD	Diets with raw *Eichhornia* leaf meal			Diets with *Eichhornia* leaf meal fermented with *B. megaterium* CI3			Diets with *Eichhornia* leaf meal fermented with *B. subtilis* CY5 + LAB		
		D1	D2	D3	D4	D5	D6	D7	D8	D9
Fish meal	30.00	25.00	22.00	20.00	25.00	22.00	20.00	25.00	22.00	20.00
Soyabean meal	35.00	30.33	33.58	34.00	30.33	33.58	34.00	28.71	30.00	31.00
Ricebran	32.00	21.66	11.41	3.00	21.66	11.41	3.00	23.28	15.00	6.00
Eichhornia leaf meal	-	20.00	30.00	40.00	20.00	30.00	40.00	20.00	30.00	40.00
Codliver oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Soyabean oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chromic oxide	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Proximate composition (%)										
Dry matter	99.75	99.90	99.90	99.90	99.91	99.93	99.94	99.00	99.90	99.93
Crude protein	31.25	29.11	29.79	29.52	29.11	28.89	32.00	28.67	30.17	31.20
Crude lipid	7.50	4.50	7.50	6.00	7.20	11.00	10.00	10.00	10.00	10.20
Ash	13.00	15.50	15.00	13.00	14.00	12.50	14.00	12.00	13.00	13.50
Crude fibre	12.00	9.89	9.50	10.00	7.20	7.50	7.80	6.70	6.90	7.20
NFE[2]	36.00	40.90	38.19	41.38	42.40	40.04	36.14	42.53	39.83	37.83
Gross energy (kJ g^{-1})	18.17	16.84	17.51	17.80	17.84	18.92	18.72	18.80	18.80	18.88
Tannin	ND	0.10	0.20	0.30	ND	ND	ND	ND	ND	ND
Phytic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[1]Vitamin and mineral mixture; [2]Nitrogen-free extract; ND=Not detectable; RD= Reference diet

conducted in a static water system. The faecal matters voided by the fish were collected everyday in the morning following the 'immediate pipetting' method outlined by Spyridakis *et al.* (1989) from the replicate of each dietary treatment. The faeces naturally released by the fish could be easily detected and were immediately removed from the water with a glass canula. Pooled samples of uneaten feed and faecal matters for each dietary treatment were dried at 55°C and stored in a refrigerator for subsequent analysis. At the termination of 80-day feeding experiment the fish from each dietary treatment were weighed and analysed for carcass composition. There was no mortality of fish during the feeding trial.

Water quality was monitored every 10^{th} day for temperature, pH, dissolved oxygen and total alkalinity. The ranges of water quality parameters were: temperature, 29-32°C, pH, 6.5-7.3, dissolved oxygen, 4.9-7.2 mg L^{-1}, alkalinity, 160-182 mg L^{-1}.

Chemical Analyses and Data Collection

Feed ingredients, experimental diets, feacal samples and fish carcass (at the beginning and after the termination of experiment) were analyzed for proximate composition according to AOAC (1990) procedures as follows: Moisture was determined by oven drying at 105°C for 24 h; crude protein (Nitrogen × 6.25) by micro Kjeldahl digestion and distillation after acid digestion using a Kjeltec 1026 Distilling Unit together with a Tecator 2000 Digestion System (Tecator, Höganäs, Sweden); lipid was determined by extracting the residue with 40-60°C petroleum ether for 7-8 h in a Soxhlet apparatus; crude fibre was estimated as loss in ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH in a Fiber cap 2021/2023 System (Foss Tecator, Sweden); and ash was determined by ignition at 550°C in a Muffle furnace to constant weight. Nitrogen free extract (NFE) was computed by taking the sum of values for crude protein, crude lipid, crude fibre and moisture and subtracting this from 100 (Maynard *et al.*, 1979). Cellulose and hemicellulose contents were determined according to Updegraff (1969) and Goering and Vansoest (1975), respectively. Estimation of total free amino acids and total free fatty acids in raw and fermented leaf meal were done according to Moore and Stein (1948) and Cox and Pearson (1962), respectively. Chromic oxide in the diets and faecal samples was estimated following the method of Bolin *et al*. (1952). Tannin content in both fermented and raw *Eichhornia* leaf meal was determined using Folin- Denis reagent (Schanderi, 1970). Phytic acid content was determined according to Wheeler and Ferrel (1971). The water quality parameters were monitored following the methods outlined by APHA (1985). Fish performance in terms of weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU, %) was calculated using the following formulae:

Weight gain (%) = [(Final body weight – Initial body weight / Initial body weight)/Initial body weight] × 100.

Specific Growth Rate = $[(\ln W_t - \ln W_i)/ T] \times 100$, (SGR; % day^{-1})

where W_t is weight of fish at time t, W_i is weight of fish at time 0, and T is the culture period in days.

Feed Conversion Ratio=Feed consumed/Weight gain. (FCR)

Protein Efficiency Ratio= Weight gain/Protein intake. (PER)

Apparent Net Protein Utilization (ANPU, %) = (Net increase in carcass protein / Protein consumed) × 100.

Apparent protein digestibility (APD, %) was calculated according to De Silva and Anderson (1995) as follows:

Apparent Protein Digestibility (%) = $100–100[100\times(I_d / I_f \times P_f / P_d)]$,

where I_d represents chromic oxide in diet and I_f, chromic oxide in faeces, P_d is protein in diet, and P_f, protein in faeces.

Statistical Analysis

Statistical analysis of data was performed by analysis of variance (ANOVA) using Microsoft software Statistica followed by Duncan's multiple range test (Duncan, 1955).

Results

Experimental diets and the proximate composition of raw and fermented *Eichhornia* leaf meal are presented in Tables 1 and 2, respectively. Fermentation of leaf meal with fish gut microbiota resulted in significant increase in the levels of free amino acids and fatty acids, whereas, there was a decrease in crude fibre content, cellulose, hemicellulose and anti-nutritional factors, tannin and phytic acid. The crude protein level in fermented leaf meal also increased.

Data on growth performance and feed utilization of *L. rohita* fingerlings in terms of percentage weight gain, SGR, FCR, PER, ANPU and APD are presented in Table 3. Growth performance was significantly affected by the type and inclusion level of *Eichhornia* leaf meal. The fish reared with raw *Eichhornia* leaf meal incorporated diets showed inferior growth performance in comparison to the group of fish reared

Table 2. Proximate composition of raw and fermented *Eichhornia crassipes* leaf meal (% dry matter basis)

Parameters	Raw *Eichhornia* leaf meal	*Eichhornia* leaf meal fermented by *B. megaterium* CI3	Fermented *Eichhornia* leaf meal by *B. subtilis* CY5 + LAB
Crude protein	13.37	14.44	16.88
Ash	17.00	14.00	12.60
Crude lipid	1.00	1.50	1.00
Crude fibre	15.00	12.00	13.50
NFE*	47.63	-	-
Gross energy (kJ g^{-1})	14.22	-	-
Free amino acids	0.36	0.79	0.98
Free fatty acids	2.80	3.30	3.80
Cellulose	11.40	7.65	5.80
Hemicellulose	0.15	0.06	0.10
Tannin	0.98	0.38	0.20
Phytic acid	0.42	0.32	0.37

*Nitrogen-free extract

Table 3. Growth performances and feed utilization efficiencies in *Labeo rohita* fingerlings fed experimental diets for 80 days. Data are mean value ± SE (n=3)

Parameters	RD	Diets with raw *Eichhornia* leaf meal			Diets with *Eichhornia* leaf meal fermented with *B. megaterium* CI3			Diets with *Eichhornia* leaf meal fermented with *B. subtilis* CY5 + LAB		
		D1	D2	D3	D4	D5	D6	D7	D8	D9
Av. initial wt (g)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
	±0.14	±0.17	±0.19	±0.20	±0.16	±0.18	±0.21	±0.15	±0.14	±0.14
Av. final wt (g)	6.59^{b}	6.42^{b}	6.40^{b}	6.38^{b}	7.19^{a}	7.25^{a}	6.83^{a}	7.09^{a}	6.98^{a}	6.76^{a}
	±0.23	±0.22	±0.23	±0.22	±0.25	±0.26	±0.24	±0.25	±0.24	±0.24
Weight gain (%)	64.75^{b}	60.50^{c}	60.00^{c}	59.50^{c}	79.75^{a}	81.25^{a}	70.75^{b}	77.25^{a}	74.50^{a}	69.00^{b}
	±2.28	±2.135	±2.21	±2.1	±2.8	±2.8	±2.5	±2.7	±2.6	±2.4
Feed intake (g $100g^{-1}$ BW of fish day^{-1})[1]	1.33	1.35	1.36	1.39	1.24	1.22	1.26	1.26	1.27	1.30
SGR (% day^{-1})	0.63^{b}	0.59^{c}	0.58^{c}	0.58^{c}	0.73^{a}	0.74^{a}	0.67^{b}	0.71^{a}	0.69^{a}	0.65^{b}
	±0.02	±0.02	±0.20	±0.02	±0.02	±0.02	±0.02	±0.03	±0.02	±0.02
FCR	2.70^{b}	2.88^{a}	2.92^{a}	2.99^{a}	2.25^{c}	2.19^{d}	2.44^{c}	2.32^{c}	2.39^{c}	2.55^{b}
	±0.09	±0.10	±0.10	±0.11	±0.08	±0.08	±0.85	±0.08	±01	±0.09
PER	1.18^{c}	1.19^{c}	1.14^{c}	1.13^{c}	1.52^{a}	1.58^{a}	1.32^{b}	1.50^{a}	1.38^{b}	1.25^{c}
	±0.042	±0.042	±0.041	±0.037	±0.055	±0.056	±0.048	±0.053	±0.049	±0.046
ANPU (%)	26.99^{e}	36.96^{d}	18.20^{f}	20.94^{f}	73.22^{c}	83.64^{a}	82.23^{a}	79.79^{b}	87.02^{a}	70.58^{c}
	±0.951	±1.304	±0.643	±0.735	±2.588	±4.18	±1.142	±2.818	±3.076	±2.510
APD (%)	80.71^{b}	80.51^{b}	79.96^{b}	79.83^{b}	81.41^{a}	86.78^{a}	82.47^{a}	81.29^{a}	82.01^{a}	82.23^{a}
	± 2.1	± 2.19	± 2.23	± 2.17	± 2.24	± 2.42	± 2.28	± 2.3	± 2.31	± 2.38

Mean values with same superscripts in the same row are not significantly different (P<0.05).
[1] Statistical analysis was not possible as determinations were performed on pooled samples.

with fermented leaf meal incorporated diets as well as reference diet (RD). A decreasing trend in growth performance was noticed with increasing level of raw *Eichhornia* leaf meal whereas, growth was significantly higher (P<0.05) with diets containing fermented leaf meal in comparison to the reference diet (RD). Highest live weight gain (%) was recorded in the group of fish fed diet D5 (30% inclusion of *Eichhornia* leaf meal fermented with *B. megaterium* CI3) which was not significantly different (P<0.05) from the fish fed other diets incorporated with fermented *Eichhornia* leaf meal. SGR was highest in the group of fish fed diet D5 which was not significantly different (P<0.05) from those in the groups of fish reared on diets D4, D7 and D8. PER value was highest for diet D5 (30% inclusion of *Eichhornia* leaf meal fermented with *B. megaterium* CI3) which was not significantly different from those with diets D4 and D7. FCR was best for diet D5 and worst for diet D3 (40% raw *Eichhornia* leaf meal incorporation). Best result regarding ANPU was observed in the group of fish fed diet D8 which was not significantly (P<0.05) different from that in the groups of fish fed diets D5 and D6.

Apparent protein digestibility (APD) value was highest in fish fed diet D5 (30% inclusion of *Eichhornia* leaf meal fermented with *B. megaterium* CI3). APD values for fermented leaf meal incorporated diets were significantly (P<0.05) higher than those for raw leaf meal incorporated diets as well as reference diet (RD). There was no significant difference (P<0.05) in APD values for raw leaf meal incorporated diets and reference diet (RD).

The results of proximate carcass composition of fish before and after the experiment are presented in Table 4. The carcass composition of the experimental fish was influenced by the type and inclusion level of *Eichhornia* leaf meal in the diets. The deposition of

Table 4. Proximate carcass composition (% wet weight) of the experimental fish at the start and end of 80 days feeding experiment. Data are mean value ± SE (n=3)

Parameters	Initial	RD	Diets with raw *Eichhornia* leaf meal			Diets with *Eichhornia* leaf meal fermented with *B. megaterium* CI3			Diets with *Eichhornia* leaf meal fermented with *B. subtilis* CY5 + LAB		
			D1	D2	D3	D4	D5	D6	D7	D8	D9
Moisture	82.0	76.75^{a} ± 2.68	78.75^{a} ± 2.78	79.00^{a} ± 2.79	80.49^{a} ± 2.84	80.00^{a} ± 2.83	79.00^{a} ± 2.79	79.20^{a} ± 2.80	78.50^{a} ± 2.77	81.00^{a} ± 2.86	79.00^{a} ± 2.79
Crude protein	9.85	10.44^{b} ± 0.37	10.60^{b} ± 0.37	10.23^{b} ± 0.36	11.29^{b} ± 0.36	11.38^{a} ± 0.40	11.57^{a} ± 0.40	10.54^{b} ± 0.37	11.49^{a} ± 0.40	11.72^{a} ± 0.41	11.40^{a} ± 0.40
Crude lipid	2.10	4.50^{a} ± 0.15	3.^{57b} ± 0.12	2.21^{e} ± 0.07	3.11^{c} ± 0.10	2.48^{d} ± 0.08	3.20^{c} ± 0.11	2.30^{d} ± 0.07	3.41^{b} ± 0.12	2.02^{e} ± 0.07	2.52^{d} ± 0.08
Ash	4.0	3.54^{b} ± 0.12	3.00^{c} ± 0.11	3.47^{b} ± 0.12	3.23^{b} ± 0.11	4.09^{a} ± 0.14	4.27^{a} ± 0.15	4.12^{a} ± 0.14	4.05^{a} ± 0.14	3.42^{b} ± 0.12	4.00^{a} ± 0.14

Mean values with same superscripts in the same row are not significantly different (P< 0.05)

protein in the carcass increased over the initial value in all dietary treatments. Highest accumulation of carcass protein was recorded in the group of fish fed diet D8 (30% inclusion of *Eichhornia* leaf meal fermented by *B. subtilis* CY5 and LAB) which was not significantly different (P<0.05) from the fish fed diets D4, D5, D7 and D9. Highest lipid deposition was observed in fish fed reference diet. The carcass ash content was highest in fish fed diet D5 (30% inclusion of *Eichhornia* leaf meal fermented with *B. megaterium* CI3).

Discussion

The results of the present study demonstrate that *Eichhornia crassipes* leaf meal fermented with enzyme producing fish gut bacteria could be exploited as an ingredient for incorporation into the formulated diets for rohu fingerlings. From the study it is evident that *Eichhornia* leaf meal fermented with both *Bacillus subtilis* CY5+*Lactobacillus* (LAB) as well as *Bacillus megaterium* CI3 could be incorporated up to 40% levels in the diets of rohu fingerling. However, the best performance and feed utilization efficiency was recorded at 30% inclusion level of *Bacillus megaterium* CI3 inoculated *Eichhornia* leaf meal and 20% inclusion level of *Bacillus subtilis* CY5+ LAB inoculated *Eichhornia* leaf meal. Growth performance of the fish fed fermented *Eichhornia* leaf meal incorporated diets was superior to those fed similar levels of raw *Eichhornia* leaf meal incorporated diets.

Eichhornia crassipes is generally considered as nuiscence to the aquatic body. There are several experiments underway to explore the potentiality of the plant's by-products as source of methane gas or animal feed. Incorporation of the *Eichhornia* leaf meal to replace the fishmeal in formulated fish feed can be attributed to achieve the goal of formulation of cost-effective fish feed. However, the presence of several anti-nutritional factors and high level of crude fibre content hinder its utilization as dietary ingredient as such. In the present study, the tannin and phytic acid contents in *Eichhornia* leaf meal were estimated to be 0.98% and 0.42%, respectively. There are several studies on the toxicity of tannins on the growth and the digestive enzyme profiles of fish (Hossain and Jauncey, 1989; Mukhopadhyay and Ray, 1996, 1999a, 1999b; Bairagi *et al*., 2002a, 2004; Maitra and Ray, 2003; Ramachandran and Ray, 2004; Mandal and Ghosh, 2010). Tannins affect the protein and dry matter digestibility either by inhibiting the activity of protease and possibly other digestive enzymes or by forming indigestible complexes with dietary protein (Krogdahl, 1989). Phytic acid acts as a chelator, forming protein and mineral-phytic acid complexes which cause reduced protein and mineral bioavailability (Spinelli *et al*., 1983; Hossain and Jauncey, 1989, 1993; Cain and Garling, 1995). In the present study, it was observed that fermentation of *Eichhornia* leaf meal with two different extracellular enzyme producing bacterial strains resulted in reduction of tannin and phytic acid. Apart from the anti-nutrients, the crude fibre content also plays a vital role in poor digestibility of plant ingredients (Hastings, 1964; Atack *et al*., 1979; Jackson *et al*., 1982; De Silva *et al*., 1990; Bairagi *et al*., 2002a, 2004; Ramachandran *et al*., 2005). The results of several feeding trials indicated that the tannin, phytic acid and crude fibre level of plant leaf meals and seed meals can be considerably reduced after being fermented with extracellular enzymes specially cellulase, amylase and protease producing bacterial strains in solid state fermentation process (Bairagi *et al*., 2002a, 2004; Ramachandran *et al*., 2005).

In the present study, two cellulolytic bacterial strains, one (*Bacillus megaterium* CI3) isolated from the gut of *Ctenopharyngodon idella* and the other (*Bacillus subtilis* CY5) from the gut of *Cyprinus carpio* were used to inoculate the *Eichhornia* leaf meal *in vitro*. During fermentation, generally an increase in the nutrient level through microbial synthesis occurs (Wee, 1991). The increased level of crude protein, free amino acids and free fatty acids in fermented *Eichhornia* leaf meal in comparison to the raw leaf meal is consistent to the findings of Bairagi *et al*. (2002a, 2004) and Ramachandran *et al*. (2005). Fermentation thus resulted in an improvement of nutritive value of *Eichhornia* leaf meal. As fermentation reduces the level of different anti-nutrients as well as crude fibre content due to the

cellulolytic activity of the bacterial strains, the protein utilization efficiency was found significantly better in the group of fish fed fermented *Eichhornia* leaf meal incorporated diets than those fed with raw leaf meal incorporated diets. The crude protein levels increased from 13.33% to 14.44% and 16.88% and the crude fibre level decreased from 15% to 12% and 13.5% in *Eichhornia* leaf meal fermented with *B. megaterium* CI3 and *B. subtilis* CY5 + LAB, respectively. Due to improved nutritional quality and better nutrient digestibility, fermented leaf meal incorporated diets resulted in better growth performance in terms of percentage weight gain, SGR, FCR and PER. Lipids contain more energy per unit weight than any other dietary component, and they are used efficiently by fish as energy sources. Moreover, lipid is easily digested and metabolized and serves as a much better source of energy for protein-sparing than carbohydrate (De Silva and Anderson, 1995). In the present study, although all the diets were isocaloric (18.23 kJ g^{-1}), diets D5 to D9 (containing fermented *Eichhornia* leaf meal) contained higher levels of lipid (10.0% to 11.0%) than diets D1 to D3 (containing unfermented or raw leaf meal) (4.5 to 6.0%). The differences in growth performance of rohu fingerlings may therefore, also be attributed to varying levels of lipid in the diets (calorie intake). While evaluating the effects of different fermentation methods on the nutritive value of water hyacinth leaf meal, El-Sayed (2003) reported that fermented water hyacinth leaf meal can be incorporated up to 20% in the formulated diets for Nile tilapia, *Oreochromis niloticus*. Bairagi *et al*. (2002a, 2004) reported that *Lemna* and *Leucaena* leaf meals fermented with two strains of *Bacillus* species could be successfully used to replace fishmeal up to 30% incorporation level in formulated diets for rohu fingerlings. Poor growth performance of fish fed raw *Eichhornia* leaf meal incorporated diets was possibly due to the presence of anti-nutritional factors and high fibre content**.**

Determination of nutrient digestibility is the first step in evaluating the potentiality of an ingredient for use in the diet of reared species (Allan *et al*., 2000). Information on digestibility coefficients of feed ingredients is very useful not only to enable formulation of diets that maximize fish growth by providing appropriate amounts of available nutrients but also to reduce fish waste products (Lee, 2002). A declining trend in APD values has been reported with higher levels of inclusion of raw mustard (Hossain and Jauncey, 1989), linseed (Hasan *et al*., 1991), sesame seed (Mukhopadhyay and Ray, 1999a), copra meal (Mukhopadhyay and Ray, 1999b), *Lemna* leaf meal (Bairagi *et al*., 2002a), *Leucaena* leaf meal (Bairagi *et al*., 2004) and grass pea seed meal (Ramachandran and Ray, 2004, Ramachandran *et al*., 2005) in formulated carp diets. In the present study, a similar trend was noticed with regard to apparent protein digestibility. Digestibility of various nutrients is influenced by the anti-nutritional factors (Lall, 1991). Brunson *et al*. (1997) proposed that differences in digestibility might be because of the nature of ingredients, not their amounts in the diets. However, the APD value was found highest in the fish fed diet D5 followed by D6. Earlier studies showed that fermentation improves the protein digestibility of plant ingredients (Bairagi *et al*., 2002a, 2004; Ramachandran and Ray, 2004).

The proximate carcass composition of the experimental fish after termination of the feeding trial showed a significant increase in protein and lipid content in all dietary treatments in comparison to the initial value. The value of carcass protein content was found to be higher in the fish fed diets containing fermented *Eichhornia* leaf meal, being highest in the fish group fed diet D8 (30% *B. subtilis* CY5 + LAB fermented leaf meal incorporation). Carcass ash content was highest in fish fed diet D4, containing 20% *Eichhornia* leaf meal fermented with *B. megaterium* CI3. These results conform to the reports of others where similar trends were noted with higher levels of inclusion of fermented seed and leaf meals in carp diets (Mukhopadhyay and Ray, 1999a, 1999b; Bairagi *et al*., 2002a, 2004; Ramachandran and Ray, 2004; Ramachandran *et al*., 2005).

The present study demonstrated the acceptable nutritional value of fermented *Eichhornia* leaf meal as an ingredient in diets for rohu fingerlings, since this product can replace the most commonly used fish feed ingredient, fish meal up to a certain level. Inclusion of fermented *Eichhornia* leaf meal in compound diets for rohu fingerlings may therefore, be a function of diet formulation. An inclusion level up to 40% fermented *Eichhornia* leaf meal in the practical diet for rohu fingerlings did not exert any adverse effect on growth, feed utilization efficiencies and body composition of the animal in comparison to raw *Eichhornia* leaf meal at the same level of inclusion. In an earlier study by Ghosh *et al.* (2004), supplementation of commercial preparation of *Lactobacillus acidophilus* (Lactobacil) in compound diets resulted better growth and feed conversion of *Labeo rohita* fingerlings. In the present study however, addition of LAB along with *B. subtilis* CY5 for fermentation of *Eichhornia* leaf meal did not show any significant improvement in growth performance of rohu fingerlings in comparison to that with diets incorporated with the leaf meal fermented with *B. megaterium* CI3 alone. However, it is too early to recommend to the industry to use fermented *Eichhornia* leaf meal in formulation of aquafeeds. It will require further experimentation with large number of fish and replication. Moreover, costs and efforts involved in fermentation of *Eichhornia* leaf meal need to be evaluated before its field application.

Acknowledgements

We are grateful to the Council of Scientific & Industrial Research (CSIR), New Delhi [Project No.

37(1139)/03/EMR-II] for financial support.

References

Allan, G.L., Parkinson, S., Booth, M.A., Stone, D.A.J., Rowland, S.J., Frances, J. and Warner-Smith, R. 2000. Replacement of fishmeal in diets for Australian silver perch, *Bidyanus bidyanus*: Digestibility of alternative ingredients. Aquaculture, 186: 293-310.

Annison, G. 1993. The chemistry of dietary fibre. In: S. Samman and G. Annison (Eds.), Dietary Fibre and Beyond – Australian Perspectives, Nutritional Society of Australia, Occasional Publications, Sydney, Australia: 1-18.

AOAC (Association of Official Analytical Chemists). 1990. In: W. Helrich (Ed.), Official Methods of Analysis of the Association of Official Analytical Chemists. Vol. I, 15th edition, Association of Official Analytical Chemists, Washington DC, 1134 pp.

APHA (American Public Health Association). 1985. Standard Methods for the Examination of Water and Waste Water, 16th Edition, American Public Health Association. American Water Works Association and Water Pollution Control federation, Washington DC, 1134 pp.

Atack, T.H., Jauncey, K. and Matty, A.J. 1979. The utilization of some single cell proteins by fingerlings minor carp (*Cyprinus carpio).* Aquaculture, 18: 337-348. doi: 10.1016/0044-8486(79)90037-6

Bairagi, A., Sarkar Ghosh, K., Sen, S.K. and Ray, A.K. 2002a. Duckweed (*Lemna polyrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu (*Labeo rohita* Ham.) fingerlings after fermentation with a fish intestinal bacterium. Bioresource Technology, 85: 17-24. doi:10.1016/s0960-8524(02)00067-6

Bairagi, A., Sarkar Ghosh, K., Sen, S.K. and Ray, A.K. 2002b. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International, 10: 109-121. doi:10.1023/A:1021355406412

Bairagi, A., Sarkar Ghosh, K., Sen, S.K. and Ray, A.K. 2004. Evaluation of nutritive value of *Leucaena leucocephala* leaf meal inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings. Aquaculture Research, 35: 436-446. doi:10.1111/j.1365-2109.2004.01028.x

Bernfeld, P. 1955. Amylase [alpha] and [beta]. In: S.P. Colowick and N.O. Kaplan (Eds.), Methods in Enzymology, Academic Press, New York: 149-150.

Bolin, D.W., King, R.P. and Klosterman, F.W. 1952. A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an index substance. Science 116: 634–635. doi: 10.1126/science. 116.3023.634

Brunson, J.F., Romaire, R.P. and Reigh, R.C. 1997. Apparent digestibility of ingredients in diets for white shrimp *Panaeus setiferus* L. Aquaculture Nutrition, 3: 9-16. doi: 10.1046/j.1365-2095.1997.00068.x

Cain, K.D. and Garling, D.L. 1995. Pretreatment of soybean meal with phytase for salmonid diets to reduce phosphorous concentration in hatchery effluents. The Progressive Fish Culturist, 57: 114-119.

Cox, H.E. and Pearson, D. 1962. The Chemical Analysis of Foods. Chemical Publishing Co. Inc, New York, 420 pp.

Denison, D.A. and Koehn, R.D. 1977. Cellulase activity of *Poronia oedipus*. Mycologia, 69: 592-601. doi: 10.2307/3758562

De Silva, S.S. and Anderson, T.A. 1995. Fish Nutrition in Aquaculture. Chapman & Hall, London, 319 pp.

De Silva, S.S., Shim, K.F. and Khim Ong, A. 1990. An evaluation of the method used in digestibility estimation of a dietary ingredient and comparisons on external and internal markers and time of faeces collection in digestibility studies in the fish *Oreochomis aureus* (Steindachner). Reproduction, Nutrition, Development, 30: 215-226. doi: 10.1051/ rnd:19900207

Devendra, C. 1985. Non-conventional feed resources in Asia and the Pacific, 2nd edition. FAO/APHCA Publication No. 6, FAO, Bangkok: 6-22.

Duncan, D.B. 1955. Multiple range and multiple *F*-tests. Biometrics, 11: 1-42. doi: 10.2307/3001478

Edwards, P., Kamal, M. and Wee, K. L. 1985**.** Incorporation of composted and dried water hyacinth in pelleted feed for the tilapia *Oreochromis niloticus* (Peters). Aquaculture and Fisheries Management, 1: 233-248. doi: 10.1111/j.1365-2109.1985.tb00312.x

El-Sayed, Abdel-Fattah M. 2003. Effects of fermentation methods on the nutritive value of water hyacinth for Nile tilapia *Oreochromis niloticus* (L.) fingerlings. Aquaculture, 218: 471-478. doi: 10.1016/S0044-8486(02)00252-1

Ghosh, K., Sen, S.K. and Ray, A.K. 2004. Supplementation of *Lactobacillus acidophilus* in compound diets for *Labeo rohita* fingerlings. Indian Journal of Fisheries, 51: 521-526.

Goering, H.D. and van Soest, P.J. 1975. Forage fibre Analysis. US Department of Agriculture, Agricultural Research Service, No: 379, Washington, DC., 20 pp.

Hasan, M.R., Moniruzzaman, M. and Farooque, A.M. 1990. Evaluation of leucaena and water hyacinth leaf meal as dietary protein sources of the fry of Indian major carp, *Labeo rohita* (Hamilton), In: R. Hirano and I. Hanyu (Eds.), Proceedings of the Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines: 275-278.

Hasan, M.R., Azad, A.K., Omar Farooque, A.M.O., Akand, A.M. and Das, P.M. 1991. Evaluation of some oilseed cakes as dietary protein sources for the fry of Indian major carp, *Labeo rohita* (Hamilton), In: S.S. De Silva (Ed.), Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society, Special Publication No. 5, Manila, Philippines: 107–117.

Hastings, W.H. 1964. Fish feed processing research. Feedstuffs, 36: 13-21.

Hossain, M.A. and Jauncey, K. 1989. Nutritional evaluation of some Bangladeshi oilseed meals as partial substitutes for fish meal in the diet of common carp, *Cyprinus carpio* L. Aquaculture and Fisheries Management, 20: 255-268. doi: 10.1111/j.1365-2109. 1989.tb00351.x

Hossain, M.A. and Jauncey, K. 1993. The effects of varying dietary phytic acid, calcium and magnesium levels on the nutrition of common carp, *Cyprinus carpio*. In: S. J. Kaushik and P. Luquet (Eds.), Fish Nutrition in Practice. INRA Editions, Paris: 705-715.

Jackson, A.J., Capper, B.S. and Matty, A.J. 1982. Evaluation of some plant proteins in complete diets for the tilapia, *Sarotherodon mossambicus*. Aquaculture, 27: 97-100. doi: 10.1016/0044-

8486(82)90129-6

Jhingran, V.G. 1997. Fish and Fisheries of India. 3rd edition. Hindustan Publishing Corporation, Delhi, India: 335-337.

Krogdahl, Å. 1989. Alternative protein sources from plants containing anti-nutrients affecting digestion in salmonids. In: M. Takeda and T. Watanabe (Eds.) Proceedings of the Third International Symposium in Feeding and Nutrition in Fish. Tokyo University of Fisheries, Tokyo: 253-261.

Lall, S.P. 1991. Concepts in the formulation and preparation of a complete fish diet. In: S.S. De Silva (Ed.), Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society, Manila, Philippines: 1-12.

Lee, K. J., Dabrowski, K., Blom, J.H., Bai, S.C. and Stromberg, P. C. 2002. A mixture of cottonseed meal and animal by-product mixture as fishmeal substitute: growth and tissue gossypol enantiomer in juvenile rainbow trout (*Oncorhynchus mykiss*). Journal of Animal Physiology and Animal Nutrition, 86: 201-231. doi: 10.1046/j.1439-0396.2002.00375.x

Maitra, S. and Ray, A.K. 2003. Inhibition of digestive enzymes in rohu, *Labeo rohita* (Hamilton), fingerlings by tannin: an *in vitro* study. Aquaculture Research, 34: 93-95. doi: 10.1046/j.1365-2109.2003.00792.x

Majid, F.Z. 1986. Aquatic Weeds: Utility and Development. Agro Botanical Publishers (India), Bikaner, India, 96 pp.

Mandal, S. and Ghosh, K. 2010. Inhibitory effect of *Pistia* tannin on digestive enzymes of Indian major carps: an *in vitro* study. Fish Physiology and Biochemistry, 36: 1171-1180. doi: 10.1007/s10695-010-9395-6

Maynard, L., Loosil, J., Hintz, H. and Warner, R. 1979. In: C.R. Zappa (Ed.), Animal Nutrition, 7th edition, McGraw-Hill, New York: 13-14.

Mondal, T.K. and Ray, A.K. 1998. Nutrient digestibility from subabul (*Leucaena leucocephala*) leafmeal in *Labeo rohita* fingerlings. In: M.S. Hameed and B.M. Kurup (Eds.), Technological Advancements in Fisheries. Publication No: 1, School of Industrial Fisheries, Cochin University of Science and Technology, Cochin, India: 129-134.

Mondal, T.K. and Ray, A.K. 1999. The nutritive value of *Acacia auriculiformis* leaf meal in compound diets for *Labeo rohita* fingerlings. In: J.M. Mohan (Ed.), The Fourth Indian Fisheries Forum Proceedings, Asian Fisheries Society, Indian Branch, Mangalore, India: 295-298.

Moore, S. and Stein, W.H. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. Journal of Biological Chemistry, 176: 367-388.

Mukhopadhyay, N. and Ray, A.K. 1996. The potential of deoiled sal (*Shorea robusta*) seed meal as feedstuff in pelleted feed for Indian major carp, rohu, *Labeo rohita* (Hamilton), fingerlings. Aquaculture Nutrition, 2: 221-227. doi: 10.1111/j.1365-2095.1996.tb00063.x

Mukhopadhyay, N. and Ray, A.K. 1999a. Effect of fermentation on the nutritive value of sesame seed meal in the diets for rohu, *Labeo rohita* (Hamilton), fingerlings. Aquaculture Nutrition, 5: 229-236. doi: 10.1046/j.1365-2095.1999.00101.x

Mukhopadhyay, N. and Ray, A.K. 1999b. Utilization of copra meal in the formulation of compound diets for rohu, *Labeo rohita* fingerlings. Journal of Applied Ichthyology, 15: 127-131. doi: 10.1046/j.1439-0426.1999.00132.x

Ramachandran, S. and Ray, A.K. 2004. Inclusion of extruded grass pea, *Lathyrus sativus* seed meal in compound diets for rohu, *Labeo rohita* (Hamilton, 1822) fingerlings. Acta Icthyologica et Piscatoria, 34(2): 205-218.

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. Bioresource Technology, 96: 1465-1472. doi:10.1016/j.biortech.2004.12.002

Ray, A.K. and Das, I. 1992. Utilization of diets containing composted aquatic weed (*Salvinia cuculata*) by the Indian major carp, rohu, (*Labeo rohita* Ham.) fingerlings. Bioresource Technology, 40: 67-72. doi: 10.1016/0960-8524(92)90121-D

Ray, A.K. and Das, I. 1994. Apparent digestibility of some aquatic macrophytes in rohu, *Labeo rohita* (Ham.), fingerlings. Journal of Aquaculture in the Tropics, 9: 335-342.

Ray, A.K. and Das, I. 1995**.** Evaluation of dried aquatic weed, *Pistia stratiotes* meal as feedstuff in pelleted feed for rohu, *Labeo rohita* fingerlings. Journal of Applied Aquaculture, 5: 35-44.

Schanderi, S.H. 1970. Method in Food Analysis. Academic Press, New York, 709 pp.

Spinelli, J., Houle, C.R. and Wekell, J.C., 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture, 30: 71-83. doi: 10.1016/0044-8486(83)90153-9

Spyridakis, P., Metailler, R., Gabaudan, J. and Riaza, A. 1989. Studies on nutrient digestibility in European sea bass (*Dicendrarchus labrax*). 1. Methodological aspects concerning faeces collection. Aquaculture, 77: 61-70. doi: 10.1016/0044-8486(89)90021-5.

Tacon, A.G.J. 1987. The Nutrition and Feeding of Farmed Fish and Shrimp – Training manual. 2. Nutrient Sources and Composition. FAO publication: GCP/RLA/075/ITA Field document S/E. Food and Agriculture Organization of the United Nations, Rome, Italy, 117 pp.

Tacon, A.G.J. and Jackson, A. 1985 Utilization of conventional and non-conventional protein sources in practical feeds. In: C.B. Cowey, A.M. Mackie and J.G. Bells (Eds.), Nutrition and Feeding in Fish. Academic Press, London: 119-145.

Updegraff, D.M. 1969. Semimicro determination of cellulose in biological materials. Analytical Biochemistry, 32: 420-424. doi:10.1016/S0003-2697(69)80009-6

Walter, H.E. 1984. Methods of Enzymatic Analysis. Verlag Chemie, Weinheim, Germany, 258 pp.

Wee, K.L. 1991. Use of non-conventional feedstuff of plant origin as fish feeds – is it practical and economically feasible? In: S.S. De Silva (Ed.), Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society, Special Publication 5, Manila, Philippines: 13-32.

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

Williams, S.T., Sharpe, M.E. and Holt, G. 1986. Bergey's Manual of Systematic Bacteriology, Vol. I. Williams and Wilkins, Baltimore, USA, 2347 pp.