Int. J. Aquat. Biol. (2015) 3(2): 89-101
E-ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Improvement of nutritive value of sesame oil cake in formulated diets for rohu, *Labeo rohita* (Hamilton) after bio-processing through solid state fermentation by a phytase-producing fish gut bacterium

Paramita Das, Koushik Ghosh*

Aquaculture Laboratory, Department of Zoology, The University of Burdwan, Golapbag, Burdwan 713 104, West Bengal, India.

Abstract: Sesame oil cake (SSC) was bio-processed through solid state fermentation (SSF) under optimized conditions by a phytase-producing fish gut bacterium, *Bacillus subtilis* subsp. *subtilis* (JX292128). SSF significantly reduced anti-nutritional factors (e.g., phytic acid, tannins and trypsin inhibitor) and crude fibre, while enhanced free amino acids, fatty acids and different minerals. Phytase production (39.72 ± 1.06 U/g) during SSF was also recorded. Along with a fish meal based reference diet (RD), 8 isonitrogenous (36% crude protein) and isocaloric (4.60 kcal g^{-1}) experimental diets incorporating raw (R1-R4) and SSF processed (F1-F4) SSC(20%, 30%, 40% and 50%, w/w) were fed to rohu, *Labeo rohita* fingerlings (mean weight 3.28 ± 0.15 g) in triplicate treatments for 70 days. In general, growth and feed utilization efficiencies in fish fed diets containing SSF-processed SSC were superior to the groups fed diets containing raw SSC. The diet F3 (40% fermented SSC) showed significantly (P<0.05) better result in terms of weight gain, feed conversion ratio, protein efficiency ratio, apparent net protein utilization, activities of digestive enzymes, carcass composition and apparent digestibility of protein, lipid and phosphorus. Faecal phosphorus discharge reduced significantly (P<0.05) in fish fed fermented diets. The results indicated that incorporation of SSF-processed SSC might be practiced as a function to replace fish meal in the diets of *L. rohita* fingerlings.

Article history:
Received 24 September 2014
Accepted 19 January 2015
Available online 25 April 2015

Keywords:
Sesame oilcake
Bacillus subtilis subsp. *subtilis*
Solid state fermentation
Labeo rohita
Fingerlings

Introduction

Sustainability of the growing aquaculture industry depends on the progressive reduction in wild fish inputs into fish feed (Naylor et al., 2000). Oil seed by-products might be the most promising alternative sources of protein and energy for formulating economic and environment-friendly aqua-feed (Hardy, 2000). However, apart from deficiencies in the essential amino acids, the use of oil cakes has been restricted by the presence of some antinutritional factors (ANFs), majority of which are polyphenols, trypsin inhibitors, non-starch polysaccharides and phytic acid (Mandal and Ghosh, 2009). Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) represents approximately 70-80% of the total phosphorus in plant seeds (Lott et al., 2000). Due to high density of negative charge, it can bind with mineral cations (Na, K, Mg, Ca, Zn, Fe, Cu, Mn etc.) forming phytates, and also forms insoluble complexes with proteins and amino acids, thereby appears as a major ANF diminishing the bioavailability and digestibility of the essential nutrients (Kumar et al., 2011). Fish cannot digest phytate compounds as they lack the intestinal phytase (Pointillart et al., 1987). Moreover, poor degradation of phytates leads to increased faecal phosphorus release and exerts detrimental effects on the aquatic environment like eutrophication (Persson, 1991). Therefore, endogenous phytate compounds reduce feed value of the protein rich oil cakes unless destroyed or inactivated.

As evidenced by an upsurge in research reports, the influence of microbial phytase supplementation on

* Corresponding author: Koushik Ghosh
E-mail address: kghoshbu@gmail.com

protein digestion and utilization is a topic of recent interest. In contrast to the success achieved in farmed animals, dietary supplementation of microbial phytase in fish diets produced contradictory and inconsistent results. Different authors have reported an increase (Vielma et al., 1998; Debnath et al., 2005a), no change (Lanari et al., 1998) or even decrease (Teskeredzic et al., 1995) in protein digestibility owing to phytase supplementation in the fish diets. The phytases have highest activity at two pH optima, i.e., 5.0-5.5 and 2.5 (Simons et al., 1990). Unlike the farmed animals (pig, poultry and swine, that do have an acidic pH within their GI tract), agastric (stomach less, e.g., carps) or even monogastric fishes do not have such pH ranges within their gut making the supplemented phytase either ineffective or less effective. Alternately, pre-treatment/processing of plant derived feed ingredients have been indicated to ameliorate feed utilization through deactivation of some ANFs (Ramachandran and Ray, 2007). Solid state fermentation (SSF) has been shown to reduce the phytate content in plant ingredients by phytases produced by the bacteria (Khan and Ghosh, 2013). Therefore, microbial deactivation (through SSF) has been considered in the present study for removal of plant derived phytate and other ANFs.

After oil extraction, processing of the oil cakes through SSF might pretend great economic feasibility to the agro-based oil production sectors providing an eco-friendly way of nutrient recycling. Efficacy of the fermented oil seed meals for partial or complete substitution of fishmeal has been suggested by several authors (Ramachandran et al., 2005). As likely incorporation of harmful metabolites during the SSF process cannot be ruled out, the use of autochthonus fish gut microorganisms might be reasonable in processing of plant feed stuffs for likely use in fish feed (Mandal and Ghosh, 2013). In this context, the major aim of the presently reported study was reclamation of plant ingredients into value added products. De-oiled Sesame oil cake (SSC) is rich source of protein and minerals, such as calcium and phosphorus (Salunkhe et al., 1991). The seeds were reported to contain 25% protein that is rich in methionine and tryptophan (Godin and Spensley, 1971). The specific objectives of the present study were value addition of SSC through bio-processing with a phytase-producing fish gut bacterium, *Bacillus subtilis* subsp. *subtilis* (JX292128) and to appraise nutritive value of the bio-processed SSC with partial replacement of fish meal and other conventional ingredients in formulated diets for rohu, *Labeo rohita* fingerlings.

Materials and Methods

Bacteria culture and optimization of solid-state fermentation parameters: The extracellular phytase-producing bacterium *Bacillus subtilis* subsp. *subtilis* (GenBank Accession no: JX292128) was isolated from the gut of a freshwater carp, *Cirrhinus mrigala* (Das and Ghosh, 2013). The culture was grown and maintained on selective modified phytase screening media (MPSM) with minor modifications (Howson and Davis, 1983). Inoculum was prepared from a freshly raised 5-d-old slant culture in MPSM broth grown at 35°C for 48 hrs. The inoculant thus obtained contained 4.8×10^7 cells ml^{-1}.

Optimization of SSF parameters influencing phytate degradation in the SSC was done following Khan and Ghosh (2013) and Das and Ghosh (2014) to detect processing conditions of the SSC. The parameters studied were: initial moisture content of the substrate (10%-90%), initial pH of the moistening media (3-8), incubation temperature (25°C-50°C), inoculum volume (1-5%, v/w), different surfactants (Tween- 20, Tween- 40, Tween-80, DMSO, SDS; 1%, v/w) and NaCl supplementation (1-5%, w/w). Further, the medium was supplemented with different carbon sources (1%, w/v) (glucose, sucrose, lactose, maltose and starch) and different organic and inorganic nitrogen sources (1%, w/v) (peptone, tyrosine, tryptophane, ammonium sulfate, ammonium nitrate and yeast extract). Impact of additional carbon and nitrogen sources were further optimized by incorporating the best source at varying levels (1-5%, w/w). Finally, a time course study was conducted to optimize the

duration of fermentation (2-12 days) incorporating the optimized physico-chemical parameters.

To determine extracellular phytase production by the bacteria during SSF enzyme extraction from the fermented material was carried out following Khan and Ghosh (2013) and quantitative phytase assay of the crude enzyme was done after Yanke et al. (1999) using sodium phytate as the substrate. One phytase unit (U) was defined as the amount of enzyme per ml of supernatant that released 1 µg of inorganic phosphorus per minute. Enzyme yield was expressed as U/g (gram dry substrate).

Analysis of proximate composition, minerals and antinutrients: Proximate composition of the raw and fermented SSC were analysed following the standard methods of Association of Official Analytical Chemists (AOAC, 1990): crude protein (N% × 6.25) by micro Kjeldahl digestion and distillation, lipid was determined by extracting the residue with 40-60°C petroleum ether in a Soxhlet apparatus, crude fiber was determined as loss on ignition of dried lipid free residue after digestion with 1.25% H_2SO_4 and 1.25% NaOH and ash was determined by ignition at 550ºC in a Muffle furnace to constant weight. Total free amino acids and fatty acids were measured according to Moore and Stein (1948) and Cox and Pearson (1962), respectively. The mineral elements were analysed by atomic absorption spectrophotometer (Perkin Elmer Aanalyst 700) using standard reference chemicals. Na and K were analysed by flame photometry. Calcium and phosphorus were estimated by biochemical methods as described by Oser (1971). Among the ANFs, tannin content in both fermented and raw SSC was determined using Folin-Denis reagent (Schanderi, 1970) and phytic acid content determined according to Vaintraub and Lapteva (1988). Trypsin inhibitor activity was determined according to Smith et al. (1980).

Formulation and processing of experimental diets: Eight isonitrogenous (36% crude protein) and isocaloric (4.60 kcal g^{-1}) experimental diets were formulated using raw (R1-R4) and fermented (F1-F4) SSC at 20%, 30%, 40% and 50% levels (w/w) replacing fish meal and other conventional ingredients. A diet with fish meal as the main protein source was used as the reference diet (RD). Each feed was formulated separately using Winfeed 2.8 software. Composition of the experimental diets has been presented in Table 2. Diets were prepared as described by Saha and Ghosh (2013).

Experimental design: The feeding trial was conducted under laboratory condition, in 27 glass aquaria, each containing 90-L of water, for 70 days, with continuous aeration. Rohu, *L. rohita* fingerlings were obtained from a local fish farm and acclimatized for 15 days. The fingerlings (mean individual weight of the 405 fingerlings: 3.28 ± 0.15 g) were randomly distributed in the glass aquaria at a stocking density of 15 fish per aquarium with three replicates for each experimental diet. The fish were fed twice daily: at 07.00 h and 13.00 h, at a feeding rate of 3% of the total body weight per day. The daily ration was adjusted every tenth day after weighing the fish from each replicate. The uneaten feed was siphoned off 6 hrs after each feeding, and oven dried at 100°C for 24 hrs to calculate the feed conversion ratio. The uneaten feeds remained almost intact due to the binder used (carboxy-methyl-cellulose, CMC) during preparation of experimental diets. The faecal samples released by the fish were collected daily from each aquarium by pipetting (Spyridakis et al., 1989). The oven dried (60°C) faecal samples were analysed for digestibility estimation. The water quality parameters, viz., temperature (°C), pH and dissolved oxygen content (mg L^{-1}) from each experimental set were monitored at regular intervals following the standard methods of American Public Health Association (APHA, 1998). The ranges of water quality parameters were 29-31°C, pH 7-7.5, dissolved oxygen 6.3-6.8 mg L^{-1} and alkalinity 148-153 mg L^{-1} (n=10).

Chemical Analysis: The proximate composition of the feed ingredients, experimental diets, faecal samples and fish carcass were analysed both prior to commencement, and on termination of experiment by following the standard methods of AOAC (1990) as described previously. Five fish from each

aquarium were sampled at the termination of the feeding experiment; they were homogenised and analysed for whole body (carcass) composition (on wet weight basis). Chromic oxide in diets and faecal samples were estimated following the method of Bolin et al. (1952). Apparent dry matter or total and nutrient digestibility values, apparent protein digestibility (APD%), apparent lipid digestibility (ALD%) and apparent phosphate digestibility (APhD%) were calculated after De Silva and Anderson (1995). Specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU%) were calculated using standard methods (Steffens, 1989).

Assay of digestive enzymes: α-amylase activity was determined following the dinitro-salicylic-acid (DNSA) method described by Bernfeld (1955). Amylase activity was expressed as mg maltose liberated h^{-1} mg protein^{-1}. Protease activity was determined by the casein digestion method of Walter (1984). One unit of enzyme activity was defined as µg of tyrosine liberated h^{-1} mg protein^{-1}. Lipase activity was measured following the method described by Bier (1955). Lipase activity was expressed as µ mole of fatty acid liberated h^{-1} mg protein^{-1}.

Statistical analysis: All experiments were performed in triplicate and the mean values were reported along with standard error (mean ± SE, n=3). Statistical analysis of the data was performed by analysis of variance (ANOVA). Mean difference between treatments were tested for significance at $P<0.05$ and comparisons were made by Tukey's test following Zar (1999) to find out which treatment differed significantly from the other in respect of growth, carcass composition, digestibility, profiles of digestive enzymes and general performance of the fish. All the statistical analyses were done using SPSS Ver11 (Kinear and Gray, 2000) software.

Results

The results revealed that phytate content in the SSC decreased from 2.58 ± 0.05 to 1.02 ± 0.04 g 100 g^{-1} dry weight (75.25% reduction) after 8 days through SSF under optimized conditions, i.e., 60% initial moisture content, pH 6, 35°C temperature, 3.5% (v/w) inoculum volume, and supplementation of Tween 80 (1%, v/w), NaCl (4%, w/w), starch (4%, w/w) and ammonium sulphate (3%, w/w). Maximum phytase production (39.72 ± 1.06 U/g) was also recorded after 8 days (Fig. 1).

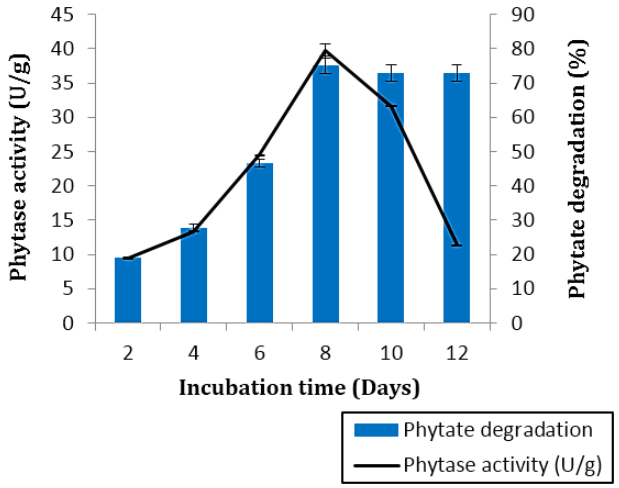

Figure 1. Phytate degradation in sesame oil cake and phytase production during solid state fermentation by *B. subtilis* subsp. *subtilis*.

Data pertaining proximate compositions of nutrients and ANFs (tannin, phytate and trypsin inhibitor) in raw and processed SSC are summarized in the Table 1. There were marginal increase (t-value significant at $P<0.05$) in the contents of protein, lipid, and minerals (Na, K, Ca, Mg, Zn, Fe, Cu, P and Mn) in the SSC after fermentation at optimal conditions by the fish gut isolate *B. subtilis* subsp. *subtilis* (JX292128).

Ingredient composition and proximate analysis of the experimental diets are presented in the Table 2. In comparison to the diets containing raw SSC, the contents of the ANFs (tannin, phytic acid and trypsin inhibitor) were lower in the diets containing fermented SSC. Although, average final weight of the fish increased considerably from the initial value in all the dietary treatments, the results clearly established that inclusion of the bio-processed oil cake in diets improved overall growth performance and nutrient utilization in *L. rohita* fingerlings (Table

Table 1. Proximate composition of nutrients, anti-nutritional factors and minerals (% dry matter) in raw and fermented sesame oil cake.

Parameters	Raw SSC	SSF processed SSC	% Increase (↑) / Reduction (↓)
Nutrients			
Crude protein	41.75 ± 0.03	46.57 ± 0.04	11.54 ↑
Crude Lipid	7.2 ± 0.04	8.9 ± 0.02	23.61 ↑
Crude Fibre	4.23 ± 0.04	2.23 ± 0.04	47.28↓
Crude Ash	5.47 ± 0.03	6.8 ± 0.04	24.31↑
Total free fatty acid	1.02 ± 0.03	1.5 ± 0.02	47.05↑
Total free amino acid	0.78 ± 0.05	1.51 ± 0.04	93.58↑
Antinutrional factors			
Tannin (mg/g)	2.8 ± 0.03	0.61 ± 0.03	68.21↓
Phytate (g %)	2.58 ± 0.05	1.02 ± 0.04	75.25↓
Trypsin inhibitor (mg/g)	9.21 ± 0.04	2.61 ± 0.03	71.66↓
Minerals			
Na (mg /g)	0.98 ± 0.51	1.14 ± 0.43	16.32↑
K (mg /g)	10.25 ± 0.43	12.45 ± 0.23	21.46↑
Ca (ppm)	0.78 ± 0.31	0.95 ± 0.42	21.79↑
Mg (mg /g)	3.11 ± 0.53	4.51 ± 0.21	44.61↑
Zn (mg /g)	1.22 ± 0.41	1.47 ± 0.55	20.49↑
Fe (ppm)	9.51 ± 0.22	11.35 ± 0.31	19.31↑
Cu (mg/g)	11.71± 0.9	14.38 ± 0.63	22.80↑
P (mg/g)	4.05 ± 0.51	4.33 ± 0.26	6.91 ↑
Mn (ppm)	11.86 ± 0.11	13.45 ± 0.9	13.40 ↑

Values are mean ± S.E of five determinations.

Figures in parentheses indicate the percent increase (↑)/decrease (↓) over the values of the corresponding raw oil cakes.

3). The performance of fish in terms of average live weight gain (%), specific growth rate (SGR, % day^{-1}) and protein efficiency ratio (PER) increased significantly (P<0.05) up to 40% incorporation (diet F3) of the SSF processed SSC and thereafter decreased. Values for ANPU and FCR were the best for fish fed the diet F3, and worst for the diet R4 containing 50% raw SSC.

Figure 2 depicts apparent digestibility of dry matter, protein, lipid and phosphate in *L. rohita* fed experimental diets. A progressive decline in the digestibility parameters with increasing level of raw SSC was observed in the present study. In comparison to all of the experimental diets, significantly (P<0.05) higher values for the digestibility parameters were noticed with the fish fed diet F3, while, apparent lipid digestibility (ALD) values did not differ significantly (P<0.05) between the diets F3 and F4. Faecal P concentrations in fish fed different experimental diets are presented in Figure 3. The highest faecal P concentration was

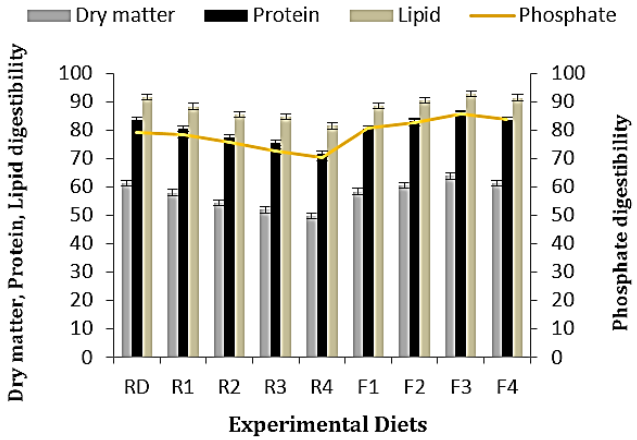

Figure 2. Apparent digestibility of dry matter and nutrients (%) of *Labeo rohita* fingerlings fed experimental diets for 70 days (error bars show deviation among three replicates).

associated in the fish fed diet R4, whereas, the lowest value was noticed in the fish fed diet F3. Higher phosphate digestibility was allied with significantly lower faecal P output in the groups of fish fed fermented SSC incorporated diets than in fish fed raw SSC incorporated diets.

Table 2. Ingredient composition and proximate composition (on % dry matter basis) of the experimental diets.

Parameters	Diets with raw SSC					Diets with bio-processed SSC			
	RD	R1	R2	R3	R4	F1	F2	F3	F4
Ingredient composition									
Fish meal	35	30	26	23	20	30	26	23	20
Mustard oil cake	30	17	13	12	7	17	13	12	7
Rice bran	32	30.0	28.0	22.0	20.0	30.0	28.0	22.0	20.0
Sesame oil cake	-	20	30	40	50	20	30	40	50
Cod liver oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Premix[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic-oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Proximate composition									
Dry matter	94.57 ± 0.58	94.32 ± 0.64	93.94 ± 0.66	93.61 ± 0.78	93.85 ± 0.46	94.32 ± 0.64	93.94 ± 0.66	93.61 ± 0.78	93.85 ± 0.46
Crude protein	36.57 ± 1.08	36.23 ± 1.05	35.25 ± 1.02	35.18 ± 1.03	36.41 ± 0.95	36.53 ± 0.89	35.85 ± 0.75	36.87 ± 0.96	36.21± 0.84
Crude lipid	7.45 ± 0.29	7.29 ± 0.24	7.45 ± 0.33	7.41 ± 0.18	7.43 ± 0.27	7.49 ± 0.38	7.27 ± 0.27	6.95 ± 0.35	7.39 ± 0.41
Ash	11.85 ± 0.62	11.34 ± 0.61	10.62 ± 0.43	10.24 ± 0.55	10.11 ± 0.54	11.75 ± 0.32	11.24 ± 0.24	10.49 ± 0.43	10.55 ± 0.32
Crude fibre	6.75 ± 0.41	6.94 ± 0.55	6.41 ± 0.28	6.15 ± 0.35	5.81 ± 0.33	5.95 ± 0.63	6.39 ± 0.25	6.81 ± 0.45	6.91 ± 0.35
NFE[2]	27.79 ± 0.43	26.57 ± 0.41	28.36 ± 0.35	28.91 ± 0.35	27.41 ± 0.4	26.57 ± 0.41	28.36 ±0.35	28.91 ± 0.35	27.41 ± 0.4
Gross energy (k cal g^{-1})	4.65 ± 0.35	4.47 ± 0.31	4.45 ± 0.29	4.37 ± 0.25	4.48 ± 0.28	4.61 ± 0.23	4.67 ± 0.31	4.82 ± 0.36	4.75 ± 0.35
Tannin	-	0.71 ± 0.03	0.89 ± 0.05	1.21 ± 0.03	1.38 ± 0.05	0.45 ± 0.02	0.52 ± 0.03	0.62 ± 0.01	0.57 ± 0.02
Phytic acid	-	0.67 ± 0.03	0.84 ± 0.01	1.18 ± 0.03	1.41 ± 0.05	0.07 ± 0.01	0.06 ± 0.03	0.09 ± 0.02	0.08 ± 0.02
Trypsin inhibitor (mg/g)	-	2.76 ± 0.05	3.71 ± 0.03	5.12 ±0.04	6.31 ± 0.06	0.88 ± 0.04	1.25 ± 0.03	1.47 ± 0.02	2.81 ± 0.03
Phosphate	94.57 ± 0.58	94.32 ± 0.64	93.94 ± 0.66	93.61 ± 0.78	93.85 ± 0.46	94.32 ± 0.64	93.94 ± 0.66	93.61 ± 0.78	93.85 ± 0.46

Values are means ± SE of three determination.

[1]Vitamin and mineral mixture (Supradyn, Bayer Consumer Care AG, Basel, Switzerland).

[2]Nitrogen-free extract; RD= reference diet

Table 3. Growth performances and feed utilization efficiencies in *Labeo rohita* fingerlings fed experimental diets for 70 days.

Parameters	RD	Diets with raw SSC				Diets with bio-processed SSC			
		R1	R2	R3	R4	F1	F2	F3	F4
Initial wt (g)	3.05±0.06	3.03±0.05	3.05±0.04	3.04±0.06	3.02±0.03	3.03±0.04	3.05±0.06	3.02±0.07	3.03±0.05
Final wt (g)	6.35±0.07^{e}	5.98±0.05^{d}	5.61±0.04^{c}	5.32±0.05^{b}	5.11±0.06^{a}	6.05±0.05^{d}	6.29±0.04^{e}	6.53±0.08^{f}	6.38±0.06^{ef}
Weight gain (%)	108.19±3.51^{e}	97.35±3.22^{d}	83.93±2.67^{c}	75.01±3.11^{b}	69.20±2.85^{a}	99.76±2.75^{d}	106.22±3.47^{e}	116.22±3.62^{f}	110.57±3.43^{e}
Feed intake$^{\#}$	1.91±0.08^{a}	1.93±0.06^{a}	1.96±0.05^{b}	1.99±0.06^{b}	2.01±0.04^{c}	2.03±0.03^{c}	2.06±0.07^{d}	1.98±0.09^{b}	2.05±0.06^{c}
SGR (% day^{-1})	4.71±0.06^{f}	4.21±0.05^{d}	3.65±0.04^{c}	3.25±0.03^{b}	2.98±0.05^{a}	4.31±0.04^{c}	4.62±0.03^{e}	5.01±0.06^{g}	4.78±0.05^{f}
FCR	2.41±0.06^{b}	3.29±0.04^{e}	3.5±0.03^{f}	3.88±0.02^{g}	4.31±0.04^{h}	3.11±0.05^{d}	2.56±0.03^{c}	2.32±0.08^{a}	2.43±0.05^{b}
PER	1.30±0.05^{e}	1.17±0.03^{c}	1.02±0.02^{b}	0.96±0.05^{b}	0.65±0.03^{a}	0.98±0.04^{b}	1.25±0.02^{d}	1.46±0.07^{f}	1.33±0.06^{e}
ANPU (%)$^{\$}$	20.51±0.58^{d}	15.95±0.61^{b}	15.27±0.52^{b}	12.63±0.49^{a}	10.71±0.48^{a}	17.47±0.47^{c}	18.69±0.63^{c}	22.65±0.65^{e}	20.57±0.56^{d}

Values are means ± SE of three determinations.

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

$^{\#}$g 100 g^{-1} body weight of fish day^{-1}.

$^{\$}$ANPU = (Net increase in carcass protein/amount of protein consumed) × 100

Table 4. Activities of protease, amylase and lipase in the gut of *Labeo rohita* fingerlings fed experimental diets for 70 days.

Diets	**Protease activity***	**Amylase activity$^{\#}$**	**Lipase activity$^{\$}$**
Initial	12.45 ± 0.21^{a}	7.14 ± 0.27^{a}	9.74 ± 0.16^{a}
RD	17.61 ± 0.19^{e}	12.35 ± 0.25^{e}	14.35 ± 0.19^{d}
R1	16.31 ± 0.18^{d}	11.26 ± 0.29^{d}	13.39 ± 0.21^{c}
R2	15.53 ± 0.23^{c}	10.63 ± 0.23^{c}	13.12 ± 0.25^{c}
R3	15.19 ± 0.17^{c}	10.41 ± 0.21^{c}	12.31 ± 0.27^{b}
R4	13.47 ± 0.25^{b}	9.35 ± 0.18^{b}	11.39 ± 0.22^{a}
F1	16.55 ± 0.17^{d}	11.36 ± 0.22^{d}	14.51 ± 0.18^{d}
F2	17.48 ± 0.22^{e}	12.61 ± 0.17^{e}	15.23 ± 0.16^{d}
F3	18.33 ± 0.16^{f}	14.26 ± 0.19^{f}	16.82 ± 0.19^{e}
F4	17.85 ± 0.21^{ef}	13.47 ± 0.18^{f}	16.41 ± 0.21^{e}

Values with the same superscript in the same column are not significantly different (P<0.05); *µg of tyrosine liberated h^{-1} mg protein^{-1}; $^{\#}$mg maltose liberated h^{-1} mg protein^{-1}; $^{\$}$µ mole of fatty acid liberated h^{-1} mg protein^{-1}

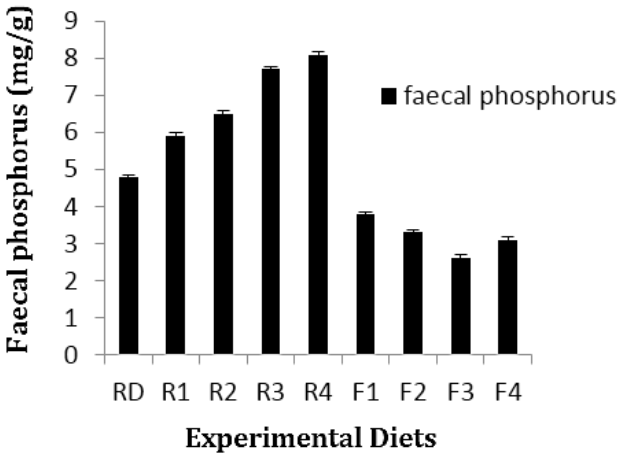

Figure 3. Faecal Phosphorus Concentration of *Labeo rohita* fingerlings fed experimental diets for 70 days (error bars show deviation among three replicates).

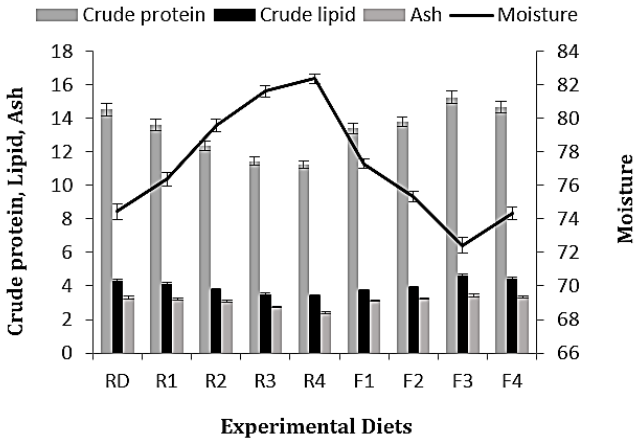

Figure 4. Proximate carcass composition (% wet weight) of experimental fish at the end of the 70 days feeding trial (Initial values: moisture, 83.48 ± 0.51; crude protein, 10.35 ± 0.35; crude lipid, 3.3 ± 0.07 and ash, 3.1 ± 0.06).

Proximate compositions of the carcass in fish fed various experimental diets are presented in Figure 4. Although all the fish were fed isonitrogenous diets, the deposition of carcass protein and lipid was significantly higher in fish fed bio-processed SSC incorporated diets than the reference diet, and an increasing level of raw SSC was associated with a decrease in carcass protein and lipid contents. The highest values for protein gain and lipid accumulation in the carcass was recorded in the group of fish reared on diet F3. Carcass ash content was also revealed the highest value in fish fed diet F3.

Activities of intestinal protease, amylase and lipase in *L. rohita* fingerlings fed experimental diets are presented in Table 4. In general, activities of all the three enzymes were significantly (P<0.05) higher in the fish fed diets containing fermented SSC as compared to the fish fed diets containing raw SSC. Maximum protease, amylase and lipase activities were noticed in the fish fed diet F3, though it was not significantly (P<0.05) different from the diet F4.

Discussion

The present investigation was intended to assess the effectiveness of a phytase-producing fish gut bacterium, *B. subtilis* subsp. *subtilis* (JX292128) in improving the nutritive value of sesame (*Sesamum indicum*) oil cake (SSC) under SSF. The results showed that fermentation was effective in reducing the crude fibre content and the ANFs, such as tannins, phytic acid and trypsin inhibitor, and enhancing available free amino acids and fatty acids. In the present study, increased level of crude protein, free amino acids and free fatty acids in fermented SSC in comparison to the raw oil cake is consistent to the findings of Roy et al. (2013). *In vitro* processing by autochthonus microorganisms might be assumed as an effective strategy as the organism itself and their metabolites would not cause harm to the fish providing the basis for mutual relationship (Khan and Ghosh, 2013).

Suitability of the SSF processed SSC as an alternate plant derived feed ingredient has been evaluated in the formulated diets for rohu, *L. rohita* fingerlings. The results revealed that inclusion of the bio-processed oil cake in diets improved significantly overall growth performance and nutrient utilization in *L. rohita* fingerlings in terms of average live weight gain, SGR and PER up to 40% incorporation of the SSF processed SSC and thereafter decreased. The results might indicate that inclusion of the 40% fermented oil cake in the diet was optimal for augmenting the bioavailability of nutrients in *L. rohita* fingerlings. Reports on the effectiveness of dietary microbial phytase and/or phytase pre-treatment were contradictory, as some authors could not detect significant effect in diverse fish species

fed plant-based diets (Cain and Garling, 1995; Sajjadi and Carter, 2004). Diet composition, methods of phytase pre-treatment/application and rearing conditions may be closely associated with the inconsistency of the experimental results. However, the present study evidenced positive effect of microbial phytase on the growth performance and nutrient utilization in rohu fingerlings, which were consistent with the results of other researchers (Vielma et al., 2002; Debnath et al., 2005a; Liebert and Portz, 2005; Sardar et al., 2007; Roy et al., 2013).

The treatment of fish feed with phytase has been reported to result in improvement of protein digestibility and retention in fish (Cheng and Hardy, 2002; Debnath et al., 2005a, b; Baruah et al., 2005). A declining trend in apparent protein digestibility (APD) values had also been reported previously with higher levels of raw plant ingredient inclusions in carp diets (Ramachandran and Ray, 2007; Saha and Ghosh, 2013; Roy et al., 2013). In the present study, fermentation of SSC by phytase-producing fish gut bacteria resulted in increased phytate hydrolysis enhancing availability of protein and minerals that are chelated by phytate. Not only protein, the apparent lipid digestibility also increased significantly in fish fed fermented SSC incorporated diets in comparison with the diets with raw SSC. Indeed, sesame proteins are amphiphilic globulins whose functional property of absorbing fat may reduce lipid digestibility in case of raw SSC incorporated diets (Johnson et al., 1979). An essential mineral nutrient for fish is phosphorus (P), a vital component of the skeletal system. It plays an important role in energy and cell metabolism, including synthesis of nucleic acids, phospholipids and some major enzymes (Luo et al., 2010). Apparent phosphate digestibility is one of the most sensitive criteria for assessing the influence of phytase on minerals utilization in fish (Sajjadi and Carter, 2004). In the present study, apparent phosphate digestibility increased with incorporation of fermented SSC, confirming the established properties of phytase with respect to dietary phosphorus availabilities. The increase in phosphate digestibility is in accordance with other studies carried out in common carp (Nwanna et al., 2008) and rohu juveniles (Baruah et al., 2005). In the present study, increased incorporation of fermented oil cake was associated with decreased faecal P output, which was in accordance with the observations made by Vielma et al. (2002) and Sugiura et al. (2001), who opined that addition of phytase in rainbow trout, *Oncorhynchus mykiss* diets reduced the faecal P excretions up to 95-98% compared with the fish fed diets without phytase. Addition of microbial phytase has been reported to be effective in improving bioavailability of phytate phosphorus due to hydrolysis of phytate to orthophosphate by phytase (Reddy et al., 1982) making the chelated phosphorus available to fish resulting in less faecal excretion (Baruah et al., 2004). The results of the presently reported study indicated that bacterial phytase was effective in enhancing the bioavailability of P considerably, thereby reducing the P output in the faeces.

In the present study, the proximate carcass composition, i.e., moisture, crude protein, ash and crude lipid, of the experimental fish was significantly influenced by the level of incorporation of raw and fermented SSC in the diets. Phytate forms compounds with a large number of minerals and also forms complexes with proteins and amino acids, thereby reduces bioavailability of minerals and decrease digestibility of proteins as phytate-protein and protein-mineral complexes are resistance to proteolytic digestion (Kumar et al., 2011). This results in lowering the gastrointestinal absorption of protein and other nutrients (Debnath et al., 2005a, b). Sesame seed α-globulin and sodium phytate use to form complex through a bi-phasic reaction (Rajendran and Prakash, 1993). At the first step phytase binds protein through strong electrostatic attractions, which is followed at the next step by slower protein-protein interactions ensuing precipitation of the protein-phytate complex. Consequently, protein utilization in fish has been reported to be reduced by phytate (Nang Thu et al.,

2011). It was apparent in the present study that the bacterial phytase could prevent the formation of protein-phytate complexes to some extent by prior hydrolysis of the phytate complex through the SSF process making nutrients and minerals bio-available for growth. Therefore, improvement in growth performance and carcass composition of rohu fingerlings could be attributed to enhanced release of the nutrients. Improved ash contents in the fish carcass indicated better mineral deposition in the fish fed SSF processed SSC incorporated diets. It has been reported that the addition of microbial phytase enhances the availability of various minerals from the plant oilseed meals and improves their absorption (Debnath et al., 2005b; Cao et al., 2008).

The results indicated that the fish was able to digest the nutrients from the diets containing fermented seed meal more efficiently. Decreased protease activities with increased raw SSC in the diets might correspond to decrease in protein availability from SSC. Similar results have been documented by Krogdahl et al. (1994), who concluded that proteases might be highly sensitive to plant ANFs. The decrease in protease activity at higher inclusion levels of raw SSC might be caused by the presence of the ANFs like tannin and phytic acid. Moreover, activity of the digestive enzymes in fermented SSC-fed groups comparable with the RD-fed group might correlate with improved nutrient availability and decreased ANFs in the fermented SSC.

Conclusions

The present study demonstrated an inclusion level of fermented SSC (up to 40%) in the practical diet for *L. rohita* fingerlings without any adverse effect on growth and feed utilization efficiencies. On the other hand, the study highlighted that dietary incorporation of bio-processed SSC improved carcass composition and apparent phosphate digestibility in *L. rohita* fingerlings. Thus, preparation of fish feed incorporating SSC after processing through SSF by phytase-producing fish gut bacteria might be expected to provide both economic and environmental benefits through decreased expenditures on supplemental minerals and mineral outputs to the aquatic ecosystem. However, further experimentation in the field condition with large number of fish and replication are essential prior to recommend it to the aquaculture industry.

Acknowledgements

This research was supported by The University Grants Commission (UGC), New Delhi, India [Major Research Project F. No. 37–383/2009(SR)]. Research facilities provided by the Department of Zoology, The University of Burdwan, West Bengal, India, The Department of Science and Technology (FIST Programme) and The University Grants Commission (UGC-SAP-DRS programme) are also gratefully acknowledged.

References

AOAC (1990). Official methods of analysis. V.A. Arlington (16th Ed), Association of Official Analytical Chemist, New York, 1134 p.

APHA (1998). Standard methods for the examination of water and wastewater, 20th edition. In: L.S. Clesceri, A.E. Greenberg, A.D. Eaton (Eds.). American Public Health Association, American Water Works Association, Water Environment Federation. Washington. DC, USA, 1220 p.

Baruah K., Pal A.K., Sahu N.P., Jain K.K., Mukherjee S.C., Debnath D. (2005). Dietary protein level, microbial phytase, citric acid and their interactions on bone mineralization of *Labeo rohita* (Hamilton) juveniles. Aquaculture Research, 36: 803-812.

Bernfeld P. (1955). Amylase (alpha) and (beta). In: S.P. Colowick, N.O. Kaplan (Eds.). Methods in enzymology, Vol. 1. Academic press, New York. pp: 149-150.

Bier M. (1955). Lipases. In: S.P. Colowick, N.O. Kaplan (Eds.). Methods in enzymology, Vol. 1. Academic press, New York. pp. 627-642.

Bolin D.W., King R.P., Klosterman E.W. (1952). A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an index substance. Science, 116: 634-635.

Cao L., Yang Y., Wang W.M., Yakupitiyage Yuan A.D.R., Diana J.S. (2008). Effect of pretreatment with microbial phytase on phosphorus utilization and

growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition, 14: 99-109.

Cheng Z.J., Hardy R.W. (2003). Effects of extrusion and expelling processing, and microbial phytase supplementation on apparent digestibility coefficients of nutrients in full-fat soybeans for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 218(1): 501-514.

Cox H.E., Pearson D. (1962). The Chemical Analysis of Foods. Chemical Publishing Co. Inc, New York, 420 p.

Das P., Ghosh K. (2013). Evaluation of phytase-producing ability by a fish gut bacterium, *Bacillus subtilis* subsp. *subtilis*. Journal of Biological Sciences, 13(8): 691-700.

De Silva S.S., Anderson T.A. (1995). Fish Nutrition in Aquaculture. Chapman and Hall, London, 319 p.

Debnath D., Pal A.K., Sahu N.P., Jain K. K., Yengkokpam S., Mukherjee S.C. (2005b). Mineral status of *Pangasius pangasius* (Hamilton) fingerlings in relation to supplemental phytase: absorption, whole body and bone mineral content. Aquaculture Research, 36:326-335.

Debnath D., Pal A.K., Sahu N.P., Jain K.K., Yengkokpam S., Mukherjee S.C. (2005a). Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. Aquaculture Research, 36:180-187.

Godin V.J., Spensley P.C. (1971). Oils and oil seeds. Tropical products institute, London, 170+xxi p. 75 p.

Hardy R.W. (2000). New developments in aquatic feed ingredients, and potential of enzyme supplements. In: L.E. Cruz-Suarez, D. Ricque-Marie, M. Tapia-Salazar, M.A. Olvera-Novoa, R. Civera-Cerecedo (Eds.), Advances en nutricion acuicola v. memorias del v simposium internacional de nutricion acuicola, 19-22 November, Merida, Yucatan, Mexico.

Howson S. J., Davis R.P. (1983). Production of phytate-hydrolyzing enzyme by some fungi. Enzyme and Microbial Technology, 5: 377-382.

Johnson L.A., Suleiman T.M., Lusas E.W. (1979). Sesame protein: a review and prospectus. Journal of the American Oil Chemists' Society, 56: 463-468.

Khan A., Ghosh K. (2013). Evaluation of Phytase Production by Fish Gut Bacterium, *Bacillus subtilis* for Processing of *Ipomea aquatica* Leaves as Probable Aquafeed Ingredient, Journal of Aquatic Food Product Technology, 22(5): 508-519.

Kinnear P.R., Gray C.D. (2000). SPSS for Windows Made Simple. Release10. Psychology Press, Sussex, UK.

Krogdahl A., Lea T.B., Olli J.L. (1994). Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology, 107: 215-219.

Kumar V., Makkar H.P.S., Becker K. (2011). Detoxified *Jatropha curcas* kernel meal as a dietary protein source: Growth performance, nutrient utilization and digestive enzymes in common carp (*Cyprinus carpio* L.) fingerlings. Aquaculture Nutrition, 17(3): 313-326.

Lanari D., D'Agaro E., Turri C. (1998). Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 161: 345-356.

Liebert F., Portz L. (2005). Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. Aquaculture, 248: 111-119.

Lott N.A., Ockenden I., Raboy V., Batten G.D. (2000). Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Science Research, 10: 11-33.

Luo Z., Tan X.Y., Liu X., Wang W.M. (2010). Dietary total phosphorus requirement of juvenile yellow catfish *Pelteobagrus fulvidraco*. Aquaculture International, 18(5): 897-908.

Mandal S., Ghosh K. (2009). Development of plant-derived low-cost fish feed through overcoming adverse effects of plant anti-nutrients. Fishing Chimes, 29(1): 156-161.

Mandal S., Ghosh K. (2013). Isolation of tannase-producing microbiota from the gastrointestinal tracts of some freshwater fish. Journal of Applied Ichthyology, 29: 145-153.

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

Nang Thu T.T., Bodin N., Saeger S.D., Larondelle Y., Rollin X. (2011). Substitution of fish meal by sesame oil cake (*Sesamum indicum* L.) in the diet of rainbow trout (*Oncorhynchus mykiss* W.). Aquaculture Nutrition, 17(1): 80-89.

Naylor R.L., Goldburg R.J., Primavera J.H., Kautsky N.,

Beveridge M.C.M., Clay J., Folke C., Lubchenco J., Mooney H., Troell M. (2000). Effect of aquaculture on world fish supplies. Nature, 405: 1017-1024.

Nwanna L.C., Kolahsa M., Eisenreich R. Schwarz F.J. (2008). Pre-treatment of dietary plant feedstuffs with phytase and its effect on growth and mineral concentration in common carp (*Cyprinus carpio* L.). Journal of Animal Physiology and Animal Nutrition, 92(6): 677-682.

Oser B.L. (1971). Power's and Levatin's oxalic acid estimation. In: Hawk's physiological chemistry (14th ed), McGraw Hill Inc, New York. 1257 p.

Persson G. (1991). Eutrophication resulting from salmonid fish culture in fresh and salt waters: Scandinavian experiences. In: C.B. Cowey, C.Y. Cho. (Eds). Nutritional Strategies and Aquaculture Waste. Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste, 2-6 June 1990, Guelph, Canada. University of Guelph, Ont., Canada. pp: 163-186.

Pointillart A., Fourdin A., Fontaine N. (1987). Importance of cereal phytase activity for phytate phosphorus utilization by growing pigs fed diets containing triticale or corn. Journal of Nutrition, 117 (5): 907-913.

Rajendran S., Prakash V. (1993). Kinetics and thermodynamics of the mechanism of interaction of sodium phytate with a-globulin. Biochemistry, 32: 3474-3478.

Ramachandran S., Bairagi A., 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.

Ramachandran S., Ray A.K. (2007). Nutritional evaluation of fermented black gram (*Phaseolus mungo*) seed meal in compound diets for rohu, *Labeo rohita* (Hamilton), fingerlings. Journal of Applied Ichthyology, 23: 74-79.

Reddy N.R., Sathe S.K., Salunkhe D.K. (1982). Phytates in legumes and cereals. In: C.O. Chichester, E.M. Mark, G.F. Stewart (Eds.). Advances in food research. Academic Press, New York. pp: 1-92.

Roy T., Banerjee G., Dan S. K., Ghosh P. Ray A.K. (2013). Improvement of nutritive value of sesame oilseed meal in formulated diets for rohu, *Labeo rohita* (Hamilton), fingerlings after fermentation with two phytase producing bacterial strains isolated from fish gut. Aquaculture international, 22(2): 633-652.

Saha S., Ghosh K. (2013). Evaluation of Nutritive Value of Raw and Fermented De-oiled Physic Nut, *Jatropha curcas* Seed Meal in the Formulated Diets for Rohu, *Labeo rohita* (Hamilton) Fingerlings. Proceedings of Zoological Society, 66(1): 41-50.

Sajjadi M., Carter C.G. (2004). Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar* L.). Aquaculture Nutrition, 10(2): 135-142.

Salunkhe D.K., Chavan J.K., Adsule R.N., Kadam S.S. (1991). World Oilseeds: Chemistry, Technology and Utilisation. Van Nostrand Reinhold. New York, 554 p.

Schanderi S.H. (1970). Methods in food analysis. Academic Press, New York, 709 p.

Simons P.C., Versteegh H.A., Jongbloed A.W., Kemme P.A., Slump P., Bos K.D., Wolters M.G., Beudeker R.F., Verschoor G.J. (1990). Improvement of phosphorus availability by microbial phytase in broilers and pigs. British Journal of Nutrition, 64: 525-540.

Smith C., Van Megen W., Twaalhoven L., Hitchcock C. (1980). The determination of trypsin inhibitor levels in foodstuffs. Journal of the Science of Food and Agriculture, 3: 341-350.

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

Steffens W. (1989). Principles of fish nutrition, Ellis Horwood, Chichester, 384 p.

Sugiura S.H., Gabaudan J., Dong F.M., Hardy R.W. (2001). Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout *Oncorhynchus mykiss* (Walbaum) fed soybean meal-based diets. Aquaculture Research, 32: 583-592.

Teskeredzic Z., Higgs D.A., Dosanjh B.S., McBride J.R., Hardy R., Beames R.M., Simell M., Vaara T., Bridges R.B. (1995). Assessment of unphytinized and dephytinized rapeseed protein concentrate as sources of dietary protein for juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 131: 261-277.

Vaintraub I.A., Lapteva N.A. (1988). Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. Analytical

Biochemistry, 175: 227-230.

Vielma J., Lall S.P., Koskela J. (1998). Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 163(3): 309-323.

Vielma J., Ruohonen K., Peisker M. (2002). Dephytinization of two soy proteins increases phosphorus and protein utilization by rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 204: 145-156.

Walter H.E. (1984). Proteinases: methods with hemoglobin, casein and azocoll as substrates. In: H.U. Bergmeyer (Ed.). Methods of Enzymatic Analysis, Volume V. Verlag Chemie, Weinheim, Germany. pp: 270-277.

Yanke L.J., Selinger L.B., Cheng K.J. (1999). Phytase activity of *Selenomonas ruminantium*: a preliminary characterization. Letters in Applied Microbiology, 29: 20-25.

Zar J.H. (1999). Biostatistical Analysis. 4th ed. Pearson Education Singapore Pte. Ltd (Indian Branch), New Delhi, India, 663 p.