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Original Article

Nutritional evaluation of soybean meal after fermentation with two fish gut bacterial strains, *Bacillus cereus* LRF5 and *Staphylococcus caprae* CCF2 in formulated diets for *Labeo rohita* fingerlings

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Abstract

Twelve isonitrogenous (35 % crude protein) and isocaloric (18.0 kJ/g) diets were formulated incorporating raw and fermented soybean meal (SBM) at 15%, 30%, 45% and 60% levels by weight. Two phytase-producing bacterial strains, *Bacillus cereus* LRF5 and *Staphylococcus caprae* CCF2 isolated from the gut of adult *Labeo rohita* and *Catla catla*, respectively were used for fermentation of SBM. Fermentation of SBM was effective in reducing the anti-nutritional factors, trypsin inhibitor and phytic acid and enhancing protein, lipid and mineral concentration. The response of *L. rohita*, fingerlings (initial weight 3.33±0.07 g) fed the experimental diets for 100 days was compared with fish fed a fish meal based diet. In terms of growth, feed conversion ratio and protein efficiency ratio, diet S7 containing 45% SBM fermented with *B. cereus* LRF5 resulted in a significantly (P<0.05) better performance of fish. The overall performance of *L. rohita* fed fermented SBM incorporated diets was better in comparison to those fed raw SBM incorporated diets. The apparent digestibility of nutrients and minerals was significantly (P<0.05) higher in fish fed diet S7. The maximum deposition of protein in the carcass was recorded in fish fed diet S7. Diets containing fermented SBM reduced fecal *P* levels. The use of this fermented feed will definitely increase the production in fish farm. Furthermore, it will also reduce the production cost, as fish meal protein is costly in the market.

Keywords: Soybean meal; fish gut bacteria; phytase; fermentation; diets; growth performance; *Labeo rohita* fingerlings

1 | INTRODUCTION

Soybean meal (SBM) is a major ingredient used in the poultry and livestock industries because of its large production, low cost, high energy content and excellent amino acid profile (Stein *et al*. 2008). Besides, it is also rich in carbohydrate, dietary fibre and minerals (Tharanathan and Mahadevamma 2003) and hence, considered promising as an important protein source in fish feeds. However, one disadvantage of SBM is its high level of anti-nutritional factors, like protease inhibitors, complex oligosaccharides and phytic acid (El-Sayed 2004). During processing of SBM the trypsin inhibitors and agglutinating lectins are inactivated by moist heat but its phosphorus storage form *i.e.* phytic acid remains unaltered (Francis *et al*. 2001). Phytate chelates metal ions, prevents digestive enzymes from contacting ingested feed and leads to poor utilization of nutrients (Erdman and Poneros-Schneier

1989; Beutler 2009; Afinah *et al*. 2010). Chelating metal ions also inhibits digestive enzyme activity (Greiner and Konietzny 2006). Thus, treating phytin-containing raw materials with phytase can result in higher growth due to higher digestion efficiency (Liu *et al*. 1998). The phytase reaction also provides phosphorus usable by the animals and leads to less phosphorus excretion to the environment (Simons *et al*. 1990; Wodzinski and Ullah 1996; Dvorˇa´kova´ 1998). In *Labeo rohita*, microbial phytase supplementation in the diet has been reported to improve phosphorus and crude protein digestibility (Baruah *et al*. 2007). In last few years, several reports have been published regarding the use of soybean meal in different animal farms such as pig (Florou-Paneri *et al*. 2014) and cattle (Giallongo *et al*. 2015). However, information on the possible nutritional benefit of incorporation of fermented SBM in the diets of *L. rohita* is lacking. Compared to phytase supplementation in fish feed, which only degrades the phytic acid, microbial fermentation has been shown to degrade the indigestible components as well as anti-nutritional factors in plant ingredients, transforms complex polysaccharides to simple forms and also eliminate large peptides (Feng *et al*. 2007). Fermentation with lactic acid bacteria has been reported to significantly improve the nutritional value of sesame seed meal and linseed meal with reduction of phytic acid content (Mukhopadhyay and Ray 1999, 2001; Roy *et al*. 2014) and also diminish the levels of non-starch carbohydrates in wheat and barley whole meals (Skrede *et al*. 2002). Presently, solid state fermentation (SSF) is the method of choice for fermentation of plant ingredients as it offers several advantages over submerged fermentation such as lower energy requirements, increased productivity, smaller effluent volumes and simpler fermentation equipment (Zambare 2010). Several strains of bacteria such as *Bacillus subtilis*, *Escherichia coli* and *Bacillus amyloliquefaciens* have been employed for phytase production in SSF systems using different substrates like coconut oil cake, mustard cake, sesame oil cake etc. (Bhargav *et al*. 2008).

The present investigation aims to assess the role of phytase-producing fish gut bacteria in improving the nutritive value of SBM incorporated diets and increasing the bioavailability of nutrients and minerals like P, Ca, Zn, Fe and Cu in the Indian major carp, rohu, *L. rohita*, fingerlings through feeding trial.

2| METHODOLOGY

2.1 | Microorganism used

Two most promising phytase producing bacterial strains, LRF5 and CCF2, isolated from the proximal intestine of adult *L. rohita* and *Catla catla*, respectively were used for fermentation of SBM. On the basis of 16S rDNA partial sequence analysis, isolates LRF5 and CCF2 were identified as *Bacillus cereus* (GenBank Accession No. KC894957.1) and *Staphylococcus caprae* (GenBank Accession no. KC894956.1), respectively (Dan *et al*. 2015).

2.2 | Fermentation of soybean meal

The low fat SBM was used in the present experiment. The SBM was sundried, finely ground and passed through a fine meshed sieve. The sieved meal was moistened with liquid basal medium containing : dextrose, 10.0; $(NH_4)_2SO_4$, 1.0; urea, 10; citric acid, 3.0; sodium citrate, 2.0; $MgSO_4.7H_2O$, 1.0; 1M Tris buffer (pH 8.0),100 ml/l; $FeSO_4.7H_2O$, 0.1 g/ l; biotin, 50 µg/ l; thiamine HCl, 20 mg/l (pH 7.5). Two bacterial strains LRF5 and CCF2, cultured in Tryptone soya broth (average viable count 10^7 cells/ ml) were added to the SBM in separate sealed containers and fermented for 10 days at 37°C in solid-state fermentation conditions. The fermented SBM was dried in hot-air oven at 60°C for 24 h, finely ground and were analyzed (Roy *et al*. 2016) for nutritional value (Table 1).

2.3 | Experimental diets

Twelve isonitrogenous (35% crude protein approximately) and isocaloric (18.0 kJ/ g) diets were formulated with raw (S1-S4) and fermented soybean meal (S5-S8 fermented by LRF5 and S9-S12 fermented by CCF2). SBM replacing other feed ingredients including fishmeal at 15%, 30%, 45% and 60% levels. Diets S5-S8 and S9-S12 were formulated incorporating SBM fermented with *B. cereus* LRF5 and *S. caprae* CCF2, respectively. A diet containing fish meal as the main protein source was used as the reference diet (RD). To each of the formulated diet, 1% chromic oxide was added as an external digestibility marker. All the diets were prepared in pelleted form using 0.5% carboxymethylcellulose as binder. The pellets were prepared by passing the slurry through a pelletizer, dried at 37°C for 72 h and stored at 4°C until used. The ingredient composition, proximate composition and mineral composition of the formulated diets are depicted in Tables 2, 3 and 4, respectively.

2.4 | Experimental design

The feeding trial was conducted in flow-through 90 L circular fibre-glass tanks for 100 days under laboratory condition. *L. rohita*, fingerlings were obtained from a local fish farm (mean initial weight 3.33±0.07 g) and acclimatized for 15 days and fed with a mixture of rice bran and mustard oil cake. The fingerlings were randomly distributed in the fibre-glass tanks at a stocking density of 15 fish per tank with three replicates for each dietary treatment. Fish were fed once daily at 10.00 h at a fixed feeding rate of 3% of total body weight per day. The digestibility study was conducted separately in static aquaria. The faecal samples were collected everyday in the morning by

siphoning 17 h after removal of the uneaten feed following the "immediate pipetting" method outlined by Spyridakis *et al*. (1989), from three replicates 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. At the termination of the 100 day feeding trial, the fish were weighed and analyzed for carcass composition. The water quality parameters from each tank were monitored each week throughout the experimental period following the methods outlined by APHA (1985). Water quality parameters ranges were temperature, 28–32 ^{o}C; pH, 6.9–7.6; dissolved oxygen, 4.6–5.5 mg/l; and alkalinity 152–169 mg/l. During the experiment, environment in all the tanks was similar.

2.5 | Chemical analysis

The experimental diets, fish carcass and faecal matter were analyzed according to the AOAC methods (1995). The crude protein content (Nitrogen × 6.25) was determined by Micro-Kjeldahl method using Tecator 2000 Digestion System (Tecator, Höganäs, Sweden) together with a Kjeltec 1026 Distilling Unit (Tecator). Crude lipid was estimated by petroleum ether extraction at 40-60°C in a Foss Tecator Soxtec system 2043 and crude fibre content was determined after acid and alkali digestion as described by Roy *et al*. (2014). Nitrogen-free extract (NFE) content was calculated by subtracting the sum of crude protein, crude lipid and crude fibre value from the dry matter content. Phytic acid and trypsin inhibitor contents in both fermented and raw SBM were estimated following Wheeler and Ferrel (1971) and Kakade *et al*. (1974), respectively. Phosphorus and calcium contents of experimental diets, faecal samples and carcass were estimated by the method of Fiske and Subbarow as described by Oser (1960) and AOAC procedure (1990), respectively. Iron, copper and zinc contents of experimental diets and fish carcass were estimated using an Atomic Absorption Spectrophotometer (Perkin Elmer 3110, Massachusetts, USA).

Apparent digestibility of nutrients and minerals, weight gain (%), specific growth rate (SGR) (%/day), feed conversion ratio (FCR), protein efficiency ratio (PER), and apparent net protein utilization (ANPU) were calculated using the standard formulae (Steffens 1989).

2.6 | Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) using MS-Excel software. Mean difference between treatments was tested and post-hoc comparisons were made by Duncan's multiple range test (Duncan 1955) for significance at $P<0.05$.

3 | RESULTS

3.1 | Effect of fermentation of SBM on proximate composition, phytic acid and trypsin inhibitor contents and mineral concentration

Fermentation of SBM resulted in increase in its nutritive value and mineral concentration and significant reduction in the anti-nutritional factors. The effect of fermentation with two phytase-producing fish gut bacterial strains LRF5 and CCF2 on nutritive value, anti-nutritional factors and mineral concentration of SBM is depicted in Table 1.

TABLE 1 Proximate composition (% dry matter basis), anti-nutritional factors and mineral contents of soybean meal (SBM) before and after fermentation with LRF5 and CCF2; *n = 3*, mean±SD

Parameters	*Raw SBM*	*SBM fermented with LRF5*	*SBM fermented with CCF2*
Dry matter	91.2±0.65	92.38±0.54	92.50±0.53
Crude Protein	48.60±1.25	51.84±1.38 (6.6)*	49.20±1.34 (1.2)*
Crude lipid	0.9±0.04	1.10±0.04 (22.2)*	1.15±0.05 (27.7)*
Crude fibre	4.5±0.15	2.85±0.16 (36.6)**	3.12±0.14 (30.6)**
Nitrogen-free extract	34.8±0.82	29.42±1.09	30.87±0.54
Ash	6.1±0.06	7.41±0.06	7.66±0.06
Anti-nutritional factors			
Phytic acid (%)	1.14±0.03	0.23±0.01 (98.8)**	0.31±0.01 (72.8)**
Trypsin Inhibitor (TIU/ mg)	3.52±0.04	2.14±0.03 (39.2)**	1.31±0.03 (62.7)**
Mineral contents			
Iron (µg/ g)	78.56±1.84	83.40±1.76 (6.2)*	80.51±1.82 (2.5)*
Copper (µg/ g)	18.20±0.35	20.56±0.37 (12.9)*	19.75±0.35 (8.5)*
Zinc (µg/ g)	48.00±1.45	51.23±1.50 (6.7)*	49.51±1.54 (3.1)*
Phosphorus (mg/ g)	7.10±0.24	9.45±0.26 (33.1)*	8.77±0.25 (23.5)*
Calcium (mg/ g)	3.10±0.11	4.53±0.14 (46.1)*	3.81±0.13 (22.9)*

*Values in the parentheses indicate percentage increase/reduction. *, % increase; **, % reduction.*

3.2 | Growth performance and feed utilization

The growth performance and feed utilization of *L. rohita* fingerlings are presented in Table 5. The average final weight of the fish increased considerably from the initial value in all dietary treatments. Fish reared on diet S7 (containing 45% SBM fermented with *B. cereus* LRF5) showed good performance in terms of live weight gain (%), specific growth rate (SGR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU). Feed conversion ratio (FCR) was lowest for diet S7 and highest for S4 containing 60% raw SBM. The reference diet (without any SBM substitution) performed significantly (P<0.05) better than diets S1-S4 containing raw SBM. The overall growth performance of *L. rohita* fingerlings fed fermented SBM incorporated diets was better in comparison to those fed raw SBM incorporated diets.

3.3 | Apparent nutrient and mineral digestibility

The apparent nutrient digestibility of the formulated diets by the *L. rohita* fingerlings is presented in Figure 1. The apparent crude protein digestibility was highest for diet S7 which was not significantly different from diets S6, S8 and S11. Though the crude lipid digestibility was highest for RD, it was not significantly (P<0.05) different from the diets S7 and S11. Ash digestibility was highest for diet S8 which was not significantly different from that for diets S6, S7, S11, S12 and the reference diet (RD). Dry matter digestibility also showed significant (P<0.05) variation among the experimental groups. The results of mineral digestibility of the experimental diets are shown in Figure 2. The apparent digestibility of phosphorus, calcium, iron, copper and zinc were better in fish fed diet S7. Digestibility of minerals was poor in fish fed diets S1-S4 containing raw SBM. The mineral digestibility in *L. rohita* fingerlings fed diet S12 containing 60% fermented SBM was also low compared to the group of fish fed 45% fermented SBM (S11).

3.4 | Proximate carcass composition

The proximate carcass composition of the experimental fish was significantly influenced by the type and level of SBM in the diets (Figure 3). An increasing level of raw sesame seed meal in the diets was associated with a decrease in carcass protein and lipid contents. The deposition of crude protein in fish carcass was highest in the group of fish fed diet S7 which was not significantly different from that with diets S11, S12 and reference diet (RD). The crude lipid value also followed a similar trend being highest in the group of fish fed diet S7. The ash content of fish carcass was highest in fish fed diets S6 and S7. The addition of microbial phytase enhanced the availability of various minerals from SBM and improved their absorption (Figure 4). The deposition of minerals (phosphorus, calcium, iron, copper and zinc) in the carcass of experimental fish increased over the initial value in the group of fish fed formulated diets containing fermented SBM. The phosphorus content was highest in the group of fish fed diet S7 (45% inclusion of SBM fermented with *B. cereus* LRF5) followed by the diets S6 and S8 (30% and 60% inclusion of SBM fermented with the same bacterial strain). The highest accumulation of carcass calcium, iron, zinc and copper was recorded in the group of fish reared on diet S7.

3.5 | Fecal phosphorus concentration

The faecal phosphorus concentration was significantly higher in fish fed raw SBM incorporated diets than the reference diet and an increasing level of fermented SBM was associated with a decrease in faecal phosphorus concentration (Figure 5). Maximum faecal phosphorus concentration was recorded in the fish fed diet S4 (60% inclusion of raw SBM) which was not significantly different from the group of fish fed diet S3 (45% inclusion of raw SBM). Minimum concentration of phosphorus was recorded in the faeces of the group of fish fed diet S7 and containing 45 % of SBM fermented with *B. cereus* LRF5.

TABLE 2 Ingredient compositions (% dry matter basis) of the diets prepared with raw and fermented soybean meal

Ingredients	*Reference diet (RD)*	*Diets with Raw soybean meal*				*Diets with soybean meal fermented with LRF5*				*Diets with soybean meal fermented with CCF2*			
		S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*	*S10*	*S11*	*S12*
Fish meal	40.0	30.0	20.0	10.0	0	30.0	20.0	10.0	0	30.0	20.0	10.0	0
Mustard oil cake	24.0	19.0	16.0	13.0	10.0	19.0	16.0	13.0	10.0	19.0	16.0	13.0	10.0
Rice bran	33.0	33.0	31.0	29.0	27.0	33.0	31.0	29.0	27.0	33.0	31.0	29.0	27.0
Soybean meal	-	15.0	30.0	45.0	60.0	15.0	30.0	45.0	60.0	15.0	30.0	45.0	60.0
Soybean oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cod liver oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Premix	1.0	1.0	1.0	1.0	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	1.0	1.0	1.0	1.0

RD= Reference Diet; S1-4 = Diets with Raw Soybean meal (15, 30, 45, 60 % soybean meal); S5-8 = Diets with fermented soybean meal by LRF5 (15, 30, 45, 60 % soybean meal); S9-12 = Diets with fermented soybean meal by CCF2 (15, 30, 45, 60 % soybean meal).

TABLE 3 Proximate composition of the experimental diets (on % dry matter basis; *n* = 3, Mean±SD)

Proximate composition (%)	*Reference diet (RD)*	*Diets with soybean meal*											
		S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*	*S10*	*S11*	*S12*
Dry matter	96.90±2.35	97.31 ±2.31	96.52 ±2.30	97.11 ±2.34	96.60 ±2.28	96.82 ±2.30	96.45 ±2.29	96.81 ±2.31	96.72 ±2.30	97.80 ±2.32	96.53 ±2.29	96.21 ±2.31	97.14 ±2.32
Crude Protein	35.91±1.24	35.50 ±1.14	35.96 ±1.15	35.91 ±1.22	36.11 ±1.25	36.25 ±1.20	35.75 ±1.18	36.40 ±1.20	36.18 ±1.19	35.77 ±1.14	35.94 ±1.16	36.10 ±1.21	36.52 ±1.23
Crude lipid	8.37±0.25	7.5 ±0.21	7.0 ±0.20	6.72 ±0.22	6.34 ±0.19	7.81 ±0.25	7.60 ±0.22	7.42 ±0.24	7.50 ±0.23	7.45 ±0.21	7.31 ±0.20	7.20 ±0.22	7.34 ±0.24
Crude fibre	9.41±0.38	8.41 ±0.32	8.7 ±0.36	8.69 ±0.30	8.53 ±0.31	8.65± 0.35	8.10 ±0.30	8.36 ±0.32	8.51 ±0.34	8.22 ±0.30	8.45 ±0.32	8.57 ±0.35	8.50 ±0.31
Ash	15.3±0.35	13.0 ±0.29	13.4 ±0.33	13.5 ±0.34	13.1 ±0.30	14.4 ±0.36	15.3 ±0.35	15.08 ±0.32	14.40 ±0.28	13.6 ±0.29	13.9 ±0.30	14.6 ±0.32	14.5 ±0.30
NFE	27.91±0.85	30.5 ±0.91	29.46 ±0.87	30.29 ±0.84	30.52 ±0.75	27.71 ±0.72	27.7 ±0.70	27.55 ±0.68	28.53 ±0.73	30.76 ±0.86	28.93 ±0.80	27.74 ±0.78	28.48 ±0.82
Gross energy (kJ/ g)	17.90±0.32	17.03 ±0.26	17.85 ±0.28	17.87 ±0.30	17.78 ±0.29	17.94 ±0.30	17.64 ±0.28	17.74 ±0.32	17.76 ±0.34	18.13 ±0.31	17.84 ±0.28	17.65 ±0.30	17.84 ±0.26
Phytic acid	0.08±0.009	0.12 ±0.02	0.20 ±0.03	0.28 ±0.04	0.36 ±0.03	ND	ND	ND	0.08 ±0.05	ND	ND	ND	0.11 ±0.08

NFE= Nitrogen-free extract; Total carbohydrate = NFE + Crude fibre. The caloric values in terms of kJ/ g were calculated using the average caloric conversion factors of 39.31, 23.50 and 17 kJ/ g for lipid, protein and carbohydrate, respectively (Cho et al. 1982). ND = Not detected.

TABLE 4 Mineral composition of the experimental diets prepared with raw and fermented soybean meal (on dry matter basis)

Minerals	*Reference diet (RD)*	*Diets with soybean meal*											
		S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*	*S10*	*S11*	*S12*
Iron (µg/ g)	38.53±0.68	36.47±0.71	35.18 ±0.74	35.78 ±0.70	35.25 ±0.75	38.21 ±0.78	37.45 ±0.70	36.89 ±0.61	37.50 ±0.72	38.03 ±0.65	37.44 ±0.71	37.87 ±0.69	37.53 ±0.76
Copper (µg/ g)	4.35±0.38	3.76±0.24	3.71 ±0.20	3.95 ±0.18	4.23 ±0.26	3.86 ±0.21	4.45 ±0.30	5.33 ±0.34	4.5 ±0.21	3.67 ±0.17	4.11 ±0.23	4.46 ±0.29	4.78 ±0.31
Zinc (µg/ g)	6.85±0.21	5.10±0.31	5.57 ±0.23	5.35 ±0.30	5.81 ±0.15	5.64 ±0.22	5.83 ±0.31	5.80 ±0.24	6.23 ±0.31	5.72 ±0.25	5.87 ±0.20	6.1 ±0.30	6.15 ±0.32
Phosphorus (mg/ g)	18.35±0.42	16.69±0.41	15.11 ±0.45	14.60 ±0.46	13.35 ±0.47	17.81 ±0.44	17.28 ±0.42	17.81 ±0.40	17.25 ±0.44	17.9 ±0.48	17.21 ±0.40	17.45 ±0.46	17.30 ±0.45
Calcium (mg/ g)	32.98±0.51	28.65±0.40	27.36 ±0.47	25.60 ±0.44	24.75 ±0.41	31.89 ±0.45	32.06 ±0.46	32.11 ±0.43	32.40 ±0.49	31.78 ±0.44	32.03 ±0.41	32.12 ±0.48	32.10 ±0.46

TABLE 5 Growth performance and feed utilization efficiencies in *L. rohita* fingerlings fed experimental diets for 100 days (mean±SD, *n* = 3)

Parameters	*RD*	*Diets with Raw soybean meal*				*Diets with soybean meal fermented with LRF5*				*Diets with soybean meal fermented with CCF2*			
		S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*	*S10*	*S11*	*S12*
Initial weight (g)	3.35 ±0.06^{a}	3.32 ±0.08^{a}	3.31 ±0.07^{a}	3.32 ±0.07^{a}	3.31 ±0.09^{a}	3.32 ±0.08^{a}	3.31 ±0.07^{a}	3.32 ±0.07^{a}	3.32 ±0.08^{a}	3.31 ±0.08^{a}	3.33 ±0.07^{a}	3.35 ±0.09^{a}	3.34 ±0.08^{a}
Final weight (g)	9.10 ±0.19^{a}	8.45 ±0.18^{b}	7.96 ±0.14^{c}	7.35 ±0.13^{d}	6.98 ±0.17^{d}	8.62 ±0.24^{b}	9.12 ±0.11^{a}	9.54 ±0.14^{a}	9.18 ±0.09^{a}	8.56 ±0.14^{b}	8.98 ±0.14^{b}	9.20 ±0.13^{a}	8.87 ±0.16^{b}
Weight gain (%)	171.64 ±2.11^{b}	154.51 ±1.76^{d}	138.32± 2.00^{f}	120.05± 1.82^{g}	108.98± 1.68^{h}	158.08± 1.09^{d}	173.05± 1.29^{b}	185.62± 1.06^{a}	174.85± 1.96^{b}	156.28± 1.95^{d}	168.86± 1.94^{c}	175.44± 1.68^{b}	165.56± 1.47^{c}
FCR	1.27 ±0.03^{e}	1.55 ±0.04^{d}	1.81 ±0.04^{c}	2.20 ±0.03^{b}	2.41 ±0.03^{a}	1.41 ±0.03^{e}	1.26 ±0.02^{f}	1.17 ±0.02^{f}	1.29 ±0.02^{e}	1.55 ±0.03^{d}	1.33 ±0.03^{e}	1.28 ±0.03^{e}	1.41 ±0.03^{e}
SGR	0.99 ±0.02^{a}	0.93 ±0.01^{b}	0.87 ±0.01^{c}	0.79 ±0.01^{d}	0.74± 0.02^{d}	0.95 ±0.01^{b}	1.01 ±0.02^{a}	1.05 ±0.02^{a}	1.017 ±0.01^{a}	0.947 ±0.01^{b}	0.995 ±0.01^{a}	1.019 ±0.01^{a}	0.982 ±0.01^{a}
PER	2.17 ±0.04^{b}	1.81 ±0.04^{e}	1.54 ±0.03^{f}	1.26 ±0.03^{g}	1.15 ±0.04^{h}	1.98 ±0.03^{d}	2.20 ±0.02^{b}	2.37 ±0.03^{a}	2.16 ±0.02^{b}	1.82 ±0.03^{e}	2.09 ±0.03^{c}	2.17 ±0.02^{b}	1.96 ±0.03^{d}
ANPU (%)	46.36 ±0.79^{b}	35.55 ±0.72^{d}	29.66 ±0.60^{e}	23.64 ±0.55^{f}	21.40 ±0.70^{f}	35.59 ±0.55^{d}	45.78 ±0.40^{b}	52.34 ±0.82^{a}	46.19 ±0.33^{b}	36.68 ±0.57^{d}	43.51 ±0.54^{c}	47.37 ±0.49^{b}	42.40 ±0.63^{c}

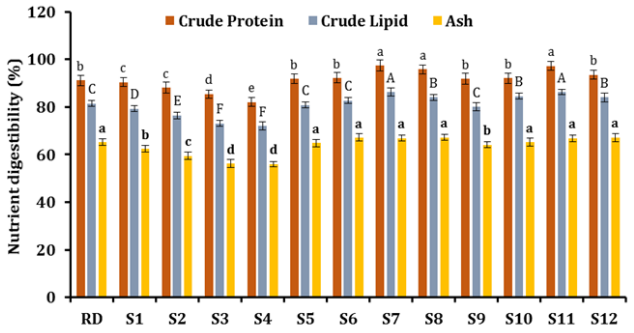

FIGURE 1 Nutrient digestibility (%) of *Labeo rohita* fingerlings. Data are mean value of three replicates ± SE; Error bars show deviation among three replicates; Means with different letters are significantly different (P<0.05).

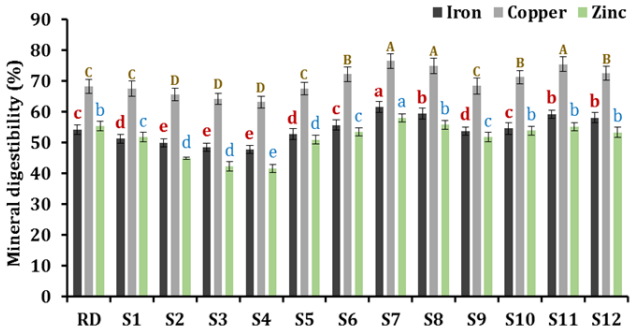

FIGURE 2A Iron, copper and zinc digestibility in *Labeo rohita* fingerlings. Data are mean value of three replicates ± SE; Error bars show deviation among three replicates; Means with different letters are significantly different (P<0.05).

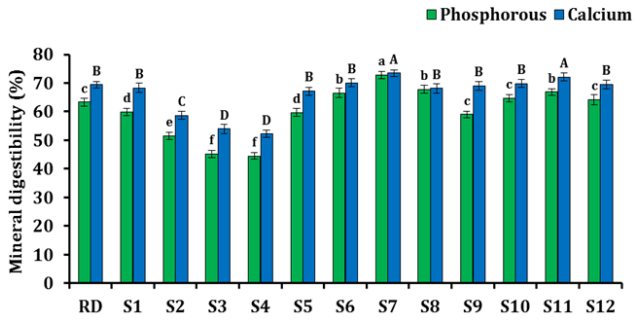

FIGURE 2B Phosphorous and calcium digestibility (%) in *Labeo rohita* fingerlings. Data are mean value of three replicates ± SE; Error bars show deviation among three replicates; Means with different letters are significantly different (P<0.05).

4 | DISCUSSION

In *L. rohita* microbial phytase supplementation in the diet has been reported to improve phosphorus and crude protein digestibility (Baruah *et al*. 2007). But information about the probable nutritional benefit of incorporation of fermented SBM in the diets of *L. rohita* is lacking. Compared to phytase supplementation in fish feed, which only degrades the phytic acid, microbial fermentation has been shown to degrade the indigestible components as well as ANFs in plant ingredients, transform complex polysaccharides to simple forms and also eliminate large peptides (Feng *et al*. 2007).

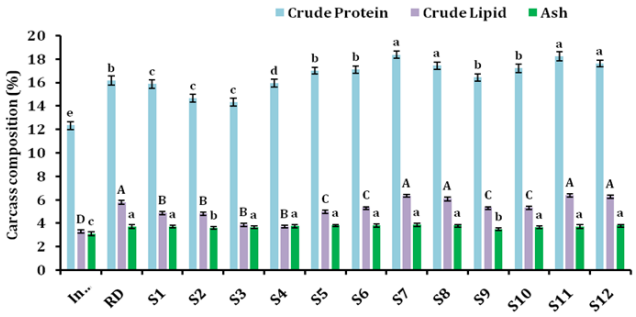

FIGURE 3 Proximate carcass composition (% wet weight) of *Labeo rohita* fingerlings at the start and end of the feeding trial. Data are mean value of three replicates ± SE; Error bars show deviation among three replicates; Means with different letters are significantly different (P<0.05).

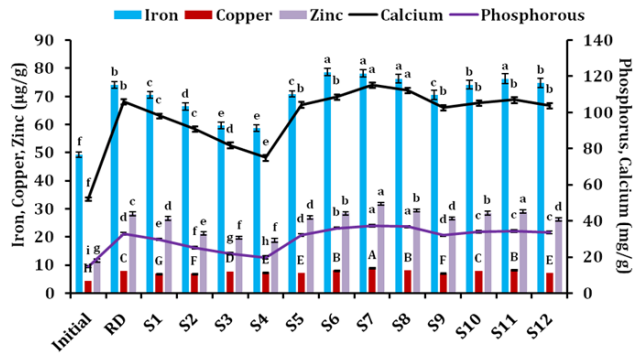

FIGURE 4 Mineral composition of the carcass of *Labeo rohita* fingerlings at the start and end of the feeding trial. Data are mean value of three replicates ± SE; Error bars show deviation among three replicates; Means with different letters are significantly different (P<0.05).

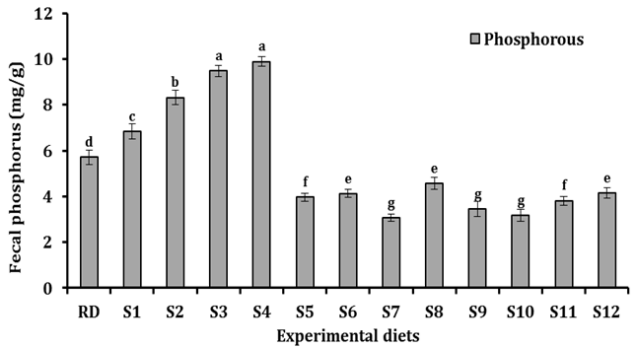

FIGURE 5 Fecal phosphorus concentration of *Labeo rohita* fingerlings. Data are mean value of three replicates ± SE; Error bars show deviation among three replicates; Means with different letters are significantly different (P<0.05)

Mukhopadhyay and Ray (2001) also reported that through fermentation with *Lactobacillus acidophilus*, inclusion of sesame seed meal in *L. rohita* diets could be increased to 400 g/ kg from 200 g/ kg for raw meal. Similarly, fermentation of duckweed, *Lemna polyrhiza* leaf meal by *Bacillus* sp. increased its inclusion limit in carp diets to 30% from 10% in raw meal (Bairagi *et al*. 2002). Incorporation of other fermented plant ingredients like, linseed, black gram, grass pea, duckweed leaf meal and sesame significantly improved the growth performance of *L. rohita* fingerlings in comparison to raw meal (Mukhophadhyay and Ray 2001; Bairagi *et al*. 2002; Ramachandran *et al*. 2005; Ramachandran and Ray 2007; Roy *et al*. 2014). It has also been reported that fermented fish offal, mustard oil cake and rice bran mixture could replace 50% of fish meal from the diets of *L. rohita* and stinging catfish, *Heteropneustes fossilis* (Mondal *et al*. 2007, 2008).

The replacement level of fishmeal with plant protein varies for different fish species and also depends on the experimental conditions. In general, high substitutions of fishmeal by plant protein sources have resulted in poor growth in fish (Abdel-Warith *et al*. 2013). It has been reported that omnivorous freshwater fishes grew well when defatted SBM was used as the sole protein source if the limiting essential amino acids were supplied (Viola *et al*. 1982). El-saidy and Gaber (2003) reported that a diet with 55% SBM supplemented with 0.5% L-lysine can totally replace fishmeal in the diet of Nile tilapia, *Oreochromis nilotica* fingerlings. Similar result was also reported by Furuya *et al*. (2004). They concluded that SBM protein is able to replace 100% of fish meal protein in diets for juvenile Nile tilapia by di-calcium phosphate and amino acid (lysine, methionine and threonine) supplementation. In *L. rohita* also SBM has been reported to fully replace fish meal as protein source with methionine and fortified minerals supplementation (Khan *et al*. 2003). It is known that plant proteins, like SBM are deficient in sulphur containing amino acids methionine and cystine, compared to the quantitative amino acid requirements of most fish species (Gatlin 2002). In juvenile cobia, *R. canadum*, 40% of dietary fish meal protein was replaced by SBM without any significant reduction in growth and protein utilization, but increasing the level to 50% had detrimental effect on fish growth (Chou *et al*. 2004). In rainbow trout (*Oncorhynchus mykiss*) diet, 60% of the fish meal protein was successfully replaced by phytase-treated SBM without compromising weight gain or feed efficiency (Yang *et al*. 2011).

The improvement of the apparent mineral digestibility and mineral bioavailability of SBM by the application of phytase has been reported in other studies. Phytate causes mineral deficiency in animals as a result of chelation of metal ions which decrease their bioavailability. Similar improvement in mineral digestibility by phytase supplementation has been reported in European seabass, *Dicentrarchus labrax* (Oliva-Teles *et al*. 1998) rainbow trout (Cheng 2004), striped bass, *Morone saxatilis* (Papatryphon and Soares 2001), Atlantic salmon (Storebakken *et al*. 1998), common carp (Nwanna and Schwarz 2007) and Australian catfish, *Tandanus tandanus* (Huynh and Nugegoda 2011). Phytate is also known to reduce the bioavailability of protein components by forming phytate–protein and protein–mineral complexes which are resistant to proteolytic digestion and results in lowering the gastrointestinal absorption of protein (Roy *et al*. 2014). This may be the reason that the digestibility of protein as well as minerals was decreased with increasing level of raw SBM in the experimental diet. Fermentation of SBM by *B. cereus* resulted in significant improvement (P<0.05) of crude protein digestibility by *L. rohita* fingerlings compared to raw meals. In other fish species, like Japanese flounder (Kader *et al*. 2012), juvenile black sea bream, (Azarm and Lee 2014), Atlantic salmon (Refstie *et al*. 2005) and grouper, *Epinephelus coioides* (Zhuo *et al*. 2014) also fermentation has been reported to improve the nutritional value and digestibility of SBM.

The phosphorus and calcium digestibility by *L. rohita* fingerlings fed diet S8 was low than the fingerlings fed diet S7, which indicates that inclusion of SBM at more than 45% level in the *L. rohita* diet reduce the digestibility. This may be due to the decreased amounts of ANFs that were not degraded by fermentation and accumulate in such level in diet S8 to hamper the nutrient utilization and digestibility. SBM generally contains high levels of various ANFs like trypsin inhibitor, phytic acid, oligosaccharides and non-starch polysaccharides (NSP). While trypsin inhibitor and phytic acid were degraded by microbial fermentation, the oligosaccharides and NSPs might not have been completely degraded. The oligosaccharides and NSPs have the ability to bind to bile acids and obstruct the action of digestive enzymes and subsequently movement of substrates in the intestine (Francis *et al*. 2001). The crude protein and crude lipid contents of fish carcass of the groups fed RD, S7 and S8 were significantly better (P<0.05) than other groups. The calcium and phosphorus deposition also significantly (P<0.05) improved in the fish fed diets S7 and S8 compared to other group. These results demonstrate that fermented SBM can effectively replace up to 100% of fishmeal protein in the diets of *L. rohita* fingerlings.

The fermentation of SBM was associated with decreased faecal release of phosphorus (P) by the fingerlings. Despite increased inclusion of fermented SBM in *L. rohita* diet, significant (P<0.05) reduction in faecal P was observed in comparison to raw SBM fed groups. In a recent

study, Roy *et al*. (2014) also reported decreased faecal phosphorus output with increased incorporation of fermented sesame oilseed meal in *L. rohita* diets. The digestibility of P in *L. rohita* fingerlings was reported to increase with increased supplementation of Natuphos phytase, although the relationship was not linear and maximum bone mineralization was found with 750 U phytase/kg diet (Barua *et al*. 2007). In juvenile rainbow trout (Yang *et al*. 2011), Tra catfish, *Pangasianodon hypophthalmus* (Hung *et al*. 2015), yellow catfish, *Pelteobagrus fulvidraco* (Zhu *et al*. 2014) and red sea bream, *Pagrus major* (Biswas *et al*. 2007) phytase supplementation was reported to increase apparent digestibility coefficient of phosphorus with reduced phosphorus discharge. The significantly (P<0.05) higher faecal P release by the fingerlings fed reference diet compared to S7, can be attributed to the significantly high amount of phosphorus in fish meal than SBM (NRC 1993) as well as fermentation of the SBM by the bacterial strain LRF5.

5 | CONCLUSION

Fermentation of the SBM with phytase producing fish gut bacterial strains, *Bacillus cereus* LRF5 and *Staphylococcus caprae* CCF2 improved its nutritional value by degrading the anti-nutritional factors and increasing the bioavailability of minerals which led to the increased inclusion of SBM in *L. rohita* diet without compromising growth. Increased use of naturally occurring minerals in plant ingredients reduces the need for mineral supplements in formulated diets and results in decreased elimination of minerals in feces. Thus, preparation of fish feed by fermented SBM by phytase-producing fish gut bacterial strains may be expected to provide both economic and environmental benefits through decreased expenditures on supplemental minerals and mineral outputs to the aquatic ecosystem.

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CONTRIBUTION OF THE AUTHORS

SKD Isolation of bacterial culture, fermentation and biochemical analysis; **AN** diet preparation and fish rearing; **GB** data analysis; **GB & AKR** manuscript preparation.