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Nutritional evaluation of groundnut oil cake in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings after solid state fermentation with a tannase producing yeast, *Pichia kudriavzevii* (GU939629) isolated from fish gut

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ABSTRACT

De-oiled groundnut oil cake (GOC) was bio-processed through solid state fermentation (SSF) for 15 days at 34 ± 1 °C by a tannase producing yeast, *Pichia kudriavzevii* (GU939629) isolated from the gut of a freshwater carp, *Cirrhinus cirrhosus*. SSF of GOC was effective in significantly ($P < 0.05$) reducing crude fibre content and antinutritional factors (e.g., tannin, phytic acid and trypsin inhibitor), whereas enhancing crude protein, lipid, ash, total free amino acids and fatty acids. Bio-processing revealed significant increase in the levels of argenine, histidine, isoleusine, lysine, methyonine and threonine in the GOC. Eight isonitrogenous (37.5%) and isocaloric (18.8 kJ g^{-1}) experimental diets were prepared incorporating raw (R1–R4) and fermented (F1–F4) GOC at 20%, 30%, 40% and 50% levels by weight replacing fishmeal and other feed ingredients into a fishmeal-based reference diet (RD) and fed to rohu, *Labeo rohita*, fingerlings (initial weight 3.24 ± 0.11 g) for 80 days. In general, diets with SSF-processed GOC produced significantly better performance than the raw GOC in terms of growth, feed utilization, nutrient digestibility, carcass composition and digestive enzyme activity. Rohu fingerlings fed the diet F3 containing 40% fermented GOC resulted in best performance in terms of weight gain (final weight 7.08 ± 0.06 g), specific growth rate (% day^{-1}), protein efficiency ratio and apparent net protein utilization. Highest protein (14.93 ± 0.51%) and lipid deposition in the carcass were also recorded in the group of fish reared on diet F3. In comparison to the FM based reference diet, the present study explored the possibility of replacing 45–55% of FM through incorporation of 40–50% (w/w) SSF-processed GOC in the diets for rohu fingerlings.

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1. Introduction

Price hike and scarcity of fish meal (FM) have necessitated replacement of FM in aqua-feeds with plant feedstuffs to sustain fish farming practices by means of reducing feed cost and environmental impact (Kumar et al., 2010; Hardy, 2010). A variety of oil cakes (*e.g.*, groundnut, sesame, linseed, soybean *etc.*) have been evaluated as plant-derived protein sources in carp diets (Hossain and Jauncey, 1989; Hossain et al., 2001; Mazurkiewicz, 2009). However, incorporation of plant protein at higher levels and/or complete substitution of animal proteins by plant proteins have resulted in poor growth and feed efficiency (Dabrowski et al., 1986; Lim, 1992), which is chiefly attributed to the presence of anti-nutritional factors (ANFs) and improper amino acid balance (Francis et al., 2001; Lim and Dominy, 1989). The endogenous plant-derived ANFs (*e.g.*, tannin, phytic acid, trypsin inhibitor, lectins, saponins *etc.*) are reported to hinder bio-availability and digestibility of the nutrients. Perhaps, the most common among the plant derived ANFs are tannins (Francis et al., 2001; Mandal and Ghosh 2010a), which are considered as the secondary compounds of various chemical structures widely occurring in the plant kingdom (Hernes and Hedges, 2000). Tannins are believed to interfere with protein and dry matter digestibility by inhibiting protease and forming indigestible complexes with dietary protein

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Table 1
Proximate composition and amino acid composition (on % dry matter basis) of raw and fermented groundnut oil cake.

Nutrients	Raw GOC	Fermented GOC	% Increase (↑)/reduction (↓)
Proximate composition			
Moisture	$8.22^b \pm 0.3$	$2.7^a \pm 0.2$	–
Dry matter[a]	$91.78^a \pm 0.3$	$97.3^b \pm 0.2$	–
Crude protein	$41.73^a \pm 0.6$	$46.37^b \pm 0.67$	11.1↑
Crude lipid	$7.7^a \pm 0.25$	$8.6^b \pm 0.36$	11.7↑
Ash	$5.76^a \pm 0.19$	$6.75^b \pm 0.17$	14.7↑
Crude fibre	$4.61^b \pm 0.33$	$2.37^a \pm 0.25$	48.6↓
NFE	$31.99^a \pm 1.32$	$33.22^b \pm 1.09$	–
Total carbohydrate[b]	$36.6^b \pm 0.99$	$35.58^a \pm 0.87$	–
Gross energy (kJ g^{-1})[c]	$19.12^a \pm 0.4$	$20.18^b \pm 0.2$	–
Total free amino acid	$0.85^a \pm 0.04$	$1.14^b \pm 0.04$	34.1↑
Total free fatty acid	$1.07^a \pm 0.06$	$1.7^b \pm 0.31$	58.9↑
Tannin	$2.7^b \pm 0.04$	$0.16^a \pm 0.02$	94.1↓
Phytic acid	$2.55^b \pm 0.07$	$1.05^a \pm 0.05$	58.8↓
Trypsin inhibitor	1.23 ± 0.08	0.41 ± 0.04	66.6↓
Amino acid composition			
Argenine	$4.6^a \pm 0.11$	$4.87^b \pm 0.15$	5.4↑
Histidine	$1.0^a \pm 0.08$	$1.3^b \pm 0.05$	23.1↑
Isoleusine	$1.49^a \pm 0.07$	$1.86^b \pm 0.06$	20↑
Leusine	$2.87^a \pm 0.15$	$2.83^a \pm 0.05$	1.4↓
Lysine	$1.69^a \pm 0.04$	$1.96^b \pm 0.06$	13.8↑
Methyonine	$0.53^a \pm 0.05$	$0.71^b \pm 0.05$	25.4↑
Phenylalanine	$2.2^b \pm 0.07$	$1.98^a \pm 0.08$	10↓
Threonine	$1.18^a \pm 0.04$	$1.33^b \pm 0.04$	11.3↑
Tryptophan	$0.48^b \pm 0.02$	$0.43^a \pm 0.03$	10.4↓
Valine	$1.86^b \pm 0.09$	$1.71^a \pm 0.05$	8.1↓

Values are means ± SE of three determinations. Values with the same superscript in the same row are not significantly different ($P < 0.05$) from each other.
[a] Includes organic matter and ash. Dried to constant weight at 100 °C.
[b] Total carbohydrate = NFE + Crude fibre.
[c] kJ = Kilojoule (Unit of energy).

(Krogdahl, 1989; Becker and Makkar, 1999). High levels of tannin in feed have been shown to encompass adverse effects on herbivorous and omnivorous fishes (Olvera et al., 1988; Al-Owafeir, 1999; Becker and Makkar, 1999). Hossain and Jauncey (1989) observed poor growth response in common carp (*Cyprinus carpio*) fed diets containing 0.57% and 1.14% tannins. The reason behind this growth inhibitory effect of tannin has been attributed to the inhibition of digestive enzymes by tannin. Inhibition of carp digestive enzymes by plant tannin has also been documented (Maitra and Ray, 2003; Mandal and Ghosh, 2010b). Although, the extents of these inhibitory effects varied in different fish species studied and also plant material used. Consequently, it has been hypothesized that tannins can seriously reduce the feed nutritional value of different plant-derived materials unless deactivated (Liener, 1989; Hossain and Jauncey, 1989). Therefore, processing of the plant ingredients through physical methods like heat inactivation and water soaking, or bio-inactivation using specific enzymes (*i.e.*, tannase) has been suggested for elimination of tannin in the plant feedstuffs to replace FM successfully (Mandal and Ghosh, 2013a,b).

Tannase (tannin acyl hydrolase, E.C.3.1.1.20) is an inducible, extracellular enzyme that catalyzes the breakdown of ester and depside bonds (ester bonds involving a phenolic OH) present in hydrolysable tannins (Mandal and Ghosh, 2013a). Although tannins are toxic and bacteriostatic compounds (Scalbert, 1991), tannase is mainly produced by a group of tolerant microorganisms, such as fungi, yeast and bacteria (Lekha and Lonsane, 1997). Tannase-producing microbiota in the gastrointestinal micro-environment of ruminants and other animals has been evidenced (Nelson et al., 1995; Goel et al., 2005). However, tannase-producing microbiota in the gut of freshwater fish has been documented recently (Mandal and Ghosh, 2013b). Preceding studies reported beneficial aspects of gut associated extracellular enzyme-producing microbiota in fish with regard to nutrition (Ray et al., 2012). Although, fermentative nutrition in aquatic animals is less understood (Esakkiraj et al., 2009), *in vitro* processing of raw plant ingredients through solid state fermentation (SSF) by exo-enzyme producing autochthonous fish gut microorganisms have been shown to reduce the ANFs and increase nutrient bioavailability because of the action of enzymes produced by the microorganisms (Bairagi et al., 2004; Ramachandran et al., 2005; Ramachandran and Ray, 2007; Saha and Ghosh, 2013; Mandal and Ghosh, 2013b).

Biotransformation of crop residues and biological detoxification of agro-industrial wastes are some of the economic applications of SSF as a way of nutrient recycling (Pandey et al., 2001). SSF can be of special interest in those processes where the crude fermented products may be used directly (Tengerdy, 1998). De-oiled groundnut oil cake (GOC) has the potential to be used as FM replacer as it is rich in protein (So and Si, 2007). Besides trypsin inhibitor and phytic acid as the ANFs (Nyina-Wamwiza et al., 2010), GOC contains high amount (27.03 ± 0.35 mg g^{-1} dry weight) of tannin (Mandal and Ghosh, 2010a). Appropriate utilization of the de-oiled GOC left after the extraction of oil is also vital for the viability and sustainability of the edible groundnut oil production industry. In the present study, heat-treated (autoclaved) GOC was processed *in vitro* through SSF using a tannase producing yeast, *Pichia kudriavzevii* (GU939629) isolated from the gut of a freshwater fish, *Cirrhinus cirrhosus* (Mandal and Ghosh, 2013a). Further, a feeding experiment was designed to evaluate the nutritive value of raw (dried ground GOC) and SSF-processed GOC to look into the possible utilization of the bio-processed GOC as a partial substitute for FM in the formulated diets for *L. rohita* fingerlings.

2. Materials and methods

2.1. Microorganism used

The tannase producing yeast, *P. kudriavzevii* (GenBank Accession No. GU939629) was isolated from the gut of a freshwater teleost, *C. cirrhosus* (Mandal and Ghosh, 2013a). The culture was grown

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

Parameters	RD	Diets with raw GOC				Diets with GOC fermented with *P. kudriavzevi*			
		R1	R2	R3	R4	F1	F2	F3	F4
Ingredient composition									
Fish meal	40.0	32.0	28.0	22.0	18.0	32.0	28.0	22.0	18.0
Mustard oil cake	13.5	7.00	5.00	6.00	7.00	4.00	1.00	0.50	–
Ricebran	43.5	38.0	34.0	29.0	22.0	41.0	38.0	34.5	29
Groundnut oil cake	–	20.0	30.0	40.0	50.0	20.0	30.0	40.0	50.0
Codliver oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Premix[a]	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	96.4 ± 0.58	96.1 ± 0.64	95.53 ± 0.66	95.3 ± 0.78	96.37 ± 0.46	96.4 ± 0.61	97.63 ± 0.24	98.03 ± 0.26	97.17 ± 0.58
Crude protein	37.17 ± 1.04	37.1 ± 0.79	37.93 ± 0.83	37.43 ± 0.43	38.1 ± 0.41	36.83 ± 0.61	37.67 ± 0.49	36.83 ± 0.84	37.8 ± 0.61
Crude lipid	7.57 ± 0.29	6.97 ± 0.24	7.37 ± 0.33	7.23 ± 0.18	7.73 ± 0.27	7.6 ± 0.38	7.43 ± 0.27	7.8 ± 0.35	8.13 ± 0.41
Ash	15.67 ± 0.62	14.1 ± 0.61	13.57 ± 0.43	11.9 ± 0.55	10.37 ± 0.54	14.87 ± 0.32	13.83 ± 0.24	13.17 ± 0.43	12.3 ± 0.32
Crude fibre	15.63 ± 0.35	13.63 ± 0.65	12.93 ± 0.26	11.63 ± 0.43	10.07 ± 0.41	14.1 ± 0.4	13.2 ± 0.44	12.03 ± 0.32	10.4 ± 0.46
NFE[b]	26.87 ± 0.43	27.67 ± 0.41	28.37 ± 0.35	29.33 ± 0.35	29.8 ± 0.4	27.4 ± 0.32	28.2 ± 0.35	29.47 ± 0.33	31.37 ± 0.41
Gross energy ($kJ\,g^{-1}$)[c]	18.94 ± 0.13	18.48 ± 0.21	18.83 ± 0.16	18.6 ± 0.23	18.77 ± 0.19	18.7 ± 0.21	18.79 ± 0.24	18.78 ± 0.17	19.18 ± 0.31
Tannin	–	$0.62^{e} \pm 0.02$	$0.87^{f} \pm 0.03$	$1.13^{g} \pm 0.04$	$1.45^{h} \pm 0.05$	$0.04^{a} \pm 0.01$	$0.05^{b} \pm 0.01$	$0.07^{c} \pm 0.01$	$0.09^{d} \pm 0.01$
Phytic acid	–	$0.6^{d} \pm 0.02$	$0.87^{e} \pm 0.02$	$1.13^{f} \pm 0.02$	$1.39^{g} \pm 0.04$	$0.25^{a} \pm 0.02$	$0.38^{b} \pm 0.02$	$0.49^{c} \pm 0.03$	$0.63^{d} \pm 0.03$
Trypsin inhibitor (mg/g)	–	$2.72^{d} \pm 0.09$	$3.96^{e} \pm 0.56$	$5.11^{f} \pm 0.08$	$6.44^{g} \pm 0.07$	$0.96^{a} \pm 0.04$	$1.38^{b} \pm 0.03$	$1.98^{c} \pm 0.04$	$3.02^{d} \pm 0.28$

Values are means ± SE of three determinations. Values with the same superscript in the same row are not significantly different ($P < 0.05$) from each other.
[a] Vitamin and mineral mixture (Supradyn, Bayer Consumer Care AG, Basel, Switzerland).
[b] Nitrogen-free extract.
[c] Kilojoule (Unit of energy), RD = reference diet.

and maintained on selective tannic acid agar (TA) medium (Bradoo et al., 1996; Mondal et al., 2001) and preserved at 4 °C.

2.2. Bio-processing of groundnut oil cake through solid state fermentation

Inoculum was prepared in 250 ml conical flasks containing 45 ml selective tannic acid broth inoculated with 5 ml of a 24 h culture. The flasks were then incubated at 35 °C for 48 h and the inoculum thus prepared was used for the inoculation of the solid substrate medium. Total viable cell count of the yeast strain in the 48 h broth culture was determined by Colony Count Technique (Mandal and Ghosh, 2013b) and the cell density was found as 7.5×10^7 cells ml^{-1}.

GOC (de-oiled) was purchased from local market, dried at 80 °C in hot air oven, grounded, passed through a fine mesh sieve (400 μm in diameter) to ensure homogeneity and the powder was used for fermentation. The powdered GOC was moistened with 60% w/v liquid basal medium containing ($g\,l^{-1}$) K_2HPO_4 (0.5); KH_2PO_4 (0.5); $MgSO_4$ (0.5); NH_4Cl (1), $CaCl_2$ (0.01) having a pH of 6.5 and autoclaved at 121 °C and 15 lbs for 20 min for sterilization. The sterilized oil cake was fermented with *P. kudriavzevii* at the rate of 3×10^7 yeast cells g^{-1} of dried oil cake for 15 days at 34 °C in an incubator (Mandal and Ghosh, 2013b).

2.3. Experimental diets

The fermented GOC was dried and analyzed for proximate composition, ANFs and amino acid composition prior to incorporation into the diets (Table 1). Eight sets of isonitrogenous and isocaloric experimental diets were formulated incorporating raw (R1–R4) and fermented (F1–F4) GOC at 20%, 30%, 40% and 50% levels by weight replacing FM, mustard oil cake and rice bran (Table 2). A diet with FM as the main protein source and without GOC was used as the reference diet (RD). The ingredients were finely powdered and sieved to obtain uniform particle size (<400 μm) and mixed thoroughly in proportion mentioned in Table 2. To each of the formulated diets, 1% chromic oxide was added as an external digestibility marker. A vitamin–mineral premix (Supradyn, Bayer Consumer Care AG, Basel, Switzerland) and cod liver oil were added (1% each) to the diets before pelletization. The mixture was made to dough using lukewarm water and 0.5% carboxy–methyl–cellulose (CMC) as binder. The dough was passed through an electrically operated semi-automatic pelletizer. The pellets (1.5 mm in diameter) thus obtained were initially sun-dried for 6 h and further in a hot air oven at 60 °C for 96 h. The dried pellets were crumbled and packed in airtight plastic bags to store in a refrigerator until use. Proximate composition of the diets is mentioned in Table 2.

2.4. Experimental design

The feeding trial was conducted under laboratory conditions, in 27 glass aquaria, each containing 90 l of water, for 80 days, with continuous aeration. Rohu, *Labeo rohita*, fingerlings were obtained from a local fish seed dealer and acclimatized for 15 days. The fingerlings (mean individual weight of the 405 fingerlings 3.24 ± 0.11 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 once daily at 10.00 h at a feeding rate of 3% (w/w) of the total body weight per day as described elsewhere (Saha and Ghosh, 2013; Roy et al., 2014). The daily ration was adjusted every tenth day after weighing the fish from each replicate. The uneaten feed was siphoned off after 6 h of each feeding, and oven dried at 100 °C for 24 h to calculate the feed conversion ratio. To determine digestibility, the faecal samples released by the fish were collected from each aquarium daily following the “immediate pipetting” method outlined by Spyridakis et al. (1989). Five fish from each aquarium were sampled at the termination of the feeding experiment and analysed for whole body composition (on wet weight basis). The ranges of water quality parameters were: temperature 23–29 °C; pH 6.5–7.8; and dissolved oxygen 6.5–7.9 $mg\,l^{-1}$.

2.5. Chemical analyses

Proximate composition of feed ingredients, experimental diets, faecal samples and fish carcass were analysed using the standard procedures described by the Association of Official Analytical Chemists (AOAC, 2005); moisture content was determined by oven

drying for 24 h at 100 ± 5 °C; crude protein ($N \times 6.25$), by the micro-Kjeldahl digestion and distillation after acid digestion, using a Tecator Digestion System together with Kjeltec 1026 Distilling Unit (Tecator, Sweden); lipids, by extracting the residue with petroleum ether (40–60 °C) for 8 h in a Soxhlet apparatus; crude fibre, as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH using Fibertec System 2021 (Foss Tecator, Sweden); ash, by ignition of samples 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, ash, crude fibre, and moisture and subtracting this from 100 (Maynard et al., 1979). Gross energy of the diets was measured with a bomb-calorimeter (Lab-X, Kolkata, India). Total free amino acids and free fatty acids in the raw and fermented groundnut oil cake were estimated according to Moore and Stein (1948) and Cox and Pearson (1962), respectively. The amino acid compositions of raw and fermented substrates were determined using an automated amino acid analyzer (Shimadzu-10 AS, Japan) after hydrolyzing the samples with 6 M HCl at 110 °C for 24 h (Bassler and Buchholz, 1993). The sulphur containing amino acids were oxidized using performic acid before the acid hydrolysis. The tryptophan contents of the above mentioned samples were determined spectrophotometrically by the method of Pinter-Szakacs and Molnar-Perl (1990).

Chromic oxide levels in the diets and in the faecal samples were estimated spectrophotometrically (Bolin et al., 1952). Proximate analyses of whole body of fish were done both at the beginning and termination of the feeding experiment (on wet weight basis) following the methods stated earlier. The water quality parameters were monitored following the standard procedures (APHA, 1995). Average live weight gain (%), 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). The apparent dry matter digestibility (ADD) and apparent digestibility of nutrients were calculated according to Cho et al. (1982).

Among ANFs, tannin content was determined using Folin–Denis reagent (Schanderi, 1970). Tannin was extracted in boiling distilled water (30 min). After centrifugation (2500 × *g*, 20 min), the supernatant was collected, mixed with Folin–Denis reagent, alkalinized by 15% (w/v) sodium carbonate and kept in the dark (30 min) at room temperature. The absorbance of the solution was read at 700 nm using a spectrophotometer (Shimadzu UV/VIS-1800, Kyoto, Japan) and the concentration of tannin in the extract was determined using pure tannic acid (Merck, Mumbai, India) as a standard. Phytic acid was extracted using 2.4% HCl, with continuous shaking (220 rpm) for 16 h. After extraction, the suspension was centrifuged (10,000 × *g*, 20 min) and the supernatant was used for phytate determination using modified Wade reagent (0.03% $FeCl_3$, $6H_2O$ + 0.3% sulfosalicylic acid) following Vaintraub and Lapteva (1988). Sodium phytate (phytic acid sodium salt, HiMedia Laboratories Pvt. Ltd., Mumbai, India) was used as the standard. Benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) was used as a substrate to determine trypsin inhibitor activity according to Smith et al. (1980) with minor modification. Trypsin inhibitor was extracted with phosphate buffer (0.15 M, pH 8.1, 4 °C). The crude extract was treated with bovine trypsin (0.004%, in 0.025 M glycine HCI buffer), added with the substrate (BAPNA) and incubated under optimized condition (37 °C, 60 min). The release of the yellow coloured *p*-nitroaniline was measured spectrophotometrically (410 nm) to determine the residual trypsin activity. The result was expressed as mg trypsin inhibited per g of sample.

2.6. Determination of digestive enzyme activity

Three fish from each aquaria (for each replicate) were dissected on an ice tray to remove the gastrointestinal (GI) tract in order to determine the digestive enzyme activities prior to commencement and at the termination of the feeding trials. A 10% homogenate (w/v) of the GI tract was prepared with chilled phosphate buffer (0.1 M, pH 7.4) containing 0.89% sodium chloride. The homogenate was centrifuged (10,000 × *g*, 30 min, 4 °C) and the supernatant was used as the enzyme extract. Protein content of the extract was determined following the method described by Lowry et al. (1951) using bovine serum albumin (BSA) as standard. Amylase activity was determined following Bernfeld (1955). The mixture of enzyme extract (1 ml) and substrate (1% soluble starch) was incubated at 37 °C for 20 min. Afterwards, 2 ml of dinitrosalicylic acid reagent (DNSA, containing 1% dinitrosalicylic acid, 30% sodium potassium tartrate in 0.4 N NaOH) was added to each tube and kept in a boiling water bath for 5 min. The tubes were then cooled and intensity of the colour developed was read at 540 nm. Blanks were obtained by adding DNSA prior to incubation. Amylase activity was expressed as mg maltose liberated h^{-1} mg $protein^{-1}$. Protease activity was measured as described by Moore and Stein (1948), using BSA as substrate. The reaction mixture containing enzyme extract (0.1 ml), substrate (0.1 ml, 10 mg ml^{-1} BSA solution) and phosphate buffer (0.5 ml, pH 7.4, 0.1 M) was incubated for 1 h at 37 °C. The reaction was stopped by adding trichloroacetic acid (TCA, 10%) and centrifuged at 4000 × *g* for 10 min. The supernatant (2 ml) was mixed with ninhydrin reagent (1 ml) and heated in a boiling water bath for 20 min, placing a marble on top of each tube. After cooling to room temperature, the developed colour was read at 570 nm in a spectrophotometer (Shimadzu UV/VIS-1800). Blanks were obtained by adding TCA to the substrate prior to incubation. Protease activity was expressed as μg of glycine liberated h^{-1} mg $protein^{-1}$. Lipase activity was measured following the method described by Bier (1955) using olive oil as substrate. The enzyme extract (0.5 ml) was mixed with olive oil emulsion (2.5 ml) in 2% polyvinyl alcohol and calcium chloride (110 mM, 0.5 ml). The reaction mixture was incubated at 37 °C for 1 h with continuous shaking (120 rpm). The reaction was stopped by acetone–ethanol mixture (1:1, 10 ml), few drops of phenolphthalein indicator were added and fatty acid liberated as a result of enzymatic action was titrated with 0.02 N NaOH till the appearance of faint pink colour. One millilitre of 0.02 N NaOH is equivalent to 100 μM of free fatty acid. Blanks were obtained using boiled enzyme. Lipase activity was expressed as μmol of fatty acid liberated h^{-1} mg $protein^{-1}$.

2.7. Microbial culture

For microbial culture, homogenate of the pooled intestinal mucosa of three fish from each aquaria was used after five serial (1:10) dilutions (Beveridge et al., 1991). Diluted samples (0.1 ml) were poured aseptically within a laminar airflow on sterilized Tryptone Soya Agar [(TSA), Himedia, India], to determine the culturable heterotrophic microbial population. To enumerate protease, cellulase, amylase, lipase and tannase producing microbial population, diluted samples (0.1 ml) were poured on peptone gelatine agar (PG), carboxymethylcellulose agar (CMC), starch agar (SA), tributyrin agar (TiA) and TA plates, respectively. Spread plate technique was employed for the purpose. Culture plates were incubated at 30 ± 1 °C for 36 h and examined for development of microbial colonies after the incubation period. It was assumed that the microflora, which had formed colonies on the PG plate, had protease activity. Similarly, the microflora grown on CMC, SA, TiA and TA plates represented cellulase, amylase, lipase and tannase activities, respectively (Ghosh et al., 2002; Ghosh et al., 2002a). Colony numbers per unit sample volume of gut homogenate were determined by multiplying the number of colonies formed on each plate by the reciprocal dilution, (Rahmatullah and Beveridge, 1993) and the data were transformed as log viable counts g^{-1} GI tract (LVC).

2.8. Media composition

TSA medium ($g\,l^{-1}$): pancreatic digest of casein 15; papaic digest of soyabean meal 5; NaCl 5; agar 15; pH 7. PG medium ($g\,l^{-1}$): beef extract 3; peptone 5; gelatin 4; agar 20; pH 7. CMC medium ($g\,l^{-1}$): beef extract 5; peptone 5; NaCl 5; carboxymethylcellulose 2; agar 20; pH 7. SA medium ($g\,l^{-1}$): beef extract 5; peptone 5; NaCl 5; starch (soluble) 2; agar, 20; pH 7. TiA medium ($g\,l^{-1}$): tributyrin-agar 10; peptone 5; agar 15; pH 7. TA medium ($g\,l^{-1}$): tannic acid 5; K_2HPO_4 0.5; KH_2PO_4 0.5; $MgSO_4$ 0.5; NH_4Cl 1, $CaCl_2$ 0.01; agar 20; pH 6.5.

2.9. Data collection

$$\text{Body mass gain (BMG, \%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{Specific growth rate } \left(\text{SGR, \%day}^{-1}\right) = \frac{\text{lnFinal weight(g)} - \text{lnInitial weight(g)}}{\text{Days on trial}} \times 100$$

where ln = natural logarithm.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry weight of feed consumed (g)}}{\text{Increased in wet weight of fish (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain of fish (g)}}{\text{Protein consumed (g)}}$$

$$\text{Apparent net protein utilization (ANPU, \%)} = \frac{\text{Net increase in carcass protein}}{\text{Amount of protein consumed}} \times 100$$

$$\text{Apparent dry matter digestibility (ADD, \%)} = 100 - 100 \times \frac{\text{\% marker in diet}}{\text{\% marker in faeces}}$$

$$\text{Apparent nutrient digestibility (\%)} = 100 - 100 \times \frac{\text{\% marker in diet}}{\text{\% marker in faeces}} \times \frac{\text{\% nutrient in faeces}}{\text{\% nutrient in diet}}$$

2.10. Statistical analysis

Statistical analysis of the data was performed by analysis of variance (ANOVA) followed by Tukey's test following Zar (1999) using SPSS v11 (Kinnear and Gray, 2000) software.

3. Results

3.1. Processing of GOC and experimental diets

Proximate composition and amino acid profile of raw and SSF-processed GOC are presented in Table 1. SSF of GOC brought about decrease in crude fibre content and ANFs (tannin, phytic acid and trypsin inhibitor) significantly ($P < 0.05$), whereas contents of crude protein, lipid, ash, total free amino acids and fatty acids were enhanced as compared to the raw GOC. Amino acid profile revealed significant increment in the levels of argenine, histidine, isoleusine, lysine, methyonine and threonine, although, phenylalanine, tryptophan and valine were decreased due to SSF.

Ingredient composition and proximate analysis of the experimental diets are presented in the Table 2. A comparison of the ANFs in the formulated diets indicated that contents of tannin, phytic acid and trypsin inhibitor were significantly low in the diets containing fermented GOC in comparison to the diets containing raw GOC.

3.2. Growth performance and nutrient utilization

Data regarding growth performance and nutrient utilization by rohu fingerlings fed experimental diets are presented in Table 3. Considerable growth was noticed in fish during the experimental period. In general, Body Mass Gain (%) and SGR (% day^{-1}) of the fish fed bio-processed GOC containing diets were significantly increased ($P < 0.05$) than those of fish fed the raw GOC containing diets. Rohu fingerlings fed diet F3 containing 40% fermented GOC had the highest weight gain, which was significantly different ($P < 0.05$) from all other experimental diet treatments. The highest values for PER and ANPU were recorded in fish fed diet F3. The value of FCR was the lowest for diet F3, and highest for diet R4 containing 50% raw GOC.

3.3. Apparent dry matter and nutrient digestibility

Table 4 presents apparent digestibility of dry matter, protein and lipid in rohu fed experimental diets. Although, the apparent dry matter digestibility (ADD) was highest for the diet F3, no significant ($P < 0.05$) differences were noticed in its values among the other fermented GOC incorporated diets (F1, F2 and F4). Apparent protein digestibility (APD) was also highest for the diet F3, though it did not show any significant difference in APD with the diets F2 and RD. Values of apparent lipid digestibility (ALD) also indicated the highest value for the diet F3, although it was not significantly ($P < 0.05$) different from all other diets except R4 and F1.

3.4. Carcass composition

The proximate carcass compositions of fish fed experimental diets are presented in Table 5. The carcass protein and lipid contents were significantly ($P < 0.05$) higher in experimental fish than the initial levels. An increasing level of raw GOC in the diets was associated with a decrease in carcass protein and lipid contents. Highest protein deposition in the carcass ($14.93 \pm 0.51\%$) was recorded in the group of fish reared on diet F3, though it did not differ significantly with the reference diet. The highest tissue lipid accumulation was also recorded in the fish fed diet F3. Moisture content was lowest in carcass of fish fed diet F3 and it was highest for the diet R4. Fish carcass ash content was the highest in fish fed diet RD, though it was not significantly ($P < 0.05$) different from the fish fed diet F1.

3.5. Digestive enzyme activity

Activities of intestinal protease, amylase and lipase in *L. rohita* fingerlings fed experimental diets are presented in Table 6. Activities of all the three enzymes increased over the initial values in all dietary treatment groups. In general, protease activity was significantly ($P < 0.05$) higher in the fish fed diets containing SSF-processed GOC and RD, as compared to fish fed diets containing raw GOC. The maximum protease and amylase activities were noticed in the fish fed diet F3, though it was not significantly ($P < 0.05$) different from the diets F2 and RD. The highest lipase activity was documented in the fish fed diet F3 and it was significantly different ($P < 0.05$) from all other dietary groups.

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 GOC				Diets with GOC fermented with *P. kudriavzevi*			
		R1	R2	R3	R4	F1	F2	F3	F4
Initial wt (g)	3.22 ± 0.04	3.28 ± 0.06	3.3 ± 0.07	3.32 ± 0.07	3.26 ± 0.05	3.18 ± 0.04	3.32 ± 0.07	3.24 ± 0.05	3.28 ± 0.07
Final wt (g)	$6.28^{d} \pm 0.08$	$5.62^{c} \pm 0.09$	$5.28^{b} \pm 0.06$	$5.1^{ab} \pm 0.07$	$4.98^{a} \pm 0.08$	$6.18^{d} \pm 0.07$	$6.62^{e} \pm 0.06$	$7.08^{f} \pm 0.06$	$6.3^{d} \pm 0.07$
BMG[a] (%)	$95.6^{de} \pm 3.21$	$71.3^{c} \pm 2.68$	$60^{b} \pm 2.23$	$53.6^{a} \pm 1.95$	$52.7^{a} \pm 1.88$	$94.3^{d} \pm 1.96$	$99.4^{e} \pm 2.58$	$118.5^{f} \pm 3.21$	$92.07^{d} \pm 2.31$
Feed intake[b]	$1.9^{a} \pm 0.09$	$1.94^{ab} \pm 0.06$	$1.98^{ab} \pm 0.12$	$2.01^{b} \pm 0.11$	$2.29^{c} \pm 0.14$	$1.99^{a} \pm 0.09$	$1.95^{a} \pm 0.08$	$2.01^{b} \pm 0.08$	$2.03^{b} \pm 0.11$
SGR (% day^{-1})	$0.84^{de} \pm 0.04$	$0.68^{c} \pm 0.03$	$0.59^{b} \pm 0.4$	$0.54^{a} \pm 0.02$	$0.54^{a} \pm 0.03$	$0.83^{de} \pm 0.02$	$0.86^{e} \pm 0.03$	$0.98^{f} \pm 0.04$	$0.81^{d} \pm 0.02$
FCR	$2.45^{b} \pm 0.09$	$3.05^{d} \pm 0.13$	$3.52^{e} \pm 0.16$	$3.85^{f} \pm 0.15$	$4.05^{g} \pm 0.19$	$2.51^{b} \pm 0.08$	$2.39^{b} \pm 0.07$	$2.18^{a} \pm 0.08$	$2.67^{c} \pm 0.09$
PER	$1.1^{f} \pm 0.06$	$0.89^{d} \pm 0.05$	$0.75^{c} \pm 0.03$	$0.69^{b} \pm 0.04$	$0.65^{a} \pm 0.02$	$1.08^{f} \pm 0.05$	$1.11^{f} \pm 0.07$	$1.25^{g} \pm 0.09$	$0.99^{e} \pm 0.04$
ANPU (%)	$20.1^{g} \pm 0.63$	$15.3^{d} \pm 0.48$	$12.9^{c} \pm 0.36$	$11.9^{b} \pm 0.38$	$10.2^{a} \pm 0.29$	$18.9^{f} \pm 0.33$	$21.1^{g} \pm 0.53$	$23.2^{h} \pm 0.55$	$16.8^{e} \pm 0.42$

Mean value with same superscripts in the same row were not significantly different ($P < 0.05$)
[a] Body Mass Gain.
[b] g 100 g^{-1} body weight of fish day^{-1}.

Table 4
Apparent digestibility of dry matter and nutrients in *Labeo rohita* fingerlings fed experimental diets for 80 days. Data are mean value ± SE (n = 3)

Parameters	RD	Diets with raw GOC				Diets with GOC fermented with *P. kudriavzevi*			
		R1	R2	R3	R4	F1	F2	F3	F4
Dry matter	$63.03^{c} \pm 0.51$	$58.99^{b} \pm 0.39$	$56.74^{b} \pm 0.31$	$52.94^{a} \pm 0.18$	$50.54^{a} \pm 0.28$	$62.45^{cd} \pm 0.48$	$63.46^{d} \pm 0.58$	$64.91^{d} \pm 0.55$	$64.57^{d} \pm 0.42$
Protein	$83.03^{cde} \pm 0.96$	$79.0^{bc} \pm 0.84$	$77.72^{b} \pm 0.78$	$72.99^{a} \pm 0.68$	$71.0^{a} \pm 0.58$	$80.99^{bcd} \pm 0.73$	$84.1^{de} \pm 0.91$	$85.8^{e} \pm 0.85$	$82.3^{c} \pm 0.79$
Lipid	$90.28^{ab} \pm 1.06$	$89.12^{ab} \pm 0.96$	$88.61^{ab} \pm 0.78$	$87.5^{ab} \pm 0.91$	$86.18^{a} \pm 0.83$	$89.58^{a} \pm 0.79$	$90.61^{b} \pm 1.12$	$91.5^{b} \pm 1.26$	$90.28^{ab} \pm 0.92$

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

3.6. Gastrointestinal microbiota

Enumeration of culturable heterotrophic (on TSA plate), proteolytic, amylolytic, cellulolytic, lipolytic and tannase producing microorganisms in the intestine of fish fed different experimental diets revealed that number (LVC) of different enzyme producing microorganisms were increased in the fish fed diets containing fermented GOC and decreased in fish fed diets containing raw GOC over their initial concentrations (Table 7). Maximum numbers of culturable heterotrophic, proteolytic, amylolytic, cellulolytic, lipolytic and tannase producing microbiota were recorded in the GI tract of fish fed diet F4.

4. Discussion

The present study demonstrated the suitability of bio-processed GOC (through SSF) as an alternate plant derived protein source in the formulated diets for rohu, *L. rohita* fingerlings. The results obtained from this study clearly indicated that fermented GOC may be incorporated up to 40% level (w/w) in the diet for rohu fingerlings. The fish reared on bio-processed-GOC incorporated diets exhibited better growth and carcass composition than the fish fed similar levels of raw GOC-containing diets. The results of the present experiment also indicated that fermentation with tannase producing yeast *P. kudriavzevii* (GU939629) isolated from fish gut

Table 5
Proximate carcass composition (% wet weight) of experimental fish at the start and end of the 80 day feeding experiment.

Parameters	Initial	RD	Diets with raw GOC				Diets with GOC fermented with *P. kudriavzevi*			
			R1	R2	R3	R4	F1	F2	F3	F4
Moisture	$83.2^{e} \pm 0.15$	$77^{abcd} \pm 0.31$	$78.5^{bcde} \pm 0.27$	$79.9^{cd} \pm 0.07$	$80.3^{de} \pm 0.24$	$81.5^{e} \pm 0.23$	$78^{abcd} \pm 0.13$	$76^{ab} \pm 0.1$	$74.5^{a} \pm 0.16$	$75.5^{ab} \pm 0.13$
Crude protein	$10.6^{a} \pm 0.36$	$14.41^{ef} \pm 0.45$	$13.36^{cd} \pm 0.32$	$13.1^{bc} \pm 0.35$	$12.86^{b} \pm 0.27$	$12.36^{b} \pm 0.22$	$13.95^{de} \pm 0.41$	$14.77^{f} \pm 0.49$	$14.93^{f} \pm 0.51$	$13.64^{cd} \pm 0.38$
Crude lipid	$2.05^{a} \pm 0.06$	$3.14^{d} \pm 0.11$	$3.11^{d} \pm 0.13$	$3.04^{d} \pm 0.09$	$2.86^{c} \pm 0.07$	$2.46^{b} \pm 0.05$	$3.06^{d} \pm 0.1$	$3.45^{e} \pm 0.14$	$4.06^{f} \pm 0.19$	$3.12^{d} \pm 0.12$
Ash	$2.16^{g} \pm 0.09$	$2.05^{f} \pm 0.06$	$1.83^{cd} \pm 0.08$	$1.76^{c} \pm 0.11$	$1.64^{b} \pm 0.13$	$1.52^{a} \pm 0.08$	$1.98^{ef} \pm 0.12$	$1.87^{d} \pm 0.09$	$1.89^{de} \pm 0.11$	$1.77^{c} \pm 0.08$

Data are mean value ± SE (n = 3). Mean value with same superscripts in the same row were not significantly different ($P < 0.05$).

Table 6
Intestinal protease, amylase and lipase activity in *Labeo rohita* fingerlings fed experimental diets for 80 days.

Diet	Protease activity (μg of glycine liberated h^{-1} mg protein^{-1})	Amylase activity (mg maltose liberated h^{-1} mg protein^{-1})	Lipase activity (μ mole of fatty acid liberated h^{-1} mg protein^{-1})
Initial	13.65 ± 0.15^{a}	7.25 ± 0.09^{a}	10.64 ± 0.16^{a}
RD	21.24 ± 0.29^{fg}	11.21 ± 0.14^{ef}	16.21 ± 0.22^{fg}
R1	19.15 ± 0.22^{e}	10.51 ± 0.12^{d}	15.24 ± 0.19^{e}
R2	17.32 ± 0.18^{d}	9.82 ± 0.13^{c}	14.08 ± 0.13^{d}
R3	15.86 ± 0.19^{c}	9.05 ± 0.08^{b}	12.91 ± 0.11^{c}
R4	14.51 ± 0.13^{b}	8.65 ± 0.11^{b}	12.06 ± 0.12^{b}
F1	20.84 ± 0.36^{f}	10.95 ± 0.13^{de}	15.64 ± 0.24^{ef}
F2	21.09 ± 0.28^{fg}	11.28 ± 0.12^{ef}	16.59 ± 0.19^{g}
F3	22.14 ± 0.38^{g}	11.59 ± 0.14^{f}	17.43 ± 0.25^{h}
F4	20.21 ± 0.24^{f}	10.85 ± 0.09^{de}	15.75 ± 0.18^{ef}

Data are mean value ± SE (n = 3). Values with the same superscript in the same column are not significantly different ($P < 0.05$).

Table 7
Gastrointestinal microbiota in *Labeo rohita* fingerlings fed experimental diets for 80 days.

Diet	Log viable counts g^{-1} intestinal tissue					
	Culturable heterotrophic	Extracellular enzyme-producing microorganisms				
		Protease	Amylase	Cellulase	Lipase	Tannase
Initial	6.26 (6.23–6.28)	4.55 (4.52–4.58)	4.79 (4.74–4.81)	4.83 (4.8–4.86)	4.34 (4.32–4.35)	1.4 (4.52–4.58)
RD	3.95 (3.93–3.96)	2.96 (2.93–2.98)	3.26 (3.2–3.27)	3.28 (3.25–3.3)	2.83 (2.81–2.85)	0. 6 (0.58–0.61)
R1	3.7 (3.69–3.71)	2.8 (4.52–4.58)	3.11 (3.08–3.13)	3.12 (3.1–3.14)	2.81 (2.8–2.82)	0.85 (0.83–0.87)
R2	3.48 (3.46–3.49)	2.71 (4.52–4.58)	3.04 (3.01–3.06)	3.02 (2.99–3.03)	2.78 (2.75–2.8)	0.9 (0.89–0.92)
R3	3.18 (3.15–3.2)	2.6 (4.52–4.58)	2.96 (2.95–2.98)	2.96 (2.94–2.98)	2.74 (2.72–2.77)	0.95 (0.93–0.97)
R4	3.11 (3.1–3.12)	2.53 (4.52–4.58)	2.88 (2.85–2.9)	2.93 (2.91–2.95)	2.72 (2.71–2.74)	0.87 (0.86–0.89)
F1	6.28 (6.26–6.29)	4.59 (4.52–4.58)	4.83 (4.81–4.85)	4.84 (4.82–4.86)	4.32 (4.3–4.35)	2.18 (2.15–2.19)
F2	6.33 (6.32–6.35)	4.62 (4.52–4.58)	4.85 (4.82–4.87)	4.85 (4.83–4.88)	4.34 (4.32–4.35)	2.28 (2.26–2.30)
F3	6.35 (6.34–6.37)	4.65 (4.52–4.58)	4.88 (4.85–4.9)	4.87 (4.52–4.58)	4.35 (4.32–4.38)	2.41 (2.39–2.43)
F4	6.37 (6.34–6.4)	4.68 (4.52–4.58)	4.9 (4.88–4.94)	4.88 (4.86–4.9)	4.37 (4.35–4.38)	2.49 (2.46–2.51)

Data represents mean values of triplicate observations with range within brackets.

could improve the nutritional potential of GOC, an efficient nutritional source of plant protein (41.7%) and energy (19.1 kJ/g). GOC is said to be highly palatable and has good binding properties for pelleting (Lovell, 1989). However, there are some impediments in using GOC as fish feed ingredient. Although, it is an excellent source of arginine, it is deficient in sulphur containing amino acids, *viz*., lysine, cystine and methionine (Green et al., 1988). Moreover, GOC has been reported to contain major ANFs, like tannin, protease inhibitor (trypsin inhibitor, TI) and phytic acid (Nyina-Wamwiza et al., 2010). Dietary tannins interfere with protein and dry matter digestibility by inhibiting protease and also forming indigestible complexes with dietary protein that may lead to growth retardation (Krogdahl, 1989). There are reports on tannin toxicity causing growth retardation and inhibition of digestive enzymes in fish (Hossain and Jauncey, 1989; Bairagi et al., 2002, 2004; Maitra and Ray, 2003; Mandal and Ghosh, 2010b). The protease inhibitors are reported to form stable complexes with target proteases (*e.g.*, TI with trypsin or chymotrypsin) and thereby block, alter or prevent access to the enzyme active site (Maitra et al., 2007). Phytic acid has been reported to act as a chelator, forming protein/mineral—phytic acid complexes reducing protein and mineral bioavailability (Hossain and Jauncey, 1989). Therefore, processing of raw GOC to remove/deactivate the ANFs is essential for its effective utilization as a protein source in animal feed formulation (Jackson et al., 1982). In the presently reported study, GOC was processed by heat treatment (autoclaving) and SSF with a tannase producing yeast strain, *P. kudriavzevii* (GU939629) isolated from fish gut, following which tannin, trypsin inhibitor and phytic acids were reduced significantly (84.30%, 66.7% and 58.8%, respectively). The total free amino acid and free fatty acid profiles were more available in the SSF-processed GOC than that of the raw. Moreover, amino acid profile of raw and fermented GOC revealed that there was significant increase in the contents of several amino acids in the bio-processed GOC including lysine and methionine (13.8% and 25.4%, respectively), which were deficient in the raw GOC. Further, fermentation of GOC resulted in an increase of crude protein content from 41.73% to 46.37% and decrease in crude fibre from 4.61% to 2.37%. Fermentation, thus, most likely led to ameliorating nutritive value of the GOC. SSF is a commercial process for the production of microbial enzymes where microorganisms grow on solid substrates in a limited amount of free water sufficient to support the growth of cells. During fermentation, an increase in the nutrient level through microbial synthesis is expected (Wee, 1991), which is in agreement with the present report.

Carps were described as vulnerable to the high level of plant-derived protein (more than 50% of FM protein replacement) in the diets because of low palatability, high fibre and ANF contents (Kumar et al., 2011). The result obtained from the present study indicated decline in weight gain of the rohu fingerlings with increase in the dietary level of raw GOC. Fish fed diets containing fermented GOC obtained superior weight gain, specific growth rate, SGR (% day^{-1}), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) than the fish fed diets containing same level of raw GOC. A similar trend was also noticed in tilapia (*Sarotherodon mossambicus*) fed high dietary levels of oil cake proteins including GOC (Jackson et al., 1982). Results of the present study were in agreement with Ramachandran et al. (2005), where in FM substitution in carp diets with fermented grass pea meal (40% of FM) did not evidence negative effect on the growth performance.

A progressive decline in the apparent dry matter, protein and lipid digestibility (ADD, APD and ALD) values were recorded with the increasing level of raw GOC in the experimental diets. Similar trends of turn down in the APD values have also 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; Roy et al., 2014; Das and Ghosh, 2015), copra meal (Mukhopadhyay and Ray, 1999b), leaf meals (Ray and Das, 1994; Bairagi et al., 2004), grass pea (Ramachandran and Ray, 2004; Ramachandran et al., 2005) and black gram (Ramachandran and Ray, 2007) seed meals in carp diets. As collection of fecal matter was done by pipetting, the possibility of over estimation of digestibility cannot be ruled out. However, dissolution of the faeces could be minimized by adding an indigestible binder to the diets that would also bind the faeces (De la Noüe and Choubert, 1986). In this study, CMC was added as a binder, which probably assisted in binding the faeces reducing the effect of leaching.

The proximate carcass composition of the experimental fish at the end of the feeding trial showed significant increase in protein and lipid in comparison to the initial values in all of the dietary treatments. The carcass protein and lipid content were higher in those fish which were fed diets containing 30% and 40% fermented GOC. Among the experimental fish, carcass moisture content was highest in fish fed reference diet containing 50% raw GOC. Carcass ash content was highest in the fish fed reference diet (RD) without GOC. The results of the present study were in accordance with the previous findings where same trends for carcass protein and lipid were noted with higher levels of fermented sesame seed, leaf meals and grass pea seed meals in carp diets (Mukhopadhyay and Ray 1999a; Bairagi et al., 2004; Ramachandran et al., 2005; Roy et al., 2014).

In general, *L. rohita* showed a significant decrease in protease, amylase and lipase activities in intestine on inclusion of raw GOC in the diet. However, the groups fed processed GOC and reference diet (RD) showed significant increase in the activity of the three digestive enzymes compared to their initial values. Decreased protease activities with increased raw GOC in the diets corresponded to decrease in protein availability from GOC. Similar results have been documented by Sandholm et al. (1976), Santigosa et al. (2008)

and Kumar et al. (2011). They noticed decline in protein digesting enzyme (trypsin) activity on increased plant protein inclusion in fish diet, and concluded that proteases might be highly sensitive to plant ANFs. Escaffre et al. (1997) observed that increasing levels of dietary soy–protein concentrate induced a significant decrease in trypsin activity in common carp. The decrease in protease activity at higher inclusion level of raw GOC might be caused by the presence of the ANFs like tannin and phytate. However, activity of the digestive enzymes in fermented GOC-fed groups comparable with the RD-fed group might correspondence with improved nutrient availability and decreased ANFs in the fermented GOC.

The presently reported study recorded to increase the population of culturable heterotrophic as well as extracellular enzyme-producing microorganisms (protease, amylase, cellulase, lipase and tannase) in the intestine of fish fed fermented GOC incorporated diets over their initial levels. Decrease in microbial population in fish fed diets containing raw GOC might be attributed to the adverse effect of tannic acid in feed as tannins were reported to inhibit gut microflora by enzyme inhibition, substrate deprivation, and action on biological membranes or metal ion deprivation (Scalbert, 1991; McSweeney et al., 2001; Smith et al., 2005). The enrichment culture method used to detect the population of heterotrophic microorganisms in the present study encompasses both bacteria and yeasts. Therefore, increase in tannase producing microbiota in the intestine of fish fed fermented GOC could be due to the fact that GOC was fermented by the tannase-producing yeast *P. kudriavzevii* (GU939629) and this species has been reported to form ascospore (Phuong et al., 2012). However, as spore forming ability and viability of the *P. kudriavzevii* strain within fish gut has not been investigated in the present study further endeavor is required to make a conclusive remark.

5. Conclusion

The present study has demonstrated the acceptable nutritional value of the SSF-processed GOC as an ingredient in carp diet. An inclusion level of up to 40% (w/w) bio-processed GOC in the diet for *L. rohita* fingerlings depicted in the present report had no adverse effect on growth, feed utilization efficiency or body composition in comparison to the FM based reference diet, and was far superior to the raw GOC at the equivalent level of inclusion. Moreover, incorporation of fermented GOC (replacing 45% of FM) would be cost effective as it is much cheaper than FM and involved low cost processing technique. However, it is early to recommend to the industry for use of fermented GOC in formulation of aquafeeds, as it demands further experimentation in the field condition with large number of fish and replicates. The potential benefit of the bioprocessing strategy adopted in the present study are also worth to further investigations to ascertain their effects on fish nutrition and health before advocating their application in commercial aquaculture.

Conflict of interest

There is no conflict of interest to be declared by the authors.

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