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Effect of Solid-state Fermented Aquafeed on Growth Performance, Digestive Enzymes and Innate Immunity of Rohu, *Labeo rohita*

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ABSTRACT

Background: There is a scope of increasing the aquaculture production by use of fish feed produced from non-conventional ingredients and also improvement in the quality of feed by solid-state fermentation. The present study was conducted to investigate the effect of solid-state fermented aquafeed on growth performance, digestive enzymes and innate immunity of rohu, *Labeo rohita.*

Methods: Fish feed containing sesame oil cake and mahua oil cake was fermented through solid-state fermentation by yeast, *Saccharomyces cerevisiae* and then compared with a non-fermented control feed with the same formula.

Result: Solid-state fermentation increased crude protein (CP), decreased crude fibre (CF) and also lowered ($p<0.05$) anti-nutritional factors (ANF) of fish feed. After five months of experimental feeding, weight gain and digestibility of nutrients like dry matter, crude protein and ether extract were significantly higher ($p<0.05$) and feed conversion ratio was significantly lower in fish of fermented feed fed group than the control group. Furthermore, non-specific immune parameters such as lysozyme, myeloperoxidase and haemolytic activities and intestinal enzyme activity of fish fed with fermented feed increased significantly ($p<0.05$) compared to fish fed control feed. It is concluded that feeding of *Saccharomyces cerevisiae* fermented feed could improve the growth, digestive enzymes activity and immunity of Rohu, *Labeo rohita*.

Key words: Anti-nutritional factors, Digestibility, Immunity, Growth performance, *Labeo rohita, Saccharomyces cerevisiae,* Solid-state fermentation.

INTRODUCTION

Solid-state fermentation is generally referred to as the process in which microbial growth and product formation occur on the surface of solid materials in the absence or near-absence of free water (Cannel and Moo-Young, 1980; Mudgett *et al*., 1986). It deals with the utilization of water-insoluble materials for microbial growth and metabolic activities. Microbial activities cease at a certain low level of moisture content and this establishes the lower limit at which solid state fermentation can take place (Moo-Young *et al*., 1983). The most regularly used solid substrates are cereal grains (Mhalaskar *et al*., 2017), legume seeds, wheat bran, lignocellulosic materials such as straws, sawdust or wood shavings and a wide range of plant and animal materials (Sadh *et al*., 2018). SSF technique has been employed intensively for the production of enzymes, antibiotics, surfactants and other value-added products.

There is a scope of increasing the aquaculture production by use of fish feed produced from non-conventional ingredients and also improvement in the quality of feed by solid-state fermentation. Appropriate technology for reduction of toxic substances in fish feed along with improvement of digestibility can provide the opportunity for entrepreneurs/farmers for fish feed production from non conventional ingredients. Many fish feed produced from non-conventional ingredients are high in anti-nutritional factors, crude fibre and digestibility of protein are low (Abowei and Ekubo, 2011). Many researchers have shown that SSF could improve the chemical composition and nutritive value of the fermented products (Yano *et al*., 2008 and Vijayan *et al*., 2009). Hence, the present study was conducted to investigate the effect of solid-state fermentation of fish feed produced from non-conventional ingredients on growth performance, nutrient digestibility and innate immunity of Rohu, *Labeo rohita.*

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MATERIALS AND METHODS

Microorganism used for SSF

The yeast *Saccharomyces cerevisiae* was obtained from National Dairy Reseach Institute (NDRI) and retrieved in Fish Nutrition and Physiology Laboratory of ICAR- Central Institute of Freshwater Aquaculture, Bhubaneswar, India using yeast extract-peptone-dextrose (YPD) medium

containing Yeast extract 10 g/L; Peptone 20 g/L; Dextrose 20 g/L; Agar 20 g/L) and stored at 4°C for further use.

Formulation and preparation of fish feed

Fish feed was formulated and prepared with available conventional and non-conventional feed ingredients *i.e.* maize, soyabean meal, de-oiled rice bran (DORB), sesame oil cake (*Sesamum indicum* L.), mahua oil cake (*Bassia latifolia Roxb*.), vegetable oil, minerals and vitamin mixture as per the requirement of rohu fingerling (Table 1) and then solid fermented with yeast, *saccharomyces cerevisiae* maintaining standard protocol. After solid state fermentation, pelleted feed (2 mm size) was produced by using extruder manufactured by Jinan Saibainuo Machinery Co. Ltd., China. The feed produced through solid state fermentation was compared with the same feed without solid state fermentation.

Solid-State Fermentation (SSF) of fish feed

1000 g of prepared ground feed was taken in an erlenmeyer flask and then it was autoclaved at 15 psi for 15-20 minutes at 121°C. 200 ml of sterilized distilled water and 500 ml of already prepared inoculum was added, the mouth was closed with a screwed wooden lid and incubated at 37°C for 48 hr. The flask was stirred and sterilized distilled water was added periodically to maintain 70% water content. Flasks containing un-inoculated culture served as controls. In un-inoculated samples, all the experimental procedures were the same as those for inoculated feed, except for the addition of sterile medium instead of inoculated bacteria. Inoculated and uninoculated samples were set up in triplicate. After 48 h of anaerobic fermentation, wet samples were collected and treated at 105°C for 30 min to prevent continuous fermentation. Then, the inoculated and un-inoculated samples were dried at 65°C for 24 h, cooled and ground.

The colony forming unit (CFU) of fermented fish feed was counted after mixing the fermented feed with sterile phosphate buffer saline and then as per the standard procedure of serial dilution and plating. The colony forming unit (CFU) of yeast in fermented fish feed was 3.2×10^6 CFU/ per gm of feed.

Pond preparation

Six earthen ponds of 0.05 ha water-spread area each were used for rearing the experimental fish. Before stocking the fish, all the ponds were dried and bleaching powder (30% active chlorine) was applied at 300 kg ha^{-1} to eradicate the unwanted predatory and weed fishes. Lime was applied at 1000 kg ha^{-1}. After 7 days of lime application, the water was filled up to 1.0 m depth and the ponds were fertilized with raw cow dung, urea and bleaching powder at 3.0 ton ha^{-1}, 40.0 kg ha^{-1} and 60.0 kg ha^{-1}, respectively (ICAR, 2009).

Fish rearing and sample collection

1200 rohu (weight 0.4 to 0.6 g) fingerlings were procured from Central Institute of Freshwater Aquaculture Farm, Bhubaneswar, India and were acclimatized in a pond for fifteen days. After acclimatization, a group of 200 fishes (average body weight of 0.5 g) were stocked randomly into triplicate earthen ponds (each 130 ft × 60 ft) for both control and treatment following a completely randomized design. The floating feed of 2 mm size was prepared and fed (3% of body weight upto 100 g and 2% of body weight afterwards) twice daily for 5 months duration. A group of fifteen numbers of fishes in each pond were batch weighed randomly once every month to estimate the average weight and biomass of fish in each pond. Physico-chemical parameters of the water were monitored routinely during the entire course of the study by using the standard method (APHA,1980). The total fish biomass per pond was measured at the beginning of the feeding trial and subsequently at the end of the trial Blood collection was done at the end of the experimental trial by randomly selecting 10 fishes from each pond. Blood and serum samples of fish were used for laboratory analyses (Swain *et al*., 2018).

The fish were anaesthetized with clove oil (50 µl of clove oil per litre of water) and blood was drawn from the caudal vein of fish using 2 ml hypodermal syringe. The collected blood (approximately 200-300 µl from each fish) was immediately transferred into two vials, where one vial was coated with a thin layer of EDTA, an anticoagulant (for blood collection) and the other one without EDTA (for serum

Table 1: Ingredients composition(g/kg) of the experimental diets fed to *Labeo rohita.*

Feed ingredients	Control (without solid state fermentation)	Treatment (with solid state fermentation)
Yellow maize	340	340
Soybean meal	200	200
Sesame oil cake	200	200
De oiled rice bran	140	140
Mahua oil cake (MOC)	100	100
Vegetable oil	10	10
Mineral and vitamin premix[a]	10	10

Note: [a]Mineral vitamin premix (PREEMIX PLUS) (quantity/2.5 kg).
Vitamin A, 5,500,000 IU; vitamin D3, 1,100,000 IU; vitamin B2, 2000 mg; vitamin E, 750 mg; vitamin K, 1,000 mg; vitamin B6, 1,000 mg; vitamin B12, 6 mcg; calcium pantothenate, 2,500 mg; nicotinamide, 10 g; choline chloride, 150 g; Mn, 27, 000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2000 mg; Co, 450 mg; l lysine, 10 g; dl methionine, 10 g; selenium, 50 ppm; Satwari, 2,500 mg.

collection). EDTA coated vials were shaken gently to prevent haemolysis and clotting of blood. Serum was separated from blood by keeping the tubes in slanting position for about 2 hr and thereafter centrifuging it at 2000 g for 15 min at 4°C and stored at 20°C until use (Das *et al*., 2015).

Digestibility study

After completion of the feeding period, a digestibility trial of 20 days duration was conducted after 10 days for acclimatization to know the digestibility of nutrients. 10 nos of fishes from each pond were randomly selected and were allocated in tanks (each 500 L capacity) for digestibility experiment. Aerators were used in each tank to provide sufficient oxygen and half of the water was changed daily. The water quality parameters were also checked and found to be optimum. During the digestibility trial, feed offered, left overfeed and faeces were collected and recorded on daily basis to determine the dry matter (DM) intake (Spyridakis *et al*., 1989). The DM percentage of each was estimated during the entire experimental period to find out the daily Dry Matter intake. The feed offered, residue left and faeces collected were analyzed at the end of the experiment for proximate composition as per AOAC (2012). The digestibility (%) for DM, CP and EE were calculated.

Analysis of proximate composition and anti-nutritional factors in feed

The proximate composition of feed *i.e*. dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), nitrogen-free extract (NFE) and total ash (TA) were analysed (AOAC, 2012) for quality evaluation. Dry matter was estimated by oven drying the samples at 105°C till a constant weight was achieved and crude protein per cent was calculated by estimating nitrogen content by the micro-kjeldahl method and multiplying with a factor 6.25. Ether extract (EE) was determined by solvent extraction with petroleum ether at the boiling point 40-60°C for 10-12 h. Total ash content was determined by incinerating the sample at 650°C for 6 h and crude fibre by acid digestion (1.25%) followed by alkali digestion (1.25%).

Total tannin of feed was estimated by titrating against potassium permanganate in the presence of indigo carmine (Sastry *et al*., 1999). 5 g of the powdered fat free sample was extracted with distilled water into 500 ml volumetric flask and then the sample was filtered. 1 ml of aliquot, 100 ml of distilled water and 2 ml of indigo carmine solution were titrated against 0.1 N $KMnO_4$ solution and tannic acid was calculated from the formula (1 ml 0.1 $KMnO_4$ = 0.006235 g tannic acid). Similarly, saponin was estimated by using the method of Obadoni and Ochuko (2001). A 20g of powdered sample was put in 250 ml conical flask and 100 ml of 20% aqueous ethanol was added. The mixture was heated for 4 hours at a constant temperature of 55°C with continuous stirring. The mixture was then filtered and the residue was again extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over water bath at a temperature of 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added with vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification was repeated twice. 60 ml of n-butanol was added and butanol extract was washed twice with 10 ml of 5% NaCl. The NaCl was discarded and the remaining solution was heated in the water bath for 30 minutes, after that the solution was transferred into a crucible and was dried in an oven to a constant weight and saponin content calculated as a percentage.

Estimation of digestive enzymes

The intestine tissues of rohu at the end of pond experiment were assessed for amylase (Rick and Stegbauer, 1974) and protease (Moore and Stein, 1948) activity after sacrificing randomly three animals in each pond. The enzyme activities were expressed for amylase as micromole of maltose released min^{-1} g $protein^{-1}$ at 37°C and protease as µg of glycine liberated min^{-1} gm $protein^{-1}$. 1 ml of sample was added to the test tube containing 1 ml of 1% starch solution prepared in 0.02 M phosphate buffer pH 7.0. This mixture was incubated at 25°C for 10 min. Two ml of 3,5-*Dinitrosalicylic acid* (DNS) was added to the test tube and placed in a water bath for 5 min. The contents of the test tubes were cooled and diluted up to 20 ml with distilled water. The absorbance of the reaction mixture was determined at 540 nm in a spectrophotometer (CECIL CE 7400) against maltose as standard. Protease activity was determined according to the method of Moore and Stein (1948). In brief, 1.5 ml buffer, 0.25 ml casein solution and 0.25 ml sample incubated for 2 hr at 39°C. The reaction was stopped by adding 2 ml trichloroacetic acid (TCA) solution. For control, TCA and casein solution were added before incubation of samples. Tubes were subjected to overnight incubation at room temperature. The centrifuge was done at 2500 rpm for 10 min in the next morning and supernatant was used to check the optical density (OD) at 625 nm in UV VIS Spectrophotometer (Thermo Spectronic, UK).

Estimation of non specific immune parameters
Lysozyme assay

A 130 µl of Lyophilized *Micrococcus lysodeikticus* (Sigma, USA) was freshly prepared (Ellis, 1990) at a concentration of 0.6 mg/ml (in 0.02 M sodium citrate buffer) and was added to a mixture containing 10 µl fish serum sample and 10 µl of 0.02 M sodium citrate buffer. The initial OD was read at 450 nm after adding bacterial solution immediately. After incubation of the sample at 24°C for 1 hr, the OD of the samples was again read at 450 nm. A standard curve was prepared using a mixture of 20 µl working standard and 130 µl of *M. lysodeikticus* solution. Lysozyme activity was expressed in units/ml where one unit is defined as the decrease in absorbance of 0.001/min.

Myeloperoxidase activity

The myeloperoxidase activity was assessed according to the method of Quade and Roth (1997). A 15 µl of fish serum was diluted in 135 µl of Hank's balanced salt solution (Ca^{2+},

Mg^{2+} free) and to it, 50 µl of 20 mM of 3, 3′, 5, 5′ tetramethyl benzidine and 5 mM of hydrogen peroxide were added. The mixture was incubated for 2 min at room temperature. Once the incubation period was over, the reaction was stopped by addition of 4 M sulphuric acid. The OD was read at 450 nm using the UV VIS Spectrophotometer (Thermo Spectronic, UK).

Bacterial agglutination activity

Bacterial agglutination test was carried out in "U" shaped microtitre plates as the method described by Swain *et al*. (2018). A 25 µl of fish serum sample was two fold serially diluted and added in each well with an equal volume of normal saline solution (NSS). A 25 µl of formalin killed *Aeromonas hydrophila* (10^7 cells/ml) suspension was added to each well. After overnight incubation at 37°C, the titre was calculated as the reciprocal of the highest dilution of serum showing complete agglutination of the bacterial cells.

Haemagglutination activity

The haemagglutination activity was carried out as described by Blazer and Wolke (1984). A 25 µl fish serum sample twofold serial diluted (inactivated at 45°C for 30 min) and mixed with an equal volume of NSS in "U" shaped microtitre plates. A 25 µl of freshly prepared 1% New Zealand white rabbit red blood cell (RBC) suspension was then added to the wells. After 2 hr of incubation at room temperature, the titre was calculated as the reciprocal of the highest dilution of serum showing complete agglutination of RBCs.

Haemolytic activity

The haemolytic assay was carried out as described by Blazer and Wolke (1984). In this case, the microtitre plates were incubated at room temperature overnight. The titre was expressed as the reciprocal of the highest dilution of serum showing complete haemolysis of rabbit RBCs.

Statistical analysis

All the data of the experiment were statistically analysed by using statistical software (Prism, version 4.0, GraphPad Software, San Diego, CA, U.S.A.). Values were expressed as mean, SEM and p values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Fish feed prepared from non-conventional ingredients has been tested in aquaculture for economizing fish production (Harter *et al*., 2017; Das *et al*., 2016; Das *et al*., 2018). However, the presence of anti-nutritional factors and poor digestibility affects the fish performance adversely thereby reducing overall production (Abowei and Ekubo, 2011; Maina *et al*., 2002). Several feed ingredients such as rape seed meal, sesame oil cake, Jatropha cake, coffee husk, tamarind seed powder, palm kernel cake and wheat bran have been used for feed formulation by employing SSF technique for the removal of anti-nutritive factors and improving the nutritional value (Hernandez *et al*., 2011; Belewu *et al*., 2011; Fazhi *et al*., 2011; Sabu *et al*., 2006). In this experiment, effort has been done on quality improvement of fish feed after inclusion of non-conventional ingredients like sesame oil cake (*Sesamum indicum* L.) and mahua oil cake (*Bassia latifolia Roxb*.) through solid-state fermentation (Table 1) considering the importance on the commercial point of view.

Proximate composition of experimental feed

The proximate composition of fish feed produced from conventional and non-conventional ingredients presented in Table 2. The control feed contained 24.76% CP whereas solid-state fermented feed contained 27.5% CP. An increase in CP was reported in this experiment whereas CF content in the diet decreased after SSF. The control feed contained 10.20% Crude Fibre (CF) whereas treatment feed contained 7.9% of Crude Fibre (CF). The chemical composition of feed for other nutrients did not show any significant difference ($p > 0.05$). The comparative analysis showed that there was a change in the chemical composition of feed after solid-state fermentation.

The anti-nutritional factors (ANF) like tannin and saponin of feed before and after solid-state fermentation has been presented in Table 3. There was a reduction in total tannin and saponin content of fish feed after solid-state fermentation showing solid state fermentation could improve the quality of fish feed.

In this experiment, the feed was formulated using both conventional and non-conventional ingredients and then fermented by using *Sacharomyces cerevisae.* The chemical composition of fermented and non-fermented feed showed

Table 2: Proximate composition (% on DM basis) of experimental diets fed to *Labeo rohita.*

Chemical characteristics	Control	Treatment	SEM	p- value
Dry matter	92.01	91.01	0.3652	0.1963
Crude protein	24.76^a	27.50^b	0.7521	0.0469
Ether extract	3.19	3.36	0.0680	0.2836
Crude fibre	10.20^a	7.90^b	0.5761	0.0166
Total ash	7.99	8.98	0.2830	0.0690
Nitrogen free extract	53.86	52.26	0.4453	0.0541
Total tannin	1.05^b	0.71^a	0.0738	<0.0001
Total saponin	4.4^b	1.0^a	0.7607	<0.0001

Note: Means with different superscript in a row differ significantly (p<0.05). SEM: Standard error of the mean.

that there was an increase in crude protein and a decrease in crude fibre content of feed stuff and same results have been reported by many workers when fish feed prepared from conventional ingredients was fermented with microbes (Lena *et al.,* 1997; Gelinas and Barrette, 2007; Qazi *et al*., 2011). The increase of crude protein may be due to the biotransformation of soluble carbohydrate to the bacterial protein (Vijayan *et al*., 2009; Rajesh *et al*., 2010), increase in microbial cell mass (Antai and Obong, 1992) and could be attributed to the ability of microorganisms to secrete some extracellular enzyme (Oseni and Akindahunsi, 2011). Similarly, a reduction of crude fiber content in fermented fish feed is an indication of higher disintegration of crude fibre by microbes (Nwanna, 2003) as observed in this experiment. Anti-nutritional factors like total tannin and saponin were reduced in the fermented feed which indicates that yeast (*Saccaromyces cerevisiae*) used in the present experiment was able to degrade anti-nutritional factors, similar to the result reported earlier (Hassan, 2015; Ghosh and Mandal, 2015, Tacon, 1993; Ray and Das, 1995; Mondal and Ray, 1999). The reduction of anti-nutritional factors by fermentation might due to the production of the relevant enzyme which causes the breakdown of anti-nutritional substrates.

Growth performance

During 5 months experimental period, the fishes of the control group varied from 0.5 to 324 g, whereas the fishes of the treated group varied from 0.5 to 391 g (Table 3). The feed conversion ratio (FCR) of the fishes was calculated from feed intake and weight gain (g/day) during the experimental period. The results showed that the final weight and weight gain were significantly higher in fish of treatment group than fish of control group ($p<0.05$). Similarly, FCR was superior in the treatment group compared to the control group.

Increase in the availability of nutrients and reduction of anti-nutritional factors as observed in this experiment might have contributed in enhancing the growth performance. Similar results have been reported by Enyidi (2018) in African catfish using fermented bambaranut feed. Increase the amount of protein in fermented fish feed in the treatment group might also be responsible for promoting growth of fish (Qazi *et al*., 2011). The improved fish growth in the treatment group may due to improved palatability as observed in this experiment. More availability of essential and non-essential amino acids due to SSF is frequently responsible for increasing the palatability of fish food items (Barnes *et al*., 2006). Sheikhzadeh *et al*. (2012) studied on Hilyses®, a fermented *Saccharomyces cerevisiae*, found that growth performance increased significantly by the dietary yeast supplement which is in line with the present investigation.

Digestibility of nutrients

The digestibility of different nutrients like DM, CP and EE were significantly ($p<0.05$) varied in treatment group having fermented feed compared to control group and presented in Table 4.

There was an increase in digestibility of nutrients in fish after feeding with fermented feed compared to non-fermented feed also reported by (Bairagi *et al.,* 2002 and

Table 3: Growth and feed utilization of *Labeo rohita* fed different experimental diets for the period of 5 months.

Growth parameters	Control	Treatment	SEM	p-value
Initial weight (g)	0.5	0.5	0.036	>0.999
Final weight (g)	324.0^{a}	391.0^{b}	15.27	0.0005
Weight gain (g/fish/5 months)	323.5^{a}	390.5^{b}	15.28	0.0005
Weight gain (g/fish/day)	2.15^{a}	2.60^{b}	0.101	0.0034
FCR	1.35^{b}	1.11^{a}	0.066	0.0438
Survivality (%)	84.0	85.0	0.8736	0.6774

Note: Means with different superscript in a row differ significantly ($p<0.05$). SEM: Standard error of the mean.

Table 4: Digestibility of nutrients in rohu, *Labeo rohita* fed different experimental diets at five months of experimental period

Nutrients	Control	Treatment	SEM	p-value
Dry matter	92.52^{b}	95.08^{a}	0.5259	0.0126
Crude protein	94.18^{b}	96.63^{a}	0.4989	0.0119
Ether extract	91.07^{b}	95.51^{a}	0.9049	0.0125

Note: Means with different superscript in a row differ significantly ($p<0.05$). SEM: Standard error of the mean.

Table 5: Digestive enzyme activities of *Labeo rohita* fed different experimental diets at five months of experimental period.

Digestive enzymes	Units	Control	Treatment	SEM	p-value
Amylase	µg/min/gm of protein	15.00^{a}	17.67^{b}	0.7149	0.0390
Protease	µg/min/gm of protein	4.67^{a}	9.4^{b}	0.1468	0.0166

Note: Means with different superscript in a row differ significantly ($p<0.05$). SEM: Standard error of the mean.

Table 6: Non-specific immune parameters of *Labeo rohita* fed different experimental diets at five months of experimental period.

Parameters	Units	Control	Treatment	SEM	p-value
Bacterial agglutination	log_2	1.603	1.793	0.0696	0.1988
Lysozyme	mg/ml serum	10.27[b]	13.70[a]	0.8459	0.0124
Myeloperoxidase	OD	0.05500[a]	0.05967[b]	0.00128	0.0488
Haemaglutination activity	log_2	2.935	3.207	0.2596	0.6554
Haemolytic activity	log_2	2.557[a]	2.810[b]	0.05846	0.0014

Note: Means with different superscript in a row differ significantly ($p < 0.05$). SEM: Standard error of the mean.

Vijayakumar *et al*., 2009). Tonheim *et al*. (2007) indicated that the increase in crude protein digestibility may be due to the increase in water-soluble protein in the solid-state fermented feed as water-soluble protein is easier to digest than water-insoluble proteins.

Enzymatic responses

Intestinal enzyme activities like amylase and protease were analysed for both treatment and control groups. Both the activities were significantly ($p<0.05$) varied with supplementation of fermented feed at the 60th day is presented in (Table 5).

Microbial fermentation is one of the most important sources of enzymes production that widely utilized in the industry for producing a variety of enzymes such as amylases, cellulases, proteases, lipases and lignocelluloses. These enzymes help to degrade the starch, non-starch polysaccharides and other polymeric forms of the molecules in the substrate to soluble monomers with a beneficial increase in total carbohydrate and protein contents (Ofuya and Nwanjiuba, 1990; Pandey *et al*., 1999; Iyayi and Losel, 2001). Prakasham *et al*. (2006) also reported a significant increase in amylase and protease activity in the intestine of fish fed formulated diet after SSF. Increase in enzyme activity may be responsible for increasing digestibility of nutrients. Qazi *et al*. (2012) reported an increase of 17.82% in protease activity by SSF of feed using yeast *S. cerevisiae*. Moura *et al*. (2012) studied on effects of enzyme complex SSF in pellet diets for Nile tilapia and found that the improvement in the growth rate of the tilapias would be related to possible increases in the bioavailability of nutrients promoted by the enzymatic action of the enzyme complex SSF which is in line with our findings.

Non-specific immune response

The non specific immune parameters of fish like myeloperoxidase, lysozyme and haemolytic activity were significantly higher ($p<0.05$) in fish of treatment group than the control (Table 6).

Fish has an advance immune system (Alexander, 1992) and non-specific immunity play a major role in combating infections in fish. The lysozyme, myeloperoxidase, haem-agglutination and haemolytic activities are main indicators of non-specific immune functions (Swain *et al*., 2007). In the present study, the fermentation process caused a significant increase in lysozyme, myeloperoxidase and haemolytic activity while there was no significant difference in bacterial agglutination and haemaglutination activity. Lysozyme, a mucolytic enzyme of leucocytic origin, is an important defence molecule of the innate immune system, which is important in mediating protection against microbial invasion (Saurabh and Sahoo, 2008). Increased lysozyme activity indicates improved immunity responses in fish (Panigrahi *et al.*, 2004; Kim and Austin, 2006). Myeloperoxidase activity is a measure of neutrophil antimicrobial activity. In a study, a significant increase in HA titre was found in *Oncorhynchus mykiss* after administration of *S. cerevisiae* in diets (Sheikhzadeh *et al*., 2012). Hence, solid-state fermented feed has ample scope to improve the aquaculture production by improving diet quality and overall physiological health status of cultured fish.

CONCLUSION

Fish feed using non-conventional ingredients and fermented through solid-state fermentation by yeast, *S. cerevisiae* increased the quality of fish feed by improving crude protein (CP), growth performance, digestibility of nutrients and intestinal enzyme activity like amylase and protease. It eliminated anti-nutritional factors like total tannin and saponin of feed. Also,non-specific immune parameters like lysozyme and myeloperoxidase activities have significantly increased in rohu. Hence, fish feed formulated with non-conventional ingredients *i.e*. sesame oil cake and mahua oil cake may be solid-state fermented with *S. cerevisiae* for improving the performance of rohu.

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