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*.

**MATERIALS AND METHODS**

**Microorganism used for SSF**

The yeast *Saccharomyces cerevisiae* was obtained from National Dairy Research 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.
Formulation and preparation of fish feed

Fish feed was formulated and prepared with available conventional and non-conventional feed ingredients i.e., maize, soybean 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 hr, 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 x 10³ CFU/gram 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 x 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 up to 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 state fermentation | Treatment 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*       | 10                        | 10                         |

Note

*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 percent 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). 5g 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 KMnO₄ solution and tannic acid was calculated from the formula (1 ml 0.1 KMnO₄ = 0.00625 g tannic acid). Similarly, saponin was estimated by using the method of Obadoni and Ochuku (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**

Effect of Solid-state Fermented Aquafeed on Growth Performance, Digestive Enzymes and Innate Immunity of Rohu...
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 analyzed 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 cerevisiae. The chemical composition of fermented and non-fermented feed showed

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**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*  | 27.50*    | 0.7521| 0.0469  |
| Ether Extract                 | 3.19    | 3.36      | 0.0680| 0.2836  |
| Crude Fibre                   | 10.20*  | 7.90*     | 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*   | 0.71*     | 0.0738| <0.0001 |
| Total Saponin                 | 4.4*    | 1.0*      | 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 (Saccharomyces 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 (Bairagi et al., 2006). Sheikhzadeh et al. (2012) studied on Hlyses®, 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<sup>a</sup> | 391.0<sup>b</sup> | 15.27| 0.0005  |
| Weight gain (g/fish/5 months) | 323.5<sup>a</sup> | 390.5<sup>b</sup> | 15.28| 0.0005  |
| Weight gain (g/ fish/day)  | 2.15<sup>a</sup> | 2.60<sup>b</sup> | 0.101| 0.0034  |
| FCR                       | 1.35<sup>a</sup> | 1.11<sup>b</sup> | 0.066| 0.0438  |
| Survival (%)               | 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 *Labeo rohita* fed different experimental diets at five months of experimental period

| Nutrients       | Control | Treatment | SEM  | p-value |
|-----------------|---------|-----------|------|---------|
| Dry matter      | 92.52<sup>a</sup> | 95.08<sup>b</sup> | 0.5259| 0.0126  |
| Crude protein   | 94.18<sup>a</sup> | 96.63<sup>b</sup> | 0.4989| 0.0119  |
| Ether extract   | 91.07<sup>a</sup> | 95.51<sup>b</sup> | 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<sup>a</sup> | 17.67<sup>b</sup> | 0.7149| 0.0390  |
| Protease          | µg/min/gm of protein | 4.67<sup>a</sup> | 9.4<sup>b</sup> | 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     | log₂          | 1.603   | 1.793     | 0.0696 | 0.1988  |
| Lysozyme                    | mg/ml serum   | 10.27a  | 13.70a    | 0.8459 | 0.0124  |
| Myeloperoxidase             | OD            | 0.05500a| 0.05967a  | 0.00128| 0.0488  |
| Haemagglutination activity  | log₂          | 2.935   | 3.207     | 0.2596 | 0.0014  |
| Haemolytic activity         | log₂          | 2.557a  | 2.810b    | 0.05846| 0.00128 |

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 Nwanjiba, 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 tilapia was 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, haemagglutination 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 haemagglutination 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 mykias 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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Effect of Solid-state Fermented Aquafeed on Growth Performance, Digestive Enzymes and Innate Immunity of Rohu...

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