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Comparative Growth Performance, *in vivo* Digestibility and Enzyme Activities of *Labeo rohita* Fed with DORB Based Formulated Diet and Commercial Carp Feed

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Abstract

A 60-day feeding trial was conducted to study the nutrient utilization and growth performance of *Labeo rohita* fed with T1 [DORB supplemented with phytase and xylanase (0.01% each)], T2 [T1+ L-lysine(1.4%), L- methionine (0.4%), combination of EPA and DHA (0.5%)] , T3 [DORB supplemented with phytase (0.01%), combination of xylanase and cellulase (0.075%), L-lysine (1.4 %), L- methionine (0.4 %), combination of EPA and DHA (0.5%)] and T4 (commercially available carp feed) diets. Weight gain%, SGR, and FCR were similar ($p>0.05$) for the fishes fed with either T3 feed or commercial carp feed (T4), whereas PER was highest for the T3 group. A significantly higher apparent dry matter and carbohydrate digestibility were observed in the T3 group. Highest crude protein digestibility was observed in T4 group, though lipid digestibility was similar in both T3 and T4 groups. The activities of digestive enzymes were similar in T2, T3 and T4 groups, whereas the lowest activity of these enzymes were observed in T1 group. Based on the findings it is concluded that T3 feed (crude protein 18.18 %) is having comparable growth performance of *Labeo rohita* as compared to T4 diet (crude protein 32.01 %) due to higher *In vivo* digestibility.

Keywords: DORB (de-oiled rice bran), exogenous enzymes, methionine, lysine, essential fatty acids

Introduction

Feed based carp culture is yet to get the momentum compared to other species due to high ingredient cost. In a recent report it is indicated that only 1.3% farmers use commercial carp feed, whereas 65.4% farmers use only mash feed (Ramakrishna *et al.,* 2013). This mash feed mainly comprises of DORB (>70%) and other ingredients like groundnut cake, mustard cake or cotton seed meal. Mash feeding is an age old practice, where farmers use locally available feed ingredients as farm-made feed. Only DORB has its limitation to be used as a complete feed as it is deficient in some nutrients. Supplementation of deficient nutrients along with exogenous enzymes appears to be an ideal strategy to improve the nutritive value of DORB.

Exogenous enzyme supplementation has been reported to improve the nutritional value of feed in terrestrial animal (Classen, 1996) along with decrease in environmental pollution. These enzymes are also widely used to reduce the anti- nutritional effects of non-starch polysaccharide (NSP) in the feed of mono-gastric animals including pigs and poultry (Alloui *et al.,* 1994; Ghazi *et al.,* 2003; Esonu *et al.,* 2005). The application of phytase has been successful in breaking down phytate to increase mineral and nutrient digestibility that in turn improves the growth performance of fish (Cao *et al.,* 2007; Cao *et al.,* 2008; Kiarie *et al.,* 2013; Adeoye *et al.,* 2016). NSP-degrading

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enzymes (e.g. cellulase, xylanase, etc.) are capable of disrupting plant cell wall integrity, this enhances rapid digestion by reducing viscosity in the gut (Zijlstra *et al.*, 2010; Bedford & Cowieson, 2012).The beneficial effects of exogenous non- starch polysaccharidases (NSPase) may be directly related to NSP hydroxylation, which improves carbohydrate digestibility, or to the improvement of other nutrients digestibility (Adeola & Bedford, 2004; Ai *et al.*, 2007; Adeola & Cowieson, 2011; Yiğit *et al.*, 2014).

It is well established that protein sources of plant origin contain some anti-nutritional factors and their amino acid profile is deficient with respect to the ingredient of animal origin (Tacon, 1994; Vandeningh *et al.*, 1996). Dietary supplementation of amino acids improves the amino acid balance of feed that can improve the growth performance and profitability of the aquaculture industry (Li *et al.*, 2009). Espe *et al.* (2006) showed that through dietary supplementation of amino acids to the plant protein based diet can reduce the use of dietary fishmeal in the diet of Atlantic salmon without impairing the feed intake and growth performance. Lysine is one of the most limiting essential amino acid in the ingredients of plant origin (Mai *et al.*, 2006; Gatlin *et al.*, 2007). Sardar *et al.* (2009) demonstrated that *Labeo rohita* fingerlings effectively utilize the supplemental amino acids (L-lysine at 4 $g.kg^{-1}$ dry diets and DL-methionine at 7 $g.kg^{-1}$ dry diets) in the soybean meal based diets. Similarly, Mukhopadhayay and Ray (1999), and Mukhopadhayay (2000) observed that carps fed soybean, sal seed, and copra meal based diets supplemented with lysine and methionine improved the growth rate, weight gain, FCR, and PER. Figueiredo-silva *et al.* (2015) also found that supplementation of soybean meal-based diet with crystalline DL-methionine has a potential to totally replace the fish meal with soybean in the diet of hybrid tilapia.

Long-chain polyunsaturated fatty acids (PUFA) play an important role for normal growth and health status of fish (Sargent *et al.*, 1993, 1995, 1999; Misra *et al.*, 2006). However, there is a paucity of information on the role of EPA and DHA on growth performance of *Labeo rohita*. Supplementation of exogenous enzymes along with the amino acids and fatty acids, which are deficient in DORB, may be an ideal strategy to improve the nutrient utilization and growth performance of *Labeo rohita*.

With this backdrop, the objective of the present study was to improve the nutrient utilization of DORB in *Labeo rohita* by exogenous enzyme supplementation along with supplementation of deficient amino acids and fatty acids.

Materials and Methods

De-Oiled Rice Bran

De-oiled rice bran samples were obtained from Vaighai Agro Products Limited, Tamil Nadu, India. Before using DORB in the feed it was very finely ground and sieved.

Exogenous Enzymes

Microbial phytase from E. coli (Quantum blue, 500 U/ kg) and xylanase (Econase® XT, 16000 U/ kg); cellulase and xylanase from *Trichoderma reesei* (Cellulase –10000 ECU/g; Xylanase –350000 BXU/g) was supplied by AB Vista, Wiltshire, UK.

Diet Preparation

Three experimental diets (T1, T2, and T3) were prepared using DORB and other ingredients as given in Table 3 (T1; DORB supplemented with phytase and xylanase, T2; DORB supplemented with phytase, xylanase, lysine,

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methionine, EPA and DHA, T3; DORB supplemented with phytase, xylanase, cellulase, lysine, methionine, EPA and DHA). Diets were prepared by blending the ingredients except for the vitamin mineral mix and other additives to make a dough. Then it was steamed for 30 min. in a pressure cooker followed by addition of vitamin-mineral mix, oil, enzymes, essential amino acid (lysine and methionine) and essential fatty acids (EPA and DHA) to the different diets as specified (Table 3). The dough was then mixed properly and pressed through a semi-automatic pelletizer (Uniextrude-Single screw extruder, S.B.Panchal& Company, Mumbai, India) to produce pellets of size 0.7-0.8 mm, which were spread over a sheet of paper and dried at room temperature. After drying, the pellets were packed in polythene bags and sealed and kept at -20 °C for storage until further use. T4 was the commercial carp feed obtained directly from the local suppliers.

Fish and Facilities

The experiment was conducted at the wet laboratory of the ICAR-Central Institute of Fisheries Education (CIFE), Mumbai over a period of 60 days from July to September 2016. Subsequently, the laboratory work was carried out in Fish Nutrition, Biochemistry and Physiology laboratory of ICAR-CIFE, Mumbai. Experimental fishes were procured from a commercial farm from Shramjivi Janta Sahayak Mandal (NGO) taluka-Mahad, district-Raigarh, Mumbai. The fishes were transported to the wet laboratory in polyethylene bags. In order to ameliorate the handling stress, the fishes were given a mild salt (4 ppm) treatment and vitamin C (4 tablets per 500 L of water; each tablet contains 400 mg of ascorbic acid), the next day. The stock was acclimatized for a period of 30 days before the start of the feeding trial. The average weight of fish used for the experiment was 6.45±0.02g. The setup consisted of 12 uniform size plastic rectangular tanks (80 cm × 57 cm × 42 cm, 150 L capacity) covered with lids. One hundred and twenty (120) fishes were randomly stocked in four distinct experimental groups in triplicates with 10 fish per tank following a completely randomized design (CRD). The total volume of the water in each tank was maintained at 120L throughout the experimental period. Round the clock aeration was provided. The aeration pipe in each tank was provided with an air stone and a plastic regulator to control the air pressure uniformly in all the tanks. The feed was fed ad libitum twice daily at 09:00 am in the morning and 04:00 pm in the afternoon.

Fish Sampling

At the end of the feeding trial, fishes fasted for 24 hrs. and then mass weight per replicate was recorded for calculating the growth and nutrient utilization parameters including weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). Survival (%) was also calculated as specified in section 2.7.

Proximate Analysis

For proximate analysis, all the dissected fishes from each replicate were collected, weighed and kept in pre-weighed petri plates. Prior to proximate analysis, the test diets and sampled fish (triplicates) were dried in a hot air oven at 100 °C ±2. After complete drying, the different test diets and fishes were ground into fine powder. Moisture, crude protein, ether extract, ash in the test diets and fish were analyzed following standard methods (AOAC 1995). Moisture (%) was calculated after drying the different test diets or fish in hot air oven at 100 °C ±2 till a constant weight is achieved. Ash content was determined after burning the dried samples in the muffle

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furnace at 550 °C for 6 hrs. Crude protein content (N% × 6.25) was estimated using semi-automatic nitrogen analyzer (2200 Kjeltec auto distillation; Foss Tecator, Hoganas Sweden). Crude lipid was determined by the ether-extraction method in a Soxhlet apparatus (Socsplus, SCS-08-AS, Pelican equipment, Chennai, India). Crude fiber content of different test diets (fat-free samples) were determined by acid (1.25% HCl) and alkali digestion (1.25% NaOH) using FibroTRON (Tulin equipments, India) followed by drying (100 °C ±2) and ashing (in muffle furnace at 550 °C for 4hrs) of the samples.

Amino Acid Analysis

Amino acid analysis of DORB was done following o-Phtalaldehyde (OPA) derivatization method using Agilent 1100 HP-HPLC through Chemstation software. For analysing amino acid of DORB, 10 µl of the digested sample was mixed in 60 µl borate buffer and 10 µl of OPA reagent in dilution vial and vortexed. From this mixture 50 µl was injected in the HPLC column. After running the sample the chromatogram was obtained and the area of the peaks were recorded, calibrated along with the standards and used for calculation.

Fatty acids (FAs) profile

Lipid of DORB was extracted following method of Folch et al. (1957). Lipids were esterified with 0.5 N NaOH methanolic solution catalysed by BF_3 solution (15 ml of 20 % BF_3 diluted in methanol 1:1). The solvent was evaporated under nitrogen stream and the residue was solubilized by 10 ml hexane, from which 1 µl was injected for GC analyses (Shimadzu Qp2010 quadrupole Gas Chromatography-Mass Spectrometer; GC-MS).

Calculations

Weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival (%) were calculated as given below

$$\text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{SGR} = \frac{\text{Log}_e \text{ Final weight} - \text{Log}_e \text{ Initial weight}}{\text{Number of days}} \times 100$$

$$\text{FCR} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

$$\text{PER} = \frac{\text{Net weight gain (wet weight)}}{\text{protein fed}}$$

$$\text{Survival (\%)} = \frac{\text{total number of harvested fish}}{\text{total number of stocked fish}} \times 100$$

Enzyme Assays

Sample preparation

At the end of the experiment, fishes were collected from each tank and anesthetized with clove oil ($50\mu L.L^{-1}$) before sampling. Fishes were then dissected and the tissues viz., liver, intestine, and muscle were immediately removed. A 5% tissue homogenate was prepared in chilled 0.25 M sucrose solution by Teflon coated mechanical homogeniser (REMI Equipments, Mumbai, India). The whole procedure was conducted in ice-cold condition.

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Homogenized samples were centrifuged at 5000 rpm for 10 min at 4 °C. The supernatant was collected in a 5ml tube and stored in a deep freezer (-20 °C) for enzyme assay. Suitable dilution of the samples was done as and when required.

Protein estimation of different tissue

Quantification of the protein of the different tissues was carried out by Bradford method (Bradford, 1976). The Bradford assay relies on the binding of the dye coomassie blue G250 to protein. Tissue homogenate (20 µl) was taken along with 80 µl 0.5M NaCl. After that 1 ml Bradford reagent (Calbiochem, USA) was added and kept for 5 min. Reading was taken at 595 nm against the reagent blank. Protein content was expressed in $mg.g^{-1}$ wet tissue.

Protease activity

Protease activity in the intestinal tissue was determined by the casein digestion method (Drapeau, 1974). The enzyme reaction mixtures consist of 1% casein in 0.05 M Tris-phosphate buffer (pH 7.8) and incubated for 5 min at 37 ^{0}C. Then, the tissue homogenate was added to the enzyme mixture. After the 10 min reaction was stopped by adding 10% TCA followed by filtration of the whole content. The reagent blank was made by adding tissue homogenate just before stopping the reaction and without incubation. One unit of enzyme activity was defined as the amount of enzyme needed to release acid-soluble fragments equivalent to $\Delta 0.001 A_{280}$ per min at 37 ^{0}C and pH 7.8.

Amylase activity

The reducing sugars produced due to the action of glucoamylase and amylase on carbohydrate was estimated using Dinitro-salicylic acid (DNS) method (Rick and Stegbauer, 1974). The reaction mixtures consist of 1% (w/v) starch solution, phosphate buffer (pH 6.9) and the tissue homogenate. The reaction mixtures were incubated at 37 ^{0}C for 30 min. DNS was added after incubation and kept in boiling waterbath for 5 min. After cooling, the reaction mixture was diluted with distilled water and absorbance was measured at 540 nm. Maltose was used as the standard. Amylase activity was expressed as mole of maltose released from starch per min at 37 ^{0}C.

Lipase activity

The lipase activity was determined by the titrimetric method of Cherry and Crandell (1932), which is based on the measurement of fatty acids released by the enzymatic hydrolysis of triglycerides present in a stabilized emulsion of olive oil. The amount of a standard sodium hydroxide solution used to titrate the fatty acids released taken as an index of lipase activity of the crude enzyme extract.

The reaction mixture consists of 1 ml of stabilized lipase substrate and 1.5 ml of 0.1 M Tris-HCl buffer at pH 8, to which 1.0 ml of the crude enzyme extract was added. The assay mixture was incubated for 24 hrs at 37 °C after which the reaction was stopped by addition of 3 ml of 95% ethyl alcohol. The mixture was then titrated against 0.01N NaOH using 0.9 % (w/v) phenolphthalein as an indicator.

Metabolic Enzymes

Aspartate aminotransferase (AST; E.C.2.6.1.1) and Alanine aminotransferase (ALT; E.C.2.6.1.2)

AST activity in liver and muscle tissues of *Labeo rohita* was assayed following method of Wooton (1964). The substrate comprised of 0.2M DL- aspartic acid and 2mM α-ketoglutarate in 0.05M phosphate buffer (pH 7.4). To

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the treatment and control tubes, 0.5ml of the substrate was added. The reaction was started by adding 0.1ml of tissue homogenate in the treatment tube. The assay mixture was incubated at 37°C for 60 min. The reaction was terminated by adding 0.5ml of 1mM 2, 4 dinitrophenyl hydrazine (DNPH). In the control tubes, the enzyme source was added after adding DNPH solution. The tubes were held at room temperature for 20 min with occasional shaking. Then 5ml of 0.4 N NaOH solution was added and the contents were thoroughly mixed. After 10 min, the OD was recorded at 540nm against the blank. The AST activity was expressed as nanomoles oxaloacetate formed / mg protein / min at 37 °C. The procedure adopted for the estimation of ALT activity was same as that for AST except that the substrate comprised of 0.2 M D, L- alanine instead of aspartic acid. The ALT activity was expressed as nanomoles pyruvate formed / mg protein / min at 37°C.

Digestibility trial

After the feeding trial, digestibility trial was conducted for a period of 30 days. The digestibility study was carried out by indirect method using the chromic oxide (Cr_2O_3) marker (Alexander *et al*., 2010; Fawole et al., 2016). The experimental feeds were prepared as specified earlier by incorporating 0.5% chromic oxide (Cr_2O_3).Before starting the digestibility trial the fishes were acclimated for a period of 10 days to the respective diet. The fishes were fed once daily at 10:00 AM. The unconsumed feeds were removed 1 h after daily feeding. Faecal matters were collected once a day at about 08:00 AM. The digestibility trial was conducted for 30 days to collect enough faecal samples, using a modified method described by Shiau and Liang (1994). Faecal samples were collected by simple siphoning from the bottom of each tank. The faeces were then freeze-dried and stored at -20 °C until analysis (Shiau and Liang, 1994; Usmani *et al.*, 2003).The faecal samples collected were analysed for crude protein, lipid and ash content following methods of AOAC (1995). Quantification of chromium oxide content of the feed and faecal matters were carried out by the method of Furukawa and Tsukahara (1966).The apparent digestibility coefficients (ADCs) of dry matter, protein, lipid and carbohydrate were calculated as follows (Cho and Slinger, 1979):

$$\text{ADC of dry matter (\%)} = 100 \times \left(1 - \frac{\%\ \text{Chromium oxide } in\ diet}{\%\ \text{Chromium oxide } in\ feaces}\right)$$

$$\text{ADC of nutrients (\%)} = 100 \times \left(1 - \frac{\%\ \text{Chromium oxide } in\ diet \times \%\ nutrient\ in\ feaces}{\%\ \text{Chromium oxide } in\ feaces \times \%\ nutrient\ in\ diet}\right)$$

Statistical analysis

The differences in the mean values of all parameters were examined following one-way ANOVA using the IBM SPSS package, version 22. Duncan's multiple range test ($P<0.05$) was performed to examine the differences in above variables between the different treatments.

Results

Proximate Composition of DORB

Proximate composition of DORB and their amino acid and fatty acid composition are presented in Table 1 & 2 respectively. Proximate composition of DORB shows that it contains 14.06±0.12 % crude protein, 0.59±0.05%

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ether extract and 14.92±0.10% crude fibre. Fatty acid composition of DORB shows that it do not contain n-3 essential fatty acid like EPA (C20:5n-3) and DHA (C22:6n-3).

Proximate Composition of the Diets

Proximate composition of the diets are presented in Table 4. Dry matter content of the diets varied from 92.43±0.02 (T1) to 94.73 % (T4). Crude protein % was highest in the commercial feed (32.01±0.01) T4, whereas it was lowest for T1 (14.16±0.01). EE % was highest for T3 (7.98±0.06) diet, whereas it was lowest (4.05±0.01) for T4 feed. Crude fibre and total ash contents of different groups varied from 13.11±0.01 to 16.53±0.01 and 11.51±0.03 to 13.60±0.06, respectively. The gross energy content was highest in the T4 (16.18 MJ/Kg) whereas it was lowest for T1 (14.86 MJ/Kg).

Growth Performance and Survival

Growth performance and survival (%) of the different test groups are presented in Table 5. Highest WG% and SGR along with lowest FCR were recorded for the fishes fed either commercial diet (T4) or T3 diet (experimental diet supplemented with phytase, xylanase & cellulase enzyme, amino acids and fatty acids). Highest PER was recorded with fishes either fed with T1 or T3 diet, whereas it was lowest for the fishes fed the commercial diet (T4). Survival (%) did not vary significantly among the groups ($p>0.05$), which ranged from 93% (T1) to 100% (T4).

***In vivo* Digestibility**

ADCs of dry matter, protein, lipid and carbohydrate of different test diets are presented in Table 6. There were significant differences in ADCs of dry matter, protein, lipid and carbohydrate of the different groups ($p<0.001$). Highest and lowest apparent dry matter digestibility were observed for the T3 (72.80±0.17) and T4 groups (64.58±0.74), respectively. Similarly, highest and lowest apparent crude protein digestibility were recorded for the T4 (88.25±0.43) and T1 (83.92±0.02) groups, respectively. Apparent lipid digestibility was found in the range of 95.18±0.06 (T2) to 96.20%±0.01 (T4). Apparent carbohydrate digestibility was highest for the T3 group (64.04±0.50), whereas, it was lowest for T1 group.

Digestive and Metabolic Enzyme Activity

Digestive and metabolic enzyme activity of *Labeo rohita* fed with different test diets are presented in Table 7. Protease, lipase and amylase activities were significantly lower in T1group than the other experimental groups (T2, T3 and T4). However, the activities of above enzymes were similar ($P>0.05$) in T2, T3 and T4 groups. AST and ALT enzyme activities in the liver varied significantly among the groups ($p<0.001$). The activity of AST in the liver was highest for the fishes fed with T4 diet, whereas it was found lowest for the T2 diet. The activity of ALT in liver was similar in T3 and T4 groups, whereas its activity was found lowest in T1 group. AST and ALT enzyme activities in the muscle also varied significantly among the groups ($p<0.001$). Activity of AST in muscle was similar in T1 and T4 & T2 and T3; T2 and T3 were found to have higher activity of AST in muscle than T1 and T4 groups. The activity of ALT in muscle was found highest for T3 group (11.89±0.02) and lowest for T1group (8.06±0.01).

Proximate Composition of the Whole Body of Fish

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Proximate composition of the whole body of fish fed with different test diets are presented in Table 8. The whole body CP% varied significantly among the groups. Highest body CP% was observed for fishes fed with the T4 diet which was not significantly different than fishes fed with T3 diet. Lowest body CP% was observed for fishes fed with T1 diet. Whole body EE % varied significantly ($p<0.05$) among the group. Lowest body EE% was observed for fishes fed with T1 diet, whereas whole body EE % did not vary significantly for fishes fed with T2, T3 and T4 diets. The whole body moisture % and ash % did not vary significantly ($p>0.05$) among the groups fed different experimental diets.

Discussion

Combinatorial supplementation of exogenous enzymes (phytase, xylanase and cellulase), essential amino acids (lysine and methionine) and essential fatty acids (EPA and DHA) appeared to have beneficial effects on nutrient utilization and growth performance of *Labeo rohita*. In the present study, it was found that when fishes were fed DORB based diet without addition of cellulase enzyme even after supplementing it with other enzymes (phytase, xylanase), essential amino acids (lysine and methionine) and essential fatty acids (EPA and DHA) did not improve the growth performance and nutrient utilization as compared to only DORB based diet. However, the growth performance and nutrient utilization of DORB based diet with the addition of cellulase enzyme along with essential amino acids (lysine and methionine) and essential fatty acids (EPA and DHA) were very much comparable with fishes fed with commercial carp diet. The better performance of T3 diet as compared to other diet is evident from the *in vivo* digestibility studies, where it was found that the supplementation of cellulase enzyme improved the apparent dry matter digestibility and apparent carbohydrate digestibility of the T3 diet as compared to the other experimental diets devoid of cellulase enzyme (T1& T2). The comparable growth performance of T3 as compared to the T4 group may be contributed by increased dry matter and carbohydrate digestibility of T3 group due to exogenous enzyme supplementation of DORB. In our previous study with DORB, we found an increased utilization of DORB when the DORB was supplemented with exogenous enzymes in comparison to diet in which the enzyme was not supplemented in *Labeo rohita* diet (Ranjan *et al*., 2017). Zhou et al. (2013) also found that cellulase supplemented duckweed based diet promoted the growth of grass carp. Present result is also supported by finding of Ai *et al.* (2007), Yildrim and Turan (2010), Ghomi *et al.* (2012) and Zamini *et al.* (2014), who also reported positive effect of various commercial multi-enzyme complex (phytase, xylanase, β-glucanase, amylase, cellulase and pectinase) on the growth performance and feed efficiency of *Lateolabrax japonicas, Clarias gariepinus, Huso huso* and *Salmo trutta,* respectively. Improved growth performance and nutrient utilization of *Labeo rohita* fed DORB based diet supplemented with exogenous enzymes may be contributed to the elimination of anti-nutritional factors and improving the utilization of dietary energy and amino acid. Similar findings were also observed by Farhangi and Carter (2007); Lin *et al.* (2007); Soltan (2009) in rainbow trout, hybrid tilapia and Nile tilapia, respectively, when they fed the diet supplemented with exogenous enzymes.

Non-starch polysaccharide (NSP) degrading enzymes (e.g. cellulase, xylanase, etc.) are capable of disrupting plant cell wall integrity, consequently, this enhances rapid digestion by reducing viscosity in the gut (Zijlstra *et al.*, 2010; Bedford & Cowieson, 2012). Apart from the potential of exogenous enzymes to enhance nutrient utilisation and growth (Adeola & Cowieson, 2011), they may alter substrates availability for specific populations of gut

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microbes, thus, altering bacterial community composition or activities (Bedford and Cowieson, 2012; Kiarie *et al.*, 2013; Zhou *et al.*, 2013; Jiang *et al.*, 2014).

Dietary enzyme supplementation enhances the nutrient digestibility in an animal including fish as reported by many workers (Lin *et al.*, 2007; Zhou *et al.*, 2009, Kumar *et al.*, 2006). An increased activities of digestive enzymes were recorded due to exogenous supplementation of enzymes in *Labeo rohita*. Xavier *et al.* (2012) also found that cellulase supplementation increases protease, lipase and amylase activity of *Labeo rohita*. Similar observation were also made by many researchers for different species, Ng *et al.* (1998) in *Ictalurus punctatus*; Van Weered *et al.* (1999) in *Clarias gariepinus*; Ng and Chen (2002) in *Clarias macrocephalus* × *C. gariepinus*., Kumar *et al.* (2006) in *Labeo rohita*, Lin *et al.* (2007) in *Oreochromis niloticus* × *Oreochromis aureus*; Zhou *et al.* (2013) in *Ctenopharyngodon idella*.

Increased endogenous enzyme activities lead to increased apparent nutrient digestibilities in *Labeo rohita* due to dietary exogenous enzyme, which is consistent with the findings of Ogunkoya *et al.* (2006), who also found that exogenous enzyme supplementation to the soybean meal based diet improves the apparent digestibility coefficient of dry matter, protein, lipid and carbohydrate in *Oncorhynchus mykiss*. This is confirmed by many workers while working with other sp. also (VanWeered *et al.* (1999) in *Clarias gariepinus*; Ng and Chen (2002) in *Oreochromis* sp. and Lin et al. (2007) in *Oreochromis niloticus* × *Oreochromis aureus*.

Whole body protein and lipid were higher in fishes fed with exogenous enzyme supplemented diets suggesting that an increased availability of non-protein energy in the enzyme supplemented diets thus allowing more dietary protein and energy for growth as higher nutrient digestibilities were found in the cocktail enzyme supplemented groups. Our finding is also in accordance with the findings of Magalhães *et al.* (2016) and Yildrim and Turan (2010), who also observed higher whole body protein and lipid in *Diplodus sargus* and *Clarias gariepinus*, respectively when fed with the diet supplemented with exogenous multi-enzymes complex (containing phytase, xylanase ,cellulase and other carbohydrases) .

AST and ALT are the two most important transaminase found in the liver of fish, which is responsible for the deamination of amino acids (Walton & Cowey, 1982; Alexis & Papaparaskeva-Papoutsoglou, 1986; Fynn-Aikins *et al.*, 1993; Enes *et al.*, 2006; Kumar *et al.*, 2009; Kumar *et al.*, 2010). In addition to liver, muscle tissues also have aminotransferase activity in fishes (Alexis & Papaparaskeva- Papoutsoglou, 1986; Kumar *et al.*, 2010). The increased activity of AST and ALT activity in liver and muscle tissues contributed to the higher protein content in T2, T3 & T4 groups as compared to the T1 group in which crude protein in the diet was significantly lower than the other groups. Fish preference to protein as the energy source may be cause for higher transaminase activity in higher protein fed groups (Kumar *et al.*, 2006; Kumar *et al.*, 2009).

Conclusion

Growth performance of *Labeo rohita* can be enhanced as compared to commercial carp feed if the DORB is supplemented with phytase (0.01%), cocktail of xylanase and cellulase (0.075%) along with the supplementation of deficient amino acids (L-lysine at 1.4% and DL-methionine at 0.4%) and ω-3 fatty acids (EPA and DHA) (0.5%). This strategy can be useful for improving the nutrient utilization from DORB. Hence, the marginal farmers, who are solely dependent on farm-made feed especially DORB instead of commercial feed may use this strategy for enhancing the aquaculture productivity.

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Table 1 Proximate composition of DORB (% DM basis)

Proximate composition	Content (%)
MOISTURE %	9.20±0.22
CP %	14.06±0.12
EE %	0.59±0.05
ASH %	11.25±0.23
CF %	14.92±0.10
Indispensable amino acid (% of total protein)	
Arginine	12.82
Histidine	2.65
Isoleucine	4.45
Leucine	8.70
Lysine	2.81
Phenylalanine	5.67
Methionine	2.17
Threonine	6.92
Valine	5.75
Dispensable amino acid (% of total protein)	
Alanine	9.12
Glycine	9.05
Aspartic acid	14.63
Glutamic acid	8.27
Serine	4.07
Tyrosine	2.85

DORB = De-oiled-rice bran, CP = Crude Protein, EE=Ether Extract, CF= Crude Fibre, DM=Dry matter.

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Table 2 Fatty acid composition of DORB

Fatty acids (% of total lipid)	DORB
C14:0	ND
C15:0	ND
C16:0	30.16
C17:0	0.12
C18:0	ND
C18:1n-9	23.77
C18:2n-6	28.9
C18:3n-3	9.57
C20:0	ND
C20:5n-3	ND
C22:6n-3	ND
C24:0	7.48
C26:0	ND
SFA	30.28
MUFA	23.77
PUFA	38.47
ω-6	28.9
ω-3	9.57
ω-6/ ω-3	3.02

DORB - De-oiled-rice bran; ND-Not detected

Table 3 Ingredient composition of Experimental diets (% DM basis)

Ingredients (%)	T1	T2	T3
DORB	90	87.66	87.595
CMC	2	2	2
Cod liver oil	3.5	3.5	3.5
Soybean oil	3.5	3.5	3.5
Vitamin- mineral mix	1	1	1
Phytase	0.01	0.01	0.01
Xylanase	0.01	0.01	0
L-Lysine	0	1.4	1.4
L-Methionine	0	0.4	0.4
Liquid enzyme (cellulase + xylanase)	0	0	0.075
ω-3 fatty acids (EPA & DHA)	0	0.5	0.5
BHT	0	0.02	0.02

DORB= De-oiled Rice Bran, CMC=Carboxy Methyl Cellulose, BHT-Butylated Hydroxy Toluene, DM=dry matter.

L-Lysine and L-Methionine were procured from HiMedia, India.

ω-3 fatty acids (EPA & DHA) were procured from MERCK, India (Maxepa tablet, each tablet contains 180 mg EPA and 120 mg DHA)

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Composition of Vitamin- mineral mix (PREMIX PLUS) (quantity.kg^{-1})

Vitamin A (55,00,000 IU); Vitamin D_3 (11,00,000 IU); Vitamin B_2 (2,000 mg); Vitamin E (750 mg); Vitamin K (1,000 mg); Vitamin B_1 (100 mg), Vitamin B_2 (200 mg), Vitamin B_6(1,000 mg); Vitamin B_{12} (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 (2,000 mg); Co (450 mg) (10g);Selenium(125mg).

Table 4 Proximate composition of Experimental diet (% Dry matter basis)

Proximate composition	T1	T2	T3	T4
Dry matter	92.43±0.02	94.40±0.01	94.32±0.01	94.73±0.01
CP	14.16±0.01	18.11±0.01	18.18±0.01	32.01±0.01
EE	7.45±0.02	7.88±0.03	7.98±0.06	4.05±0.01
NFE	48.36±0.08	44.28±0.02	43.91±0.01	39.32±0.02
CF	16.43±0.03	16.53±0.01	16.39±0.03	13.11±0.01
Ash	13.60±0.06	13.20±0.01	13.54±0.06	11.51±0.03
GE (MJ/Kg)	14.86±0.02	15.26±0.01	15.25±0.02	16.18±0.01

All values are Mean ± SE, obtained from three replicates.
T1 -DORB supplemented with phytase and xylanase ; T2 -DORB supplemented with phytase, xylanase, L-lysine , L-methionine , combination of EPA and DHA ; T3 -DORB supplemented with phytase , combination of xylanase and cellulase , L-lysine , L- methionine and combination of EPA and DHA ; T4 : commercially available carp feed (Procured from Godrej Agrovet Pvt. Ltd. India).
CP = Crude Protein, EE=Ether Extract, CF= Crude Fibre, NFE=Nitrogen free extract, GE=Gross Energy, SE=Standard error.

Table 5 Growth performance, Body indices and Survival (%) of *Labeo rohita* fed with different test diets

Treatments	T1	T2	T3	T4	p-value
WG%	78.99^{a}±0.36	81.96^{b}±0.57	100.83^{c}±0.72	102.59^{c}±0.51	<0.001
SGR	0.97^{a}±0.00	1.00^{b}±0.01	1.16^{c}±0.01	1.18^{c}±0.00	<0.001
FCR	2.89^{c}±0.03	2.74^{b}±0.04	2.24^{a}±0.01	2.24^{a}±0.02	<0.001
PER	2.47^{c}±0.02	2.03^{b}±0.03	2.48^{c}±0.01	1.40^{a}±0.01	<0.001
HSI	0.86^{a}±0.03	0.99^{b}±0.06	0.98^{b}±0.05	1.04^{b}±0.02	<0.05
ISI	4.43±0.09	4.30±0.11	4.29±0.08	4.34±0.10	>0.05
Survival %	93	93	96	100	>0.05

All values are Mean ± SE, obtained from three replicates. Values in the same row with different superscript letters are significantly different (P< 0.05).
T1 -DORB supplemented with phytase and xylanase ; T2 -DORB supplemented with phytase, xylanase, L-lysine , L-methionine , combination of EPA and DHA ; T3 -DORB supplemented with phytase , combination of xylanase and cellulase , L-lysine , L- methionine and combination of EPA and DHA ; T4 : commercially available carp feed.
WG %= Weight Gain %, SGR= Specific Growth Rate, FCR=Feed Conversion Ratio, PER= Protein Efficiency Ratio.

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Table 6. Apparent digestibility coefficient of nutrients in different experimental diet

Treatments	DMD (%)	CPD (%)	LD (%)	CHOD (%)
T1	69.28^{b}±0.34	83.92^{a}±0.02	95.62^{b}±0.05	56.42±0.04
T2	70.43^{b}±0.21	85.25^{b}±0.19	95.18^{a}±0.06	55.54±0.01
T3	72.80^{c}±0.17	86.10^{b}±0.48	96.16^{c}±0.08	64.04±0.50
T4	64.58^{a}±0.74	88.25^{c}±0.43	96.20^{c}±0.01	59.44±0.41
p-value	<0.001	<0.001	<0.001	<0.001

All values are Mean ± SE, obtained from three replicates. Values in the same column with different superscript letters are significantly different (P< 0.05).
T1 -DORB supplemented with phytase and xylanase ; T2 -DORB supplemented with phytase, xylanase, L-lysine , L-methionine , combination of EPA and DHA ; T3 -DORB supplemented with phytase , combination of xylanase and cellulase , L-lysine , L- methionine and combination of EPA and DHA ; T4 : commercially available carp feed.
DMD: Dry matter digestibility; CPD: Crude protein digestibility; LD: Lipid digestibility; CHOD: Carbohydrate digestibility

Table 7 Digestive and metabolic enzyme activity of *Labeo rohita* fed with different experimental diets

Treatments	T1	T2	T3	T4	p-value
Protease	0.15^{a}±0.01	0.25^{b}±0.02	0.28^{b}±0.01	0.27^{b}±0.01	0.001
Amylase	0.63^{a}±0.03	0.87^{b}±0.01	0.94^{b}±0.03	0.87^{b}±0.03	<0.001
Lipase	0.73^{a}±0.03	0.90^{b}±0.06	0.93^{b}±0.03	0.83^{ab}±0.03	0.036
AST Liver	10.20^{b}±0.01	10.07^{a}±0.01	10.31^{c}±0.03	10.71^{d}±0.04	<0.001
AST Muscle	29.07^{a}±0.02	29.47^{b}±0.06	29.43^{b}±0.02	29.03^{a}±0.02	<0.001
ALT Liver	5.45^{a}±0.07	8.02^{b}±0.01	10.52^{c}±0.06	10.60^{c}±0.01	<0.001
ALT Muscle	8.06^{a}±0.01	10.90^{b}±0.02	11.89^{d}±0.02	11.59^{c}±0.01	<0.001

All values are Mean ± SE, obtained from three replicates. Values in the same row with different superscript letters are significantly different (P< 0.05).
Protease activity expressed as micromol of tyrosine released min $^{-1}$mg protein -^{1}
Amylase activity expressed as micromol of maltose released min $^{-1}$mg protein -^{1}
Lipase activity expressed as units mg protein^{-1}
AST: activity expressed as nanomoles of sodium pyruvate formed min $^{-1}$mg protein -^{1}at 37 °C
ALT: activity expressed as nanomoles of oxaloacetate released min $^{-1}$mg protein -^{1} at 37 °C

Table 8 Carcass composition of *Labeo rohita* fed with different experimental diets

Treatments	Moisture (%)	CP (%)	ASH (%)	EE (%)
T1	75.93±0.03	13.96^{a}±0.02	3.72±0.01	3.10^{a}±0.01
T2	75.78±0.20	14.81^{b}±0.02	3.78±0.01	3.28^{b}±0.01
T3	75.56±0.37	15.09^{c}±0.02	3.76±0.02	3.35^{b}±0.06
T4	75.02±0.17	15.14^{c}±0.03	3.77±0.01	3.35^{b}±0.03
p-value	0.089	<0.001	0.103	0.002

All values are Mean ± SE, obtained from three replicates. Values in the same column with different superscript letters are significantly different (P< 0.05).
T1 -DORB supplemented with phytase and xylanase ; T2 -DORB supplemented with phytase, xylanase, L-lysine, L-methionine, combination of EPA and DHA ; T3 -DORB supplemented with phytase, combination of xylanase and cellulase, L-lysine, L- methionine and combination of EPA and DHA ; T4 : commercially available carp feed.
CP- Crude protein, EE- Ether extract, SE- Standard error.