Evaluation of Growth Performance in *Mugil cephalus* L. Juveniles Fed Diets Incorporated with Fermented Plant Feedstuffs Replacing Fishmeal or Diets Supplemented with Fish Gut Bacteria

De D1,2*, Ghoshal TK1, Biswas G1, Mukherjee S1, Kumar S1, Anand PSS2, Raja RA1 and Vijayan KK2

1ICAR-Kakdwip Research Centre of Central Institute of Brackish Water Aquaculture, South 24 Parganas, West Bengal, India
2ICAR-Central Institute of Brackish Water Aquaculture, Chennai, India

Abstract

An experiment was conducted to evaluate utilization of fermented ingredients for replacing fish meal and examine the efficacy of live bacterial supplement in diets of *Mugil cephalus* juveniles. The experiment had six treatment groups (I, II, III, IV, V and VI) of *M. cephalus* juveniles (0.12 ± 0.001 g) with triplicate. A control diet (group I) was prepared with plant feedstuffs (wheat flour, rice bran, mustard cake) and fish meal. For other diets, plant feedstuffs (rice bran, mustard cake, sunflower cake, sesame cake, azolla meal, leucaena leaf meal) were fermented with two gut bacteria i.e., Bacillus sp. DDKRC1, Bacillus subtilis DDKRC5., isolated from Lates calcifer and Chanos Chanos, respectively. Diets for groups II, III, IV and V were formulated with fermented ingredients replacing 25%, 50%, 75% and 100% of fish meal by weight. Diet for group VI was prepared by supplementing the control feed with mixture of Bacillus subtilis DDKRC5 and Bacillus sp. DDKRC1, (1:1) at 1% (w/w) of feed. The Diets were offered twice daily for a period of six weeks. Supplementation of live bacterial mixture with control diet could improve (P<0.01) nutrient digestibility, growth rate, feed conversion ratio (FCR), protein efficiency ratio (PER), body protein and lipid content and immune response and fermented ingredients could replace 75% of fish meal in diet of *M. cephalus* without affecting growth rate, FCR, PER, survival, body protein and lipid content and hematological indices.

Keywords: *Mugil cephalus*; Fish meal; Fermented ingredients; Growth; Digestibility; Bacterial supplement; Plant ingredients

Introduction

Global aquafeed production is expected to increase over 80 million tons by 2020 [1]. This development shows a trend towards intensification of aquaculture and dependency on formulated aquafeed. Fish meal is a major ingredient in aquafeed to meet up high protein requirement of fish. Because of high cost and uncertainty in supply of good quality fish meal, present research is more focused to find out some alternative plant protein sources for replacing it in fish diets [2,3] to minimize the dependency on fishmeal and reduce the cost of formulated feeds. Notable emphasis was put on the use of different plant protein sources which are not directly usable for human consumption [4]. But these alternative plant protein sources are low in protein and rich in fibre and being monogastric animal, fish cannot efficiently utilize fibre rich plant ingredients. In addition to that, these plant proteins are deficient in certain essential amino acids, minerals and contain anti-nutritional factors and complex carbohydrates [5,6]. Several strategies are available to optimally utilize the plant feedstuffs to reduce the dependence on fish meal. Solid state fermentation (SSF) is an ideal approach for the utilization of plant protein sources for animal [7-9]. As cellulosytic microbes are capable of breaking complex ligno-cellulosic bonds present in the cell wall of plant protein ingredients, those microorganisms can be cultivated on agro-industrial products with production of large amounts of cells rich in proteins that commonly contain all the essential amino acids, in addition to favourably high vitamin and mineral levels [10]. Another approach to increase the nutrient utilization of plant protein-based diet is the supplementation of live bacterial supplement with diet [11]. Supplementation of beneficial bacteria improves enzyme activity, feed digestibility and feed utilization, health and performance in aquatic animals [12-14]. *Bacillus* has been widely used as potential probiotics [15-18] since they secrete a variety of antimicrobial compounds and exoenzymes [19,20]. Grey mullet (*Mugil cephalus*) is a reasonably significant aquaculture species contributing to a quite large fisheries of estuarine and coastal regions in many countries such as China [21], Egypt [22,23] India [24-26], Israel [27] Italy [28], New Zealand [29], Nigeria [30], Sri Lanka [31], Taiwan [32,33], Tunisia [34] etc.. Formulated feed for this species has been developed by Central Institute of Brackish Water Aquaculture using locally available feed ingredients, fish meal and is being used for culture [35]. To make the feed more cost-effective attempt has been made to reduce fish meal content of the feed using fermented plant ingredients. Potential cellulosyltic (*Bacillus* sp. DDKRC1) and amylolytic bacteria (*Bacillus subtilis* DDKRC5), isolated from the gut of brackish water fish, were used for fermentation of plant ingredients. In the present study, we aimed to evaluate the potential of these nutrients enriched plant protein ingredients to replace fish meal in diet of grey mullet and to compare the efficacy of approaches for using bacterial culture (*Bacillus* sp. DDKRC1 and *Bacillus subtilis* DDKRC5.), either for fermentation or as feed supplement (probiotics), for better utilization of fibre rich plant ingredients in diet of grey mullet.

Materials and Methods

The present study on replacement of fish meal with fermented ingredients and the effect of cellulosyltic and amylolytic gut bacteria as a feed supplement on growth performance, apparent nutrient digestibility and digestive enzyme activity of grey mullet, *M. cephalus*,...
was conducted in fish nutrition laboratory at Kakdwip Research Centre of ICAR-CIBA, Kakdwip (Lat. 21°51' 15.01" to 21°51' 30.77" N, Long. 88°10' 58.44" to 88°11' 12.69" E), West Bengal, India.

**Fermentation of ingredients**

*Bacillus* sp. DDKRC1. (JN641289), a potential cellulyotic bacterium and *Bacillus subtilis* DDKRC5. (JN641293), a potential amylolytic bacterium, were isolated from the gut of adult Asian seabass, *Lates calcarifer* and milkfish, *Chanos Chanos*, respectively and identified through 16S rDNA sequencing and the fermentation has been done according to De et al. [36]. These isolates were used for fermentation of plant ingredients (Rice bran, mustard cake, sesame cake, sunflower cake, *Leucaena leucocephala* leaf meal and Azolla meal). All the ingredients were ground and sterilized in autoclave at 15 lb pressure for 15 min. Sterile distilled water was added to ingredients to bring the desired (50%) moisture content in feed ingredients. Then a mixture of both bacterial culture (× 10⁷ cfu mL⁻¹) was added in equal ratio (1:1) at 1% (v/w) of feed ingredients in conical flask and mixed thoroughly and incubated at 34°C in orbital shaker incubator (shaking speed 120 rpm).

Both the bacteria were grown in nutrient broth (Himedia, India) for 24 h at 34°C prior to fermentation. Incubation was carried out for 48 h, as found optimum considering the dry matter (DM) loss and protein enrichment of feed in earlier study. After fermentation feed ingredients were dried at 60°C for 24 h and used for preparation of diet.

**Experimental diets preparation**

Different plant ingredients and fish meal were used for preparation of control diet (crude protein ~ 28%) for *M. cephalus*. All the feed ingredients (including fermented ingredients), except bacterial culture, mineral-vitamin mixture, amino acid mixture and fish oil, were mixed with water and cooked in autoclave for 20 minutes with exposure at 15 psi, 121°C for 5 minutes. The remaining ingredients including bacterial culture were mixed after cooling the dough using dough mixer. The dough was passed through a mincer with a die (2 mm diameter) to get spaghetti-like strings. Feed strings were air dried for 1 hr. at ambient temperature and fed to fishes [11]. The control diet (group I) had no bacterial supplementation. The diets for groups II, III, IV and V were prepared using fermented ingredients replacing 25, 50, 75 and 100% of fishmeal by weight, respectively (Table 1). Diet for group VI was prepared using similar ingredients as of control diets but supplemented with the culture of *Bacillus subtilis* DDKRC5. (14.25 × 10⁷ cfu mL⁻¹) and *Bacillus* sp. DDKRC1. (2.94 × 10⁷ cfu mL⁻¹) in equal ratios (1:1) at 1% (v/w) at the feeding time. Feed for group V1 was prepared twice daily before feeding to maintain the efficacy of bacterial culture. Chronic oxide was added at 0.5% in each diet as a marker.

**Experimental set up**

The feeding trial was conducted for 42 days under laboratory condition in the circular FRP tank (Diameter 120 cm, Height 70 cm) containing 600 L dechlorinated brackish water with continuous aeration. Grey mullet juveniles initially weighing 0.12 ± 0.001 g (mean ± SE) were randomly distributed in eighteen FRP tanks with 15 juveniles per tank for six groups with triplicate. Fish were acclimatized about 7 days before the start of the feeding trial. The daily ration was offered twice daily at 10:00 and 16:00 hours at a feeding rate of 10% of the total biomass [11]. The feed given was adjusted at 7-day intervals after calculating the biomass through intermediate sampling and counting the numbers of fish survived. The left-over feed was siphoned off after 2 h of feeding, dried in an oven at 105°C for 16 h. Daily feed consumption was estimated by the difference between feed offered and uneaten feed in dry weight. Fifty percent of brackish water in the experimental tanks was replaced at 2-day intervals with fresh dechlorinated brackish water. The faecal samples were collected twice daily after 4 h of offering feed from each tank by pipetting [37]. The oven-dried (60°C) faecal samples were analyzed for digestibility estimation. Ten fish from each tank were collected at the end of the experiment for gut bacterial population, intestinal enzyme and hematological study.

**Water quality**

Water quality parameters (pH, temperature, DO, salinity and alkalinity) were measured daily and maintained throughout the study period at appropriate levels for the growth of fish. Water temperature was measured using a thermometer. Dissolved oxygen (mg L⁻¹), pH and salinity (g L⁻¹) were measured using HACH HQ30d portable multi-

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control (D1)</th>
<th>Fish meal replaced (25%) (D2)</th>
<th>Fish meal replaced (50%) (D3)</th>
<th>Fish meal replaced (75%) (D4)</th>
<th>Fish meal replaced (100%) (D5)</th>
<th>Mixture of live bacterial supplement (D6)</th>
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<tr>
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<tr>
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<td>110</td>
<td>55</td>
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<td>220</td>
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<tr>
<td>(Min vit mix + amino acid)*</td>
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<tr>
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<td>5</td>
<td>5</td>
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</tbody>
</table>

*Ingredients were purchased from Kamala Khadya Bhandar, Kakdwip, South 24 Parganas, West Bengal, India

*Vitamin mix (g Kg⁻¹):* Vitamin A: 40.8 million IU; Vitamin D: 8 million IU; Vitamin E: 40 gm; Vitamin K: 8 g; Riboflavin: 16 gm; Vitamin B1: 6 gm; Vitamin B2: 40 gm; Pantothenic acid: 40 gm; Nicotinic acid: 80 gm; Folic acid: 4 gm

*Mineral mix (g Kg⁻¹):* CaCO3: 28.0; K2SO4: 10.0; MgSO4·7H2O: 12.5; CuSO4·5H2O: 0.2; FeCl3·6H2O: 0.5; MnSO4·H2O: 0.5; KI: 0.01; ZnSO4·7H2O: 1.0; CoSO4·7H2O: 0.01; Cr2(SO4)3·2H2O: 0.05

*Mineral mix (g Kg⁻¹):* L-Arginine HC: 120.35; L-Cystine: 69.8; L-Tryptophan: 2; L-Histidine HC: 2; DL-Alanine: 22.6; L-Aspargine Na: 10.0; L-lysine HC: 193.3; L-Valine: 7; Glycine: 75.2; Glutamic acid: 494.6

Table 1: Composition of experimental diets for *Mugil cephalus*.
parameter meter. Alkalinity of water was measured as per the method of APHA [38]. Total microbial count and *Vibrio* count of water was recorded at 14-day intervals using tryptone soya agar (TSA) and thiosulphate citrate bile salts sucrose (TCBS) agar, respectively.

**Sample collection, chemical analysis and data collection**

The proximate analyses of feed and faecal samples were determined following AOAC [39]. Hemicellulose and cellulose contents of feed and faecal were measured according to the Van Soest methods [40,41]. Chromic oxide in the diets and faecal samples were estimated by the wet digestion method of Furukawa and Tsukahara [42]. Average live weight gain (%), specific growth rate (SGR; % day⁻¹), apparent feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated using standard methods [43].

The apparent digestibility coefficient (ADC) of nutrients was calculated according to De Silva and Anderson [44], using the following formula:

\[
\text{Percentage of weight gain (g/kg)} = \left( \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial weight}} \right) \times 100
\]

**Microbial culture**

Five fish from each replication of all the treatments were dissected on an ice tray to remove the gastrointestinal (GI) tract to determine the intestinal microbial population at the end of the feeding trial. The entire GI tract was homogenized with five times (w/v) of sterile chilled phosphate-buffered saline with 0.9% NaCl (pH 7.2). The homogenate of each intestine of each fish was 10-fold serially diluted and 0.1 mL of each dilution was poured aseptically under laminar flow on sterilized TSA, carboxy methyl cellulose agar (CMC agar), starch agar (SA) and peptone gelatin agar (PGA) media containing plates, in duplicate for total heterotrophic population, cellulolytic, amylolytic and proteolytic bacterial count, respectively. These culture plates were incubated at 30°C for 48 h and examined for the development of bacterial colonies [11]. By multiplying the number of colonies formed on each plate by the reciprocal of dilution, colony numbers per unit sample volume of gut homogenate were determined [45].

**Media composition**

I. **TSA medium (gL⁻¹):** Pancreatic digest of casein, 15; papain digest of soybean meal, 5; NaCl, 5; agar, 15; pH 7.3.

II. **TCBS agar (gL⁻¹):** Protease peptone, 10; yeast extract, 5; sodium thioulate, 10; sodium citrate, 10; Oxgall, 8; sucrose, 20; NaCl, 10; ferric citrate, 1; bromothymol blue, 0.04; thymol blue, 0.04; agar, 15; pH 8.6 ± 0.2 at 25°C.

III. **CMC-agar medium (gL⁻¹):** CMC, 10; KH₂PO₄, 1.5; starch soluble, 2.0; sodium chloride, 5; agar, 15; pH 7.4.

IV. **Starch-agar medium (gL⁻¹):** Peptone, 5; yeast extract, 1.5; beef extract, 1.5; starch soluble, 2.0; sodium chloride, 5; agar, 15; pH 7.4.

V. **Peptone–gelatin agar medium (gL⁻¹):** Peptone, 5; gelatin, 4; beef extract, 3; agar, 20; pH 7.

**D Digestive enzyme assay**

Homogenate of GI tracts prepared as described in microbial culture section was centrifuged at 10,000 rpm for 1 h at 4°C, and the supernatant was collected and used for enzyme assay. Cellulase activity was assayed using 1% carboxy methyl cellulose in citrate buffer (0.1 M, pH 6.75) as substrate [46]. Amylase activity was measured using 1% soluble starch in phosphate buffer (0.02 M; pH 6.9 containing 0.0067 M NaCl) as substrate [47]. Protease activity was detected by the caseinase assay method using 0.5% casein in Tris-HCl buffer (0.02 M, pH 7.0) as substrate [48].

**Whole body composition**

To determine the whole-body composition of grey mullet fed with different diets, fish were minced in a meat grinder, homogenized and immediately frozen for further analysis. The proximate chemical composition was determined according to the methods of AOAC [39].

**Analysis of haematological parameters**

Blood was collected from fish of all the experimental groups from caudal vessels and by tail ablation method with 1 mL syringe moistens with EDTA (100 IU mL⁻¹) at the end of the experiment. Haemoglobin (Hb) concentration was determined using the cyanomet-haemoglobin method according to Stokkopf [49]. Erythrocyte (RBC) and leucocyte (WBC) count was done using haemocytometer counting chamber according to Stokkopf [49]. Serum was separated by centrifugation of blood at 3000 rpm for 20 min. The serum was then stored at -20°C for analysis of total protein, albumin and globulin. Total serum protein was estimated as per Lowry et al. [50]. Serum albumin was estimated by a colorimetric method at 550 nm according to Dumas and Biggs [51]. Globulin was calculated by mathematical subtraction of albumin value from total proteins.

**Statistical analysis**

The experimental data were subjected to analysis of variance (ANOVA) to test the significance among the treatments. One-way analysis of variance, followed by Duncan’s multiple range tests [52] was applied to find the significant difference between the treatments, using IBM SPSS, Version 20 for Windows (IBM SPSS Inc., Chicago, IL, USA).

**Results**

**Nutrient composition and cost of experimental diets**

All experimental diets were isonitrogenous and isocaloric sinking pellet type and remain intact in water for about 30 min. The proximate composition of ingredients (pre and post fermentation) and experimental diets were presented in Tables 2 and 3, respectively. Crude protein in the experimental diet of groups I, II, III, IV, V and VI were almost similar (p>0.05) ranging from 276.5 to 282.7 g Kg⁻¹. The lipid content was similar (p>0.05) in all 6 diets ranging from 80.8 to 86.2 g Kg⁻¹. Fiber percent ranges from 84.6 to 110.5 g Kg⁻¹. When fishmeal was replaced with fermented ingredients at different level (25%, 50%, 75% and 100%) feed cost per kg reduced from INR 26.99 to INR 25.23, 23.98, 23.01 and 22.19 (Table 3).
Water quality parameters

During the experimental period, water temperature, dissolved oxygen, pH and alkalinity were monitored on a daily basis. Data presented (Table 4) were mean of every 14-day readings during experiment. The average temperature ranged from 28.00°C to 29.47°C. Dissolved oxygen, pH, and alkalinity levels of water ranged from 8.2 ± 0.12 to 8.37 ± 0.16 ppm, 7.84 ± 0.03 to 8.27 ± 0.00, and 120.0 ± 4.00 to 137.13 ± 0.06 ppm, respectively. In the present study, all the water quality parameters were within the acceptable ranges for fish culture.

Growth performance

There was no significant difference (p>0.05) in the initial fish body weight between treatments (Table 5). At the end of the 42-day trial, total weight gain, average daily gain weight gain percent and SGR were significantly (p<0.01) higher in group VI. There were no significant differences in total weight gain, average daily gain weight gain percent and SGR between groups I, II, III and IV. The lowest weight gain and
SGR were observed in group V. Survival (%) of fish was similar among the groups I, II, III, IV and VI. Survival (%) of fish was significantly lower in group V where fishmeal replacement with fermented ingredients was 100%.

Feed conversion ratio (FCR) was significantly (p<0.01) lower in group VI (1.90 ± 0.01) compared to that of other groups (Table 5). There was no significant difference in FCR between groups I, II, III and IV. FCR was the highest in group V (4.29 ± 0.04). PER was significantly (p<0.01) higher in group VI than that of other groups. PER was similar (p>0.05) in groups I, II, III and IV. PER was found to be significantly (p<0.01) higher in group VI as compared to that of other groups. PER was significantly (p<0.01) lower in group V. There was no significant difference in FCR between groups I, II, III and IV.

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**Nutrient utilization**

Dry matter (DM), organic matter (OM), crude fibre (CF), hemicellulose, cellulose, crude protein, lipid and nitrogen free extract (NFE) digestibility were significantly (p<0.01) higher in group VI (Table 6) supplemented with live bacterial mixtures (Bacillus subtilis DDKRC5. and Bacillus sp. DDKRC1.). Crude fibre, cellulose, hemicellulose, NFE digestibility in group II, III and IV was significantly (p<0.01) higher than that of group I fed with control diet but lower than that of group VI. Crude protein and lipid digestibility was similar among groups I, II, III and IV. Digestibility of nutrients (DM, OM, crude protein and lipid) was significantly lower (p<0.01) in group V as compared to that of other groups.

**Gut microbial counts and digestive enzyme activity**

Total heterotrophic bacterial count, amylolytic, cellulolytic and proteolytic bacterial count in the gastrointestinal tract were significantly (p<0.05) higher in group VI as compared to that of other groups (Figure 1). No significant difference (p>0.05) in total heterotrophic count, cellulolytic count, amylolytic count and proteolytic bacterial count in GI tract was observed among the groups II, III, IV and V. Total count, amylolytic, cellulolytic and proteolytic bacterial counts in the gastrointestinal tract were significantly lower (p<0.05) in fishes of group I than that of other groups.

**Amylase, cellulase and protease activity in the fish gastrointestinal tract**

Amylase, cellulase and protease activity in the fish gastrointestinal tract were significantly (p<0.05) higher in group VI followed by group II, III, IV, V and the lowest enzyme activity was found in fish gut of group I (Figure 2). No significant difference was observed in enzyme activity among groups II, III, IV and V.

**Whole body composition**

A significant difference in crude protein and lipid contents (p<0.01) was observed in grey mullet fed different dietary treatments (Table 7).
Crude protein and lipid were higher in fish group VI. No significant difference in protein and lipid content was found in fish groups I, II, III and IV. Protein and lipid contents were significantly (p<0.01) low in fish group V.

Hematological parameters

Hb, RBC and WBC count were significantly higher (p<0.01) in group VI than that of other groups (Table 8). The lowest value was found in blood of group V. There was no significant difference in Hb, RBC and WBC counts among groups I, II, III and IV.

Serum protein and globulin contents were significantly higher (p<0.01) in group VI than that of other groups and were the lowest in group V. Albumin-Globulin ratio (A/G) was significantly decreased (p<0.01) in group VI as compared to other groups. No significant difference in A/G ratio was observed among the groups I, II III and IV. A/G ratio of group V was significantly higher than that of other groups.

Microbial load in water

As the days of experiment progressed Vibrio count decreased and total microbial load increased in all the groups except group I (control) where both Vibrio count and total microbial load increased (Figure 3). At the end of the 42-day trial, Vibrio count was significantly lower (p<0.01) in group VI than other groups. Vibrio count was also significantly decreased (p<0.01) in groups II, III, IV and V, than that of control group but was higher than that of group VI. Total microbial load increased in water as the experiment progressed.
load did not differ significantly (p>0.05) among groups I, II, III, IV, V and VI at the end of the experiment.

Discussion

In the present study, grey mullet juveniles fed with mixture of live Bacillus sp. DDKRC1, and Bacillus subtilis DDKRC5, were found to have increased growth performances than that of fish without microbial supplementation. Bacillus used as a probiotic was probably able to adhere to the intestinal mucosa and colonize in digestive tract of fish due to their higher multiplication rate than the rate of expulsion [13]. Furthermore, the bacterial supplemented group had significantly higher (p<0.01) PER, improved nutrient digestibility and significantly lower (p<0.01) FCR compared with control. Improved growth performance was reported after supplementation of probiotics in different fish species like Asian seabass [11], common carp [16], rohu [53], Japanese flounder [54], gilthead seabream [55], Persian sturgeon and beluga [56]. Weight gain, FCR, nutrient digestibility of fish without bacterial supplement and fish fed with feed containing fermented ingredients replacing up to 75% fishmeal were similar. But when 100% fishmeal was replaced by fermented ingredients, weight gain and PER significantly (p<0.01) reduced and FCR became higher. Causes of the reduction in performance in fish fed diets with more than 75% fishmeal replacement in the present study might be the variations in amino acid profiles and digestibility of feed [57-59]. Different studies on fishmeal replacement in other species using various alternative protein sources suggest that fishmeal could be successfully replaced by alternative protein sources up to certain levels without any adverse effects on growth performance/ nutrient utilization, but beyond such levels, growth and nutrient utilization could be impaired [57,60-67]. Sun et al. [59] reported 30% fishmeal replacement with fermented fishery by-products and soybean curd residues mixture in olive flounder without any adverse effect. Katya et al. [68] showed that fermented mushroom, Pleurotus ostreatus, by-product can be used up to 6.3% level as a dietary fishmeal replacer in juvenile Amur catfish, Sillurus asotus. Jananathulla et al. [69] reported fermented SBM and fermented SFC could substitute 60% and 20% of dietary fishmeal, respectively in P. vannamet with no negative effect on growth. In the present study, total heterotrophic count, cellulolytic, amylolytic and proteolytic bacterial counts in gut were higher in live bacterial supplemented group VI as compared to fish fed control diet and diet prepared with fermented ingredients (group II, III, IV and V). Increased beneficial bacterial populations in gut of bacterial supplemented group lead to increased digestive enzyme (cellulase, amylase and protease) activity in the intestinal tract, better nutrient digestibility and nutrient absorption [11,70], which in turn contributed to the improved weight gain and improved FCR (p<0.01) in M. cephalus. Nutrient digestibility of grey mullet fed with live bacterial mixture supplemented feed was higher as because the extracellular enzyme produced by the bacteria complement the digestive enzymes of fish [71,72]. When the fishmeal was replaced with fermented ingredients it was observed that up to 75% replacement there was no significant difference in crude protein and lipid digestibility compared to control. But CF, cellulose and hemicellulose digestibility were higher in fish fed diets containing fermented ingredients replacing up to 75% fishmeal. This increase in fibre digestion might be due to increased population of cellulolytic bacterial population and increased cellulase secreted in gut of those groups in the present study, in addition to supply of additional nutrients such as vitamins, essential amino acid and fatty acids in feed ingredients during fermentation by cellulolytic and amylolytic bacteria [18]. Overall, increased digestive enzyme activities and availability of additional nutrients from microbes may have enhanced the nutrient digestibility and growth performance in microbial supplemented groups. The bacteria could also have improved digestive activity via synthesis of vitamins and cofactors or via enzymatic improvement [72]. The increased populations of these favorable cellulolytic, amylolytic and proteolytic bacteria in the gut, apart from secreting the digestive enzymes and essential nutrients, colonize within the gut and so prevent the colonization of pathogenic microbes [73]. The higher (p<0.01) serum protein, globulin and lower (p<0.01) A/G ratio in live Bacillus supplemented fish indicated their higher immune response as compared to the group receiving no bacterial supplement. Albumin and globulin could exert immunomodulatory effect in the liver cells which activate the anabolic capacity of the hepatocytes to produce blood protein particularly globulin [74]. Therefore, when globulin content of serum was more or A/G ratio was less, fish were having better immunity that was reflected in fish group VI having no mortality. Any unhealthy condition caused by poor nutrition could affect the serological parameters of fish as observed in grey mullet of group V fed diet with 100% fishmeal replacement by fermented ingredients. However, results in the present experiment do not indicate any obvious adverse effects of fishmeal replacement up to 75% with fermented ingredients, suggesting that this fishmeal replacement does not adversely affect the serological parameters and thereby does not affect the immune status of grey mullet. Vibrio is dominant in the gut and aquatic environment of marine fish [75] and is also reported as a major bacterial pathogen in the brackish water fish and shrimp [72]. In the present study, Vibrio count of water in bacteria supplemented group was significantly lower (p<0.01). Although count of supplemented cellulolytic and amylolytic bacteria was not measured in tank water of bacteria supplemented groups, it seems that reduced number of Vibrio resulted from the competitive exclusion by other bacteria fed to fish [11].

Whole body composition of grey mullet fry revealed that protein and lipid contents were significantly (p<0.01) higher in bacterial supplemented group compared with the control. This might be due to higher conversion of feed nutrients to the carcass nutrient as reflected by lower FCR, better protein digestibility and PER [11,76,77]. No significant difference was observed in whole body crude protein and lipid content between control and groups fed with diets containing fermented ingredient replacing up to 75% fishmeal as no difference in FCR and PER was observed among the same groups. When fishmeal was completely (100%) replaced by the fermented ingredients, lipid and protein contents of whole body were significantly reduced because of low nutrient digestibility, PER and FCR in the same group. When fishmeal was replaced to the tune of 75% in the feed with fermented ingredients feed cost could be reduced from INR 26990 in control feed to INR 23010 and it could save INR 3980 per ton of feed.

Conclusion

Supplementation of live bacterial mixture with feed augmented the growth and feed utilization in fish. Supplementation of live bacterial culture with diet was found to show better performance than using the fermented ingredients in diet of M. cephalus replacing fishmeal.

Fermented ingredients could able to replace up to 75% of fishmeal in diet of grey mullet without affecting growth, nutrient digestibility and serological parameters, and opened an avenue to reduce the feed cost. The findings of the present study have practical significance towards low cost feed formulation and development of feed probiotics for brackish water aquaculture.

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