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EFFECT OF DIETARY PROBIOTICS SUPPLEMENTATION ON GROWTH PERFORMANCE OF ROHU, LABEO ROHITA FINGERLINGS

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ABSTRACT

The effect of dietary supplementation of commercial probiotics Bifilac® on growth of rohu, *Labeo rohita* fingerlings were evaluated. Experimental diet (ED) was supplemented with Bifilac® (3g/kg diet). Reference diet (RD) was not supplemented with Bifilac®. The feeding trial was conducted using *L. rohita* fingerlings (5.30±0.01 g) for 60 days in triplicates. The groups D1 and D2 were fed daily with RD and ED, respectively, while the D3 group was fed alternately with RD and ED at an interval of 7 days. All experimental groups were fed *ad libitum* twice daily at 8:00 am and 2:00 pm. The growth performance and feed utilization efficiencies were highest for D2. Carcass protein content was found highest for D2. The digestive enzyme activity and the heterotrophic bacterial count of gut were highest for D2 fish group. So, daily dietary supplementation of Bifilac® resulted in improved growth and feed utilization efficiencies of *L. rohita* fingerlings.

KEYWORDS: Probiotics, Bifilac®, Growth performance, Rohu, *Labeo rohita* fingerlings

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INTRODUCTION

Aquaculture is now recognized as the fastest growing food producing sector ensuring both nutritional and livelihood security of millions. Carp is the mainstay of freshwater aquaculture, produced mostly through semi-intensive farming using farm made feed based on regional availability of ingredients, in the country. India being agrarian, the agricultural by-products is commonly used as ingredients in farm made fish feed to support the aquaculture sector. The conversion efficiency of such farm made feed being low, several attempts in the past was made to improve efficiency by fortifying with nutritive supplements, such as, probiotics, prebiotics, amino acids[1-3] etc.

Incorporation of monocultures or mixed cultures of probiotic microorganisms in fish feed has been reported to benefit the host by increasing population of indigenous micro flora that improves digestive enzyme activity, feed utilization, health and growth performance[4-10]. It has been supposed that regular administration of probiotics is required for their optimal functioning[11].Considering the likely beneficial role of commercially available probiotics, attempt was made in the present experiment, to evaluate the efficiency of the probiotics Bifilac® in the diet for *Labeo rohita* fingerlings. Effort was also made to understand the feeding strategy of Bifilac®, a commercial probiotic preparation, in term of its daily or alternative feeding requirement.

It is claimed that commercially available Bifilac® if supplemented in diet helps in proliferation and colonization of both lactobacilli and bifidobacteria in one hand and prevents the colonization of the potentially pathogenic organisms in the gastrointestinal tract by lowering the intestinal pH on the other hand. All the bacteria in Bifilac® colonize in the intestinal tract only and do not enter into systemic circulation, thus it is safe to use.

Herbivorous carp*, Labeo rohita* of Cyprinidae family is considered as one of the important aquaculture species because of its taste and growth. Among the farmed Indian major carp, *L. rohita* ranks second in terms of biomass produced[12]. Hence, the experiment was carried out involving *L. rohita* fingerlings.Therefore, in the present study an attempt was made to assess the effect of daily and alternate dietary Bifilac® supplementation on growth performance of rohu, *L. rohita* fingerlings.

MATERIALS AND METHODS

(i) Collection of feed ingredients

Brewery waste (BW), a distillery by-product of rice fermentation using yeast (*Saccharomyces cerevisiae*), was collected from IFB AGRO Industries Limited, Noorpur, near Diamond Harbour at South 24 Parganas, West Bengal, India. Other feed ingredients were procured from local market.

(ii) Experimental diets formulation and preparation

Two isonitrogenous (CP= 36%) and isocaloric (4.42 Kcal/g) diets were formulated. Combination of BW and Soybean oil cake (1: 1) was used as the protein source in the diet. The reference diet (RD) was not supplemented with any Bifilac®, while the experimental diet (ED) was supplemented with Bifilac® at the rate of 3g/kg diet[13]. Bifilac® is a commercial probiotic preparation containing *Streptococcus faecalis* T-110 (30 million), *Clostridium butyricum* TO-A (2 million), *Bacillus mesentericus* TO-A (1 million) and *Lactobacillus sporogenes* (50 million) as revealed by the manufacturer, Tablets (India) Limited, Puducherry, India. The required quantities of each finely pulverized, sieved and sterilized ingredients were weighed by electronic weighing balance, mixed thoroughly and steam cooked. After the cooked mixture cooled down to room temperature, Bifilac® and the vitamin - mineral mixture[14] was added. The dough thus prepared was passed through an electrically operated semiautomatic pelletizer (pellet size: 2 mm in diameter) to obtain pellets. The pellets were then oven dried, grinded and finally sacked in plastic bags for storage in refrigerator until used. The ingredients composition (% dry weight) and proximate composition of diets are given in Table 1.

(iii)Experimental design

The feeding trial involving hatchery bred *L. rohita* fingerlings was conducted in 55 liter troughs fitted with flow through system (flow rate 2 L/min) using un-chlorinated fresh tap water in the indoor feeding trial facility of Central Inland Fisheries Research Institute (CIFRI), Barrackpore, Kolkata, India, for 60 days. After acclimatization, average initial weight of 20 fingerlings (5.30± 0.01 g each) was recorded and stocked in triplicates for each dietary treatment. D1 and D2 groups were fed daily with RD and ED, respectively, while D3 group was fed alternatively with RD and ED at an interval of 7 days. All the fish groups were fed twice daily *ad libitum* at 8:00 am and 2:00 pm. To determine feed consumption, leftover feed was collected after 2 hr of each feeding and pooled samples from each trough were weighed after oven drying. At the termination of the feeding trial, the fish were weighed and analyzed for carcass composition. The water quality parameters, viz., temperature (°C), pH and conductivity (µs/cm) of water were monitored at regular intervals following the standard methods of American Public Health Association[15].

(iv)Chemical analysis

The proximate composition of the feed ingredients and experimental diets prior to commencement of the experiment was analysed. Ten fish were taken for initial proximate carcass composition (% dry weight). On the termination of the experiment, ten fish from each trough were taken for carcass analysis (% dry weight). The proximate analyses was carried out by following the standard methods[16] as follows; moisture was determined by oven drying at 105°C for 24 hour; crude protein (nitrogen×6.25) by micro Kjeldahl digestion, followed by distillation and titration; lipid by extracting the residue with petroleum ether (40- 60°C) for 7-8 hr in a Soxhlet apparatus and ash by ignition at 550°C in a Muffle furnace to a constant weight. Crude fibre was determined through sequential acid-alkali hydrolysis of fat-free sample, followed by ignition for 2 hr at 550°C. The nitrogen-free extract was calculated by difference. Gross energy (Kcal/g) was calculated using the factor 5.65, 9.45 and 4 for crude protein, ether extract and carbohydrate, respectively[17].

(v) Assay of Digestive Enzymes

Prior to commencement of the experiment, eight fish as initial sample and at the termination of the experiment eight fish from each trough was sacrificed and the gut was aseptically dissected out. Blood and other debris were washed with chilled phosphate buffer saline or PBS (0.1 M, pH 7.4). A 5% homogenate was prepared with the same buffer and centrifuged in a refrigerated centrifuge at 10,000 rpm (g value = 12.063) for 10 min. The supernatant was collected for enzyme assay. α-amylase activity was determined following the dinitrosalicylic acid (DNSA) method[18]. Protease activity was determined by the casein digestion method[19]. Lipase activity was measured by titrimetric method[20].

(vi)Screening of heterotrophic bacteria

Two fish as initial sample prior to commencement of the experiment and two fish from each trough at the termination of the experiment were starved for 24 hours in order to clean their intestinal tract before being sacrificed. The gut was aseptically dissected out both prior to commencement and at the termination of the experiment. Then the gut was homogenized with sterilized and chilled 0.1 M PBS, pH 7.4 (10:1; volume: weight). The homogenate, after five serial 1:10 dilutions, was plated on Tryptone Soy Agar (TSA) and de Man, Rogosa and Sharpe agar (MRS agar; selective for *Lactobacillus)* plates in duplicates. The TSA and MRS plates were then incubated at 37± 1°C for 24 and 48 hrs[21] respectively. The bacterial load of fish gut was expressed as number of colony forming units per g gut tissue (CFU/g).

(vii) Evaluation of performance

Specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR) and protein efficiency ratio (PER) was calculated according to standard methods[22] using the following formulae: FCR= Dry weight of feed consumed/ Increase in wet weight of fish, PER = Wet

weight gain of fish/ Protein consumed and SGR= [(*ln* Final weight - *ln* Initial weight)/ days on trial] x 100.

(viii) Statistical analysis

Statistical analyses were done by one way analysis of variance (ANOVA) using MS-Excel software. Mean difference between different treatments was tested for significance at $P<0.05$ and comparisons was made by Duncan's multiple range test[23] to find out significant difference between different treatments in respect of growth, carcass composition and general performance of the fish.

RESULTS

The data regarding growth performance and feed utilization by *L. rohita* fingerlings fed experimental diets are presented in Table 2. Dietary supplementation of Bifilac® resulted in better growth performance and feed utilization efficiencies over the control. Live weight gain (%) was highest for the D2 fish group which differed significantly ($P<0.05$) than that of D1 and D3. SGR showed the similar trend. FCR and PER was best for D2 fish group which showed significant difference ($P<0.05$) than that of D1 and D3. Survival was 100% for every fish group.

The proximate carcass composition of fish is presented in Table 3. Carcass protein and lipid deposition for all fish group increased over the initial value. Protein deposition in carcass was the highest for D2 fish group which showed significant difference ($P<0.05$) than that of D1 and D3. Carcass lipid deposition was the highest in D1 fish group which differed significantly ($P<0.05$) than that of D2 and D3. The ash content was the lowest for D2 fish group which showed significant difference ($P<0.05$) than that of D1 and D3.

Activity of the digestive enzymes in fish is presented in Table 4. α-amylase, protease and lipase activities were found to be the highest for D2 fish group that differed significantly ($P<0.05$) than that of D1 and D3. The heterotrophic bacterial count has been presented in Table [5]. The count of heterotrophic bacteria was found to be highest for D2 fish group which was followed by D3 and D1.

The experiment was conducted under optimal water temperature 26- 29^0C and other water quality parameters such as, conductivity (406-416 µs/cm) and pH (7.5-7.9) respectively.

Table 1

Ingredients composition (%) and proximate composition (%) of diets on dry matter basis

Ingredients composition (%)	RD	ED
Soybean oil cake	20	20
Brewery waste	20	20
Mustard oil cake	39	39
De-oiled rice bran	12	11.7
Fish meal	5	5
[a]Vitamin premix	1	1
[b]Mineral mixture	1	1
Oil mixture	2	2
Bifilac®	-	0.3
Proximate composition (%)		
Dry matter	94.32±0.01	94.26±0.01
Crude protein	36.60±0.15	36.46±0.15
Crude lipid	7.16±0.01	7.16±0.01
Crude fibre	11.29±0.01	11.23±0.02
Ash	8.79±0.01	8.74±0.01
[c]NFE	30.48±0.14	30.67±0.09
[d]GE (Kcal/g)	4.42±0.01	4.41±0.01

Data are mean values ± SE (n=3). [a]Vitamin premix and [b]mineral mixture[12], [c]Nitrogen- free extract, [d]Gross energy.

Table 2

Growth and feed utilization efficiency in L. rohita fingerlings fed with diets for 60 days

Parameters	D1	D2	D3
Initial average weight	5.30±0.03[a]	5.31±0.03[a]	5.29±0.03[a]
Final average weight	10.60±0.03[c]	12.03±0.07[a]	11.29±0.04[b]
Weight gain (%)	99.83±0.64[c]	127.48±1.14[a]	112.56±0.56[b]
Survival	100	100	100
Feed intake (g/kg body weight of fish/day)	34.27±0.25[a]	30.08±0.19[c]	32.25±0.3[b]
FCR	2.06±0.01[a]	1.80±0.01[c]	1.93±0.02[b]
PER	1.33±0.01[c]	1.52±0.01[a]	1.42±0.01[b]
SGR(% day^{-1})	1.16±0.004[c]	1.37±0.009[a]	1.26±0.004[b]

Data are mean values ± SE (n = 3). Values in the same row with the same superscripts are not significantly different (P< 0.05).

Table 3

Proximate carcass composition (% dry weight) of L. rohita fingerlings fed with diets for 60 days.

Parameters	Initial	D1	D2	D3
Crude protein	50.31±0.25[d]	52.5±0.25[c]	57.17±0.39[a]	54.25±0.25[b]
Crude lipid	8.12 ±0.1[d]	23.89±0.28[a]	19.96±0.53[c]	21.94±0.4[b]
Ash	27.61±0.32[a]	11.33±0.18[b]	10.13±0.18[c]	10.9±0.49[b]

Data are mean values ± SE (n = 3). Means in the same row with the same superscripts are not significantly different (P< 0.05).

Table 4
Activity of digestive enzymes in the gut of L. rohita fingerlings fed with diets for 60 days

Digestive enzymes	Initial	D1	D2	D3
α-amylase	0.49±0.01^{d}	0.90±0.02^{c}	1.56±0.03^{a}	1.19±0.03^{b}
Lipase	1.75± 0.05^{d}	4.25±0.05^{c}	6.25±0.05^{a}	5.17±0.1^{b}
Protease	13.9±0.34^{d}	15.55±0.32 ^{c}	20.05±0.29^{a}	17.85±0.38^{b}

Data are mean values ±SE (n=3). Means in the same row with the same superscripts are not significantly different (P< 0.05).

α-amylase activity = mg maltose liberated/min/ml enzyme extract
Lipase activity = µmol free fatty acid liberated/min/ml enzyme extract
Protease activity = µg tyrosine liberated/min/ml enzyme extract

Table 5
Heterotrophic bacterial count (CFU/g gut tissue) in L. rohita fingerlings fed with diets for 60 days

[a]CFU/g gut	Initial	D1	D2	D3
Bacterial count on TSA	1.95± 0.1 × 10^{8}	1.05± 0.1 × 10^{8}	8.3±0.1 × 10^{8}	4.9±0.1 × 10^{8}
Bacterial count on MRS	0.85 ± 0.1 × 10^{8}	0.45 ± 0.1 × 10^{8}	3.05± 0.1 × 10^{8}	1.7± 0.1 × 10^{8}

[a]Colony forming units per gram gut tissue

DISCUSSION

After 60 days of feeding trial, fish growth and conversion efficiency of both feed and protein were assessed to understand the impact of incorporating of probiotics in the diets for *L. rohita* fingerlings. The study revealed that the commercial probiotics Bifilac® has beneficial effect on the growth of the experimental fish. The beneficial activity of Bifilac® is ascribed to the beneficial activity of its component bacteria such as, *Lactobacillus sporogenes, Streptococcus faecalis* T-110, *Clostridium butyricum* and *Bacillius mesentericus* TO-A. *Lactobacillus sporogenes* produces enzymes required in the digestion of various carbohydrates, fats, and proteins and aids in their absorption. When consumed by the host, they produce lactic acid thereby creating an acidic environment, which will inhibit the growth of potentially pathogenic organisms. It also helps in synthesis of vitamin B complex and Vitamin K. *Streptococcus faecalis* T-110 and *Clostridium butyricum* TO-A also create an acidic environment to inhibit of the growth of harmful bacteria and thus helps to maintain the health of intestinal flora. *Bacillius mesentericus* TO-A is responsible for production of a nutrient which helps in increasing the count of bifidobacteria.

The dietary supplementation of Bifilac® also influenced digestion and assimilation of nutrients in *L. rohita* fingerlings, measured in terms of FCR and PER compared to group fed with RD, irrespective of its daily or alternate feeding strategy. The beneficial role of commercial probiotics in improving growth, feed utilization and carcass protein deposition was reported by several other workers for both warm water herbivore Nile tilapia (*Oreochromis niloticus*), juvenile common carp (*Cyprinus carpio*) and carnivore African catfish (*Clarias gariepinus*)[24-29]. The better weight gain of fish group fed probiotic supplemented diet in the present experimental condition, however, found inferior to its growth under natural or farm condition. Such phenomenon of reduced growth in confined environment was not reported in

tilapia and common carp[30].The better growth performance of fish group fed Bifilac® incorporated diet daily (D2) differed significantly from other two treatments where Bifilac® was either absent or fed alternatively. The better growth performance of *L. rohita* fed with D2 thus may be attributed to daily dietary supplementation of Bifilac®.

The beneficial effect of Bifilac® incorporation also reflected in terms of FCR and PER. Commercial probiotics, such as, Biogen® and Pronifer® has been already proved to improve the growth performance, FCR and PER for monosex tilapia (*Oreochromis niloticus*) fingerlings compared to fish fed the control diet[24]. Biogen® dietary supplementationis also reported to improve growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) respectively[25, 26] in comparison to the control group. It has been also reported that dietary probiotic Biogen® had significantly ($P \leq 0.05$) increased all growth performance parameters of Nile tilapia (*Oreochromis niloticus*) compared to the control group[27]. Use of either Premalac® or Biogen® in diets for Nile tilapia (*Oreochromis niloticus*) was reported to improve growth performance over the control diet[28]. Dietary supplementation of Biogen® improved growth performances and feeding efficiency when fed to juvenile common carp (*Cyprinus carpio*)[29]. The alternate withdrawl of Bifilac® in diet led to poor growth compared to daily supplementation which strengthened the fact that colonization and proliferation of probiotics is a continuous process, thus necessitating the daily supplementation of probiotics in fish gut[11].

The carcass protein was highest in D2 fish group that significantly differed than that of D1 and D3. Similarly, Biogen® dietary supplementation was also reported to improve carcass protein deposition in Nile tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) respectively[25,26]. Dietary supplementation with yeast extract powder also reported improved carcass protein content in rohu (*Labeo rohita*) fingerlings[31]. The higher carcass protein content can be attributed to the colonization of probiotics in the gut that produces protease enzymes for hydrolyzation of complex protein molecules, facilitating their better digestion and absorption resulting ultimately in higher protein retention in body.

The protease and α-amylase activity was higher for D2 fish group that differed significantly than that of D1 and D3. Similar observation was reported in rohu (*Labeo rohita*) fingerlings fed with yeast extract powder supplemented diet[31]. Lipase activity also showed the similar trend and was also reported for Nile tilapia (*Oreochromis niloticus*) fed with dietary probiotics[32]. These observations may be attributed to the fact that probiotics adhere and colonise in the gut of host and produce extracellular digestive (proteolytic, lipolytic and amylolytic) enzymes[33]. The heterotrophic bacterial count was highest for D2 fish group. It indicates that a regular supply of probiotics is required to improve its proliferation, colonization and extracellular digestive enzymes production in the gut of *L. rohita* fingerlings.

CONCLUSION

Daily dietary supplementation of probiotic Bifilac® resulted in enhanced growth and feed utilization efficiencies of *L. rohita* fingerlings. So, daily dietary supplementation of probiotic Bifilac® as feed additive could be recommended to improve growth performance and nutrient utilization efficiencies of *L. rohita* fingerlings. However, the authors have no conflict of interest.

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