Growth Performance of *Labeo rohita* Fingerlings Fed with Probiotic Added Plant By-Products Based Feeds

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**ABSTRACT**

The current study was carried out to investigate growth performance of *Labeo rohita* fingerlings fed with *Lactobacillus rhamnosus* supplemented plant by products based feeds. The study comprised of two experimental and one control groups. In treatment 1 (T₁), fish were fed with soybean meal along with *L. rhamnosus*. In treatment 2 (T₂), fish were fed with sunflower meal along with *L. rhamnosus*. The control group was served with commercial feed. Initial mean weights of the experimental fish was recorded as 40.00 ± 1.00, 40.33 ± 0.76 and 40.01 ± 1.00 g for T₁, T₂, and control groups, respectively. The highest weight gain was found in T₁ (747.96 ± 1.9 g) and control group (479.35 ± 1.8 g). Significant differences were found in the feed conversion ratio between control group (2.02 ± 0.07), T₁ (1.48 ± 0.03) and T₂ (1.47 ± 0.02). Survival remained 100% in all the treatments. Water quality parameters differed insignificantly for different treatment groups and remained in favorable range throughout the study period. All the treatments showed lower values of fat contents and higher values of crude protein. No negative change were found in the histological parameters of the liver and intestinal tissues for the fishes fed with plant based feeds added with probiotics. Conclusively, fish production can be enhanced with the addition of probiotics in the feed derived from plants.

**INTRODUCTION**

Aquaculture sector is facing difficulty in development due to expensive feed stuff which is vital to increase the fish production. Feed cost in aquaculture industry represents over 50% of the total cost which in turn depends on different factors like source, kind of ingredients, crude protein level and formulation practices which affect the lipid profile, vitamins and mineral content of fish produced and ultimately the consumer demand (Prabhu et al., 2017). Cost-effective feed ingredients which are easily available on demand from market and well suited for fish types to be cultured can play vital role in the development of aquaculture sector (Iqbal et al., 2015; Mishra et al., 2022).

Suitable plants-based by-products can replace expensive animal-based feed ingredients without compromising the growth, enzymatic efficiency, survival rate of fish and its resistance against various infectious diseases (Fraga-Corral et al., 2022). Probiotics as biotherapeutic agents have been used to promote health benefits since long. Reported health benefits associated with daily consumption of probiotics include their anticancer, antioxidant, hypoglycaemic, immunomodulatory potential amelioration of arthritis and cholesterol reduction (El-Kady et al., 2022; Thatcher et al., 2022; Tran et al., 2022).

Probiotics can reduce the cost and can easily improve the growth and feed conversion efficiency of fish (Swain et al., 1996; Bogut et al., 1998; Ghiobh et al., 2003; Carnevali et al., 2006; Yanbo and Zirong, 2006; Mazurkiewicz et al., 2007; Kesarcodi-Watson et al., 2008). Many probiotics are being used in aquaculture but lactic acid bacteria (LAB) are the most prominent one as they build natural intestinal microflora of a healthy fish (Noh et al., 1994; Brunt and Austin, 2005; Nayak et al., 2007; Wang, 2007, 2011; Yin et al., 2007; Ramakrishnan et al., 2008). Keeping in view the health benefits and productive utility of probiotics in aquaculture, the current study was planned to explore benefits of *Lactobacillus rhamnosus* (NR-113332.1)
supplementation in plant based feeds on growth, feed conversion ration and intestinal health of *Labeo rohita* fingerlings. The findings of this study will be helpful for replacing the commercial expensive feed with the experimentally validated feed with higher yields.

**MATERIALS AND METHODS**

**Experimental design**

The fish fingerlings for the experimental trial were obtained from Fisheries Research Institute, Manava, Lahore and the experimental trial was carried out in Microbial Biotechnology Laboratory, Institute of Zoology, University of the Punjab, Lahore. The fishes were divided into two different treatment groups on the basis of feeding with and without different diets made from different kinds of plant based ingredients with the addition of probiotic (*L. rhamnosus*) in soybean and sunflower based feeds. Control group was fed with commercial feed. One hundred and eighty fish individuals were stocked in 9 aquaria (60.96 × 55.88 × 40.64 cm). Twenty fishes (weighing between 25 to 40 g) were stocked in each group. Before stocking, the fish were treated with KMnO₄. In treatment group 1 (T₁), fish were fed with soybean meal along with *L. rhamnosus* (NR-113332.1). In treatment group 2 (T₂), fish were fed with sunflower meal along with *L. rhamnosus*. The control group was served with commercial feed. All the fish were carefully weighed during the trial to verify the result of feed on growth. Feed was readjusted after every fortnight sampling.

**Feed formulation**

Plant based feeds; soybean meal and sunflower meal having 30% CP level with the addition of probiotics *L. rhamnosus* (NR-113332.1) were used (Table I). The control group was fed with commercial feed. The fish were fed for three months at rate of 3% fish wet body weight twice a day.

**Feeding procedure**

Fish were fed twice a day (morning and afternoon) at the rate of 3% of body weight of fish. Fish feed quantity was recalculated after fortnight sampling.

**Growth study**

Before stocking the fish, initial weight and length were measured. At the end of the experimental trial, final gain in weight, feed conversion ratio (FCR), percentage weight gain, net gain in weight and specific growth rate (SGR) were calculated.

**Table I. Percentage ingredient contribution of different plant based experimental feeds.**

| Ingredients       | Control group (Commercial feed) | Treatment 1 (Soybean based feed + *L. rhamnosus*) | Treatment 2 (Sunflower based feed + *L. rhamnosus*) |
|-------------------|---------------------------------|---------------------------------------------------|---------------------------------------------------|
| Fish meal         | 25                              | 12                                                | 12                                                |
| Sunflower meal    | --                              | -                                                 | 60                                                |
| Soybean meal      | 56                              | -                                                 | -                                                 |
| Rice polish       | 30                              | 12                                                | 8                                                 |
| Wheat flour       | 30                              | 10                                                | 10                                                |
| Fish oil          | 5                               | 3                                                 | 3                                                 |
| Vitamin premix    | 2                               | 1                                                 | 1                                                 |
| Minerals          | 2                               | -                                                 | 1                                                 |
| Ascorbic acid     | 2                               | 1                                                 | 1                                                 |
| Chronic oxide     | 2                               | 1                                                 | 1                                                 |
| Citric acid       | 2                               | 3                                                 | 3                                                 |
| *L. rhamnosus*    | -                               | 3 × 10⁸ C.F.U/g of feed                          | 3 × 10⁸ C.F.U/g of feed                          |
| Total             | 100                             | 100                                               | 100                                               |

The mixed feed ingredients were formulated in pelleted form to a particulate size of 2mm by a pelleting machine. The air-dry product was packed in opaque and well-sealed plastic zipper packets till further use.

**Histological analysis**

The fish were dissected to get liver and gut (intestine). Both these samples were preserved in 4% formalin. Histology was carried out to determine the effects of probiotic on liver and intestine of fish. The tissues were routinely dehydrated through different ethanol grades, absolute ethanol and xylene. The tissue sections were prepared following Qin et al. (2018). The wax embedded tissues’ sections (8 µm) were cut with the help of a microtome. The sections were subsequently stained with hematoxylin and eosin and studied microscopically. Photomicrographs of the representative sections were taken with the help of a camera fitted microscope.

**Periodic physicochemical analysis**

Dissolved oxygen, temperature and pH were recorded on daily basis by using multi meter. Nitrates and phosphates were recorded using HANNA Nitrate Test Kit HI3874.

**Statistical analysis**

Analysis of variance ANOVA was applied on the data obtained to compare the means by using statistical software SAS 9.1.

**RESULTS AND DISCUSSION**
**Fish growth analysis**

The highest weight gain 747.96 ± 1.9 was observed in T1, followed by T2 (657.01 ± 1.6) as compared to the control value of 479.3 ± 1.8 (Table II). Maximum gain in length was measured as 5.33 ± 0.29, 5.67 ± 0.43, 3.83 ± 0.29 for T1, T2, and control group, respectively. Statistically significant differences of length increase were observed among the control group, T1 and T2 (Table II). Our results were similar with Saghaei et al. (2015) who described the effect of garlic (*Allium sativum*) on Oscar fish (*Astronotus ocellatus*) growth performance and body compositions. They reported that highest protein content was attained in the fish fed with 10 g/kg of garlic powder. Our results were also supported by Tiamiya et al. (2016) who reported that feeding *Oreochromis mossambicus* with 55% substitution of *Moringa oleifera* meal for soybean meal presented the best growth results and nutrients utilization. Gradual decrements in the growth performance suggested that *M. oleifera* should be added less than 7.34%. Mohanty et al. (1996) showed feeding with addition of probiotics and yeast resulted in higher survival rate, better immunity and increase in body weight gain and nutrient utilization in thaila (*Catla catla*). Yano and Zirong (2006) reported that the survival of fish, gain in weight and nutrient utilization of fish depend on the type of probiotics used.

**Table II. Growth of *L. rohita* fed with *L. rhamnosus* supplemented soybean based meal and sunflower based meal.**

| Parameter (%) | Control | Treatment 1 | Treatment 2 |
|---------------|---------|-------------|-------------|
| Initial weight (g) | 40.01±1.00 | 40.00±1.00 | 40.33±0.76 |
| Final weight (g) | 231.67±1.08 | 339.00±1.07 | 305.00±0.78 |
| Gain in weight | 479.35±1.8 | 747.96±1.9 | 657.01±1.6 |
| Initial length (cm) | 13.17±0.29 | 13.83±0.28 | 13.33±0.58 |
| Final length (cm) | 17.00±0.01 | 19.17±0.02 | 19.01±0.01 |
| Increase in length (cm) | 29.16±1.79 | 38.58±2.2 | 42.67±1.03 |
| FCR (%) | 2.02±0.07 | 1.48±0.03 | 1.47±0.02 |
| SGR (%) | 0.64±0.02 | 0.81±0.01 | 0.75±0.01 |

Values represent Mean ± S.D. of triplicates. Those having different superscripts in a row are significantly different from each other P≤0.05

**Physicochemical parameters**

Non-significant differences were recorded in all the treatment groups. Temperature in all the treatment groups as well as control group fluctuated between 25 to 27°C throughout the experimental period. Throughout the experiment, DO remained within favorable range from 6.5 to 7 mg/L. During the whole experimental period, pH remained neutral. Non-significant differences were also observed for the nitrates and phosphates contents of water among all the treatment groups.

**Body composition of fish**

At the end of the experimental trial, fish were processed for proximate analysis. Maximum crude protein (66.49 ± 0.14) was observed in T2 and was remarkably different from that of control group. Significantly higher fat contents up to 9.15 ± 0.05 were found in control group, whereas the respective values for T1 and T2 were 6.23 ± 0.15 and 6.67 ± 0.12. Ash contents were appeared as 23.40 ± 0.06, 21.33 ± 0.21 and 21.57 ± 0.06 in control group, T1 and T2, respectively. Moisture contents were appeared as 8.74 ± 0.21, 8.66 ± 0.17 and 9.35 ± 0.09 in control group, T1 and T2, respectively. Dry matter of fish sample showed non-significant (P > 0.05) differences among all the treatment groups (Table III).

**Table III. Whole-body composition of *L. rohita* fed with *L. rhamnosus* supplemented soybean based meal and sunflower based meal.**

| Parameter (%) | Control | T1 | T2 |
|---------------|---------|----|----|
| CP | 63.87±0.40 | 66.22±0.02 | 66.49±0.14 |
| Fat | 9.15±0.05 | 6.23±0.15 | 6.67±0.12 |
| Ash | 23.40±0.06 | 21.33±0.21 | 21.57±0.06 |
| Moisture | 8.74±0.21 | 8.66±0.17 | 9.35±0.09 |
| Dry matter | 91.26±0.21 | 91.34±0.17 | 90.65±0.09 |

Values represent Mean ± S.D. of triplicates. Those having different superscripts in a row are significantly different from each other P≤0.05

**Histological analysis**

Normal pattern of hepatocytes was observed in the control as well both the experimental groups. Whereas, normal appearance of tunica mucosa, serosa layers and villi made the folds thicker in the probiotics supplemented fish. Intestines of the fish fed with probiotics showed relatively well-developed villi and epithelial layers (Figs. 1-3).

In control group, the intestine showed normal appearance of serosa layers and villi structure (Fig. 3). Bamidele et al. (2015) observed the liver and intestine health by replacing soybean meal (T1) 0, (T2) 25, (T3) 50, (T4) 75 and (T5) 100% with moringa meal at 40 % CP. He reported T1 group showed no visible degeneration with normal intestine and liver. T2 and T3 showed degeneration of hepatocytes in liver. T4 and T5 showed severe degeneration and intestinal necrosis which had been explained due to excessive work required by the fish liver to get rid of the plant toxicant from its body during the process of detoxification. It is well known that liver is
the main organ for detoxification. No negative change in the histological parameters of the liver tissue as well as the intestinal tissue for the fishes fed with probiotic plant based feeds was observed in the present study.

Fig. 1. Cross section of intestine of L. rohita fed with Soybean based feed supplemented with L. rhamnosus showing normal appearance of tunica mucosa, serosa layers and villi (100X).

Fig. 2. Cross section of intestine of L. rohita fed with Sunflower based feed supplemented with L. rhamnosus showing normal appearance of connective tissues of villi, tunica mucosa, muscularis and serosa layers (100X).

CONCLUSIONS

Our research suggested that L. rhamnosus can be used safely for employing plant based products feeds for sustaining or even escalating growth without any harmful and damaging effect on body composition of L. rohita.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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