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Short Communication

# Growth Performance of *Labeo rohita* Fingerlings Fed with *Lactobacillus rhamnosus* Supplemented Canola Meal and Corn Gluten-Based Feeds

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## ABSTRACT

The present study was carried out to record growth respose of *Labeo rohita* fingerlings fed with *Lactobacillus rhamnosus* supplemented feed. In a completely randomized design fishes were divided into two different treatments based on feeding with and without probiotics in canola meal and corn gluten meal. The control group was fed with commercial feed. Before stocking, all the aquaria were dried for 3 days. One eighty hundred and eighty fish (*Labeo rohita*) were stocked in 9 aquaria. Twenty fishes were kept in each group. Before stocking, the fish was treated with $KMnO_4$. In treatment 1 ($T_1$) fish were fed with canola meal along with *Lactobacillus rhamnosus* (NR-113332.1). In treatment 2 ($T_2$) fish were fed with corn gluten meal along with *Lactobacillus rhamnosus*. The fish were fed at the rate of 3% body weight. All the fish were carefully weighed during the trial to verify the result of feed on growth. Feed was readjusted after every fortnight's sampling. The initial mean weight of the experimental fish (*Labeo rohita*) was recorded as 40.01±1.00g, 39.67±0.76 g, and 40.01±1.00 g for $T_1$, $T_2$ and control group respectively. After 90 days the highest weight gain was found in case of $T_1$, (683.64$^c$ ±1.93) and the lowest one (479.35 ±1.8g) was recorded in the control group. Significant differences were found in the feed conversion ratio between the control group (2.02±0.07), $T_1$ (1.65±0.01) and $T_2$ (1.74±0.01). Survival remained 100% in all the treatments. Water quality parameters remained in a favorable range throughout the study period, and non-significant differences were recorded for the different treatment groups. All the treatments showed low fat contents and good crude protein values of the fish meat. It is concluded that fish production can be enhanced with the addition of the probiotic in the feed derived from plant origin ingredients. This practice will economize, promote growth and prevent feed associated stress in the aquaculture industry in this country in an environmentally sustainable way.

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**Authors' Contribution**
**SA,YFC and JIQ planned and designed the study. RU and FA analyzed the data.**

**Key words**
**Canola meal, Corn gluten meal, *Lactobacillus rhamnosus*, Fish growth, Probiotics, *Labeo rohita***

Aquaculture is not a new concept, rather, it is the most ancient source of animal culturing. Fish are the primary component of aquaculture and are a vital component of human di*et al*l over th world. Fish meat supplies a significant resource of nutrients, essential for a good health (World Aquaculture, 2010). Fishes have great significance in the life of humankind, for being an important natural source of protein and providing some other useful products as well.

Fish meal is generally considered the golden standard protein source for many species but today it is considered both anviornmentally and ecologically unstable. It has generally been claimed that upto 50% fishmeal protein can be replaced by plant proteins in fish feed.However, while employing plant based feeds once has to limit the %age of

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plant origin protein source.

Among the leading causes of mortalites in fish hatcheries are bacterial infections, which are major problems for the industry. When looking at probiotics intended for aquatic usage, it is important to consider certain factors that are fundamentally different from the applications of probiotics in environment. Most probiotics products contain bacteria from the genera *Lactobacillus* spp., *Bifidobacterium* spp. Lactic acid bacteria and *Bifidobacterium* inface these genera have been established as safe and reliable probiotics. However, *Bacillus* spp. have been relatively less studied as probiotics (Patel *et al.*, 2009).

Aquatic animals have a much closer relationship with their external environment. Potential pathogens are able to maintain themselves in the external environment of the animal and proliferate independently of the host animal in the water (Hansen and Olafsen, 1999; Verschuere *et al.*, 2000). Probiotics are microorganisms that have beneficial effects on their hosts. Kozasa (1986) made the first empirical application of probiotics in aquaculture following the applications of probiotics in humans and poultry. Probiotics have been used in aquaculture to increase the growth of cultivated species. However, it is important to determine whether probiotics taste good for aquaculture species (Irianto and Austin, 2002). Probiotics are living microorganisms that play a vital role in improving fish health and immunity (Gatesoupe,1999) and increase digestibility rate in fish by adding plant meal-based diets (Irianto and Austin, 2002). Probiotics also help in the fast metabolic rate of nutrients, which helps in the digestion of the breakdown of starch, protein, lipids, and cellulose which makes the digestion process easy (Hoyoux *et al.*, 2009; Suzer *et al.*, 2007). Das *et al.* (2017) reported when probiotics are added to fish diets, the growth rate of fish increased. Many studies have showen that probiotic yeast (*Saccharomyces cerevisiae*) when incorporated in Nile tilapia improved the growth rate and also increased its immunity (Abdel-Tawwab *et al.*, 2008; Iwashita *et al.*, 2015; Abass *et al.*, 2018; Sutthi and Thaimuangphol, 2020).

Probiotics are microorganisms which applied in sufficient amounts render health benefit to the host. They have been documented to promote growth of animals and to protect them from certain diseases especially gastrointestinal infection.They can colonize in the gastrointestinal tract when administered over a long period addition of probiotics continuous to fish cultures, they will colonize there, and adhere to the intestinal mucosa, developing and exercising their multiple benefits (Balcazar *et al.*,2006). The present study was carried out to improve growth performance in fish by incorporating *Lactobacillus rhamnosus* in the plant based feeds.

*Materials and methods*

The experimental trial was carried out in the Fisheries and Microbial Biotechnology Lab, Institute of Zoology, University of the Punjab, Lahore. The experiment was carried out in glass aquaria each of (60.96 × 55.88 × 40.64 cm) in diamensions for 90 days. There were two replicates of each treatment. The design of experiment was CRD.

The fish was collected from Fisheries Research Institute, Manawan, Lahore. Fingerlings weighing 25 to 40 g were stocked in 9 aquaria, each aquarium with 20 fingerlings. Before stocking the fish, wet body weight and length of the fish were measured.

The control group was fed with commercial feed, wherase the treatment groups were fed with plant based feeds. The treatment groups $T_1$ and $T_2$ were fed with canola meal and corn gluten meal each supplemented with probiotic *Lactobacillus rhamnosus* (NR-113332.1), respecteviely. The control as well as experimental feeds had 30% crude protein (CP) levels. Fish were fed twice a day (morning and afternoon) with 3% of the body weight of fish for 6 days a week. Fish feed quantity was re-calculated after fortnightly sampling.

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

Before stocking the fish, initial weight and length were measured. At end of the experiment, final gain in weight, feed conversion ratio, percentage weight gain, the net gain in weight, and specific growth rate (SGR %) were calculated. Dissolved oxygen, temperature, and pH were recorded on a daily basis by using multimeter. In addition, nitrates and phosphates were recorded using HANNA Nitrate Test Kit HI3874.

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

*Results and discussion*

The fish fed with the plant product-based diet supplemented with *L. rhamnosus* (NR-113332.1) showed a remarkable increase in weight. Highest % weight gain upto 683.64 ±1.93 was observed in the case of $T_1$, followed by $T_2$ 660.49±0.80 as compared to the control value of 479.35±1.8 (Table I). Likewise, maximum % gain in length was measured as 35.90 ±1.4, 37.73±4.4, 3.83±0.29 for $T_1$, $T_2$ and control groups, respectively. Statistically notable differences in % length increase were observed among the control group, $T_2$ and $T_1$ groups (Table I).

**Table I. Growth of *Labeo rohita* fed with *Lactobacillus rhamnosus* supplemented canola based meal and corn gluten based feeds.**

| Parameters | Control | *L. rhamnosus* supplemented canola meal ($T_1$) | *L. rhamnosus* supplemented corn gluten meal ($T_2$) |
|---|---|---|---|
| Initial weight (g) | 40.01±1.00$^a$ | 40.01±0.27$^a$ | 39.67±0.29$^a$ |
| Final weight (g) | 231.67±1.08$^a$ | 313.33±1.09$^c$ | 301.67±1.07$^c$ |
| % gain in weight | 479.35±1.8$^a$ | 683.64±1.93$^c$ | 660.49±0.80$^c$ |
| Initial length(cm) | 13.17±0.29$^a$ | 13.00±0.56$^a$ | 13.33±0.56$^a$ |
| Final length (cm) | 17.00±0.01$^a$ | 17.67±0.53$^b$ | 18.33 ±0.55$^b$ |
| Increase in length (cm) | 29.16±1.79$^a$ | 35.90±1.4$^b$ | 37.73±4.4$^a$ |
| FCR | 2.02±0.07$^c$ | 1.65±0.01$^a$ | 1.74±0.01$^a$ |
| SGR (%) | 0.64±0.02$^a$ | 0.76±0.02$^c$ | 0.75±0.02$^c$ |

Values represents Mean ± S.E.M of triplicates. Values having different superscripts in a respective row are significantly different from each other p≤0.05. FCR, feed conversion rate; SGR, specific growth rate.

Carnevali *et al.* (2006) reported that growth was observed in groups fed with *Lactobacillus delbrueckii* compared to the control. The present results indicate that fish health and growth performance improved, despite the different feeding methods and species in the current study.

Non-significant differences in the value of physico chemical parameters were recorded in all the treatment groups. Throughout the experiment the temperature in all the treatment groups, as well as the control group fluctuated between 27.1°C to 27.9°C throughout the experimental period. Likewise, DO remained within a favorable range from 6.4mg/l to 6.8mg/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 treatments.

At end of the experiments, fish were processed for proximate analysis. Maximum CP upto 65.94 ±0.03was observed in the case of $T_2$ which was remarkably different from the control group. Significantly higher fat content up to 9.15 ±0.05 was found in the control group, whereas the respective values of $T_1$ and $T_2$ were 6.57±0.13 and 6.22±0.01. Ash content of the fishes were 20.15±0.01, 21.27±0.15, and 21.57±0.06 in the control group, $T_1$ and $T_2$, respectively. Moisture contents of fishes were 9.31±0.07, 9.22±0.01, and 9.35±0.09 in the control group, $T_1$ and $T_2$ groups, respectively. Dry matter of the fish sample showed a non-significant (p>0.05) differences between the treatments (Table II).

**Table II. Whole body composition (%) fed with *Lactobacillus rhamnosus* supplemented canola based meal and corn gluten based feeds of *Labeo rohita.***

| Parameter (%) | Control | *L. rhamnosus* supplemented canola meal ($T_1$) | *L. rhamnosus* supplemented corn gluten meal ($T_2$) |
|---|---|---|---|
| CP | 63.87±0.40$^a$ | 65.79±0.13$^c$ | 65.94±0.03$^b$ |
| Fat | 9.15±0.05$^c$ | 6.57±0.13$^a$ | 6.22±0.01$^a$ |
| Ash | 23.40±0.06$^c$ | 20.15±0.01$^a$ | 21.27±0.15$^a$ |
| Moisture | 8.74±0.21$^b$ | 9.31±0.07$^b$ | 9.22±0.01$^b$ |
| Dry matter | 91.26±0.21$^b$ | 90.69 ±0.07$^a$ | 90.78±0.01$^a$ |

Values represent Mean ± SD of triplicates. These were having different superscripts in a row are significantly different from each other p≤0.05. CP, crude protein.

*Conclusion*

The present results suggested that *Lactobacillus rhamnosus* (NR-113332.1) is a promising candidate generates useful information for aqua feed and fish industry regarding the possible combination of plant-based feeds and probiotic usage for enhancing the growth of *Labeo rohita* under control conditions. This blending of probiotic and plant based feed will likely replace expensive feed ingredients with low-cost ingredients that will economize aquaculture production through optimum survival rate and enhanced growth with good fish yield.

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*Ethical statement*

No animals were harmed while carrying out this research.

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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