Boosting Growth and Immunity in Fish Variety Rohu Fingerlings (Labeorohita): The Role of Bacillus Subtilis as a Probiotic in Semi-Intensive Aquaculture

Muhammad Kashif Hameed [1], Waqas Ameen[2](Corresponding Author)**, Tabay Hassan[3], Mukhtar Ahmad[4], Asma Naqia[5]**

[1,] MS Student, Department of Zoology, Punjab University, Lahore, Punjab, Pakistan
[2,] MS Student, Department of Zoology, Islamia University of Bahawalpur, Punjab, Pakistan. Email: waqasameen814@gmail.com
[3, 4, 5,] MS Student, Department of Zoology, Islamia University of Bahawalpur, Punjab, Pakistan

Abstract

Aquaculture plays a significant role in rapidly increasing fish production to meet the world's growing protein needs. The goal of the current study was to determine the response of Indian major carp Rohu fingerlings (Labeo rohita) in terms of growth, Haematological, and immune parameters after the dietary supplementation with probiotic Bacillus subtilis. An 8-week feeding trial was carried out under a semi-intensive culture system. During the trial, fish were fed with three diets, including a control diet and two experimental diets with varying concentrations of probiotics. Probiotic concentrations in experimental diets were 103 and 106 CFU g-1, respectively. All these treatments were applied in triplicate. After the experiment, the growth, hematological, and immune parameters of the fish were evaluated. One-way ANOVA followed by post hoc LSD Tukey test based results indicated that growth rate, weight gain, and specific growth rate were significantly ($P<0.5$) higher in fish fed with a probiotic-containing diet, with the highest growth observed with a diet containing 106 CFU/g of probiotic. Leucocyte, erythrocyte count, and glucose content were also high in experimental groups as compared to control groups. Total protein content and Peroxidase activity were significantly higher ($P<0.5$) in fish fed with the highest concentration (106 CFU/g) of probiotic as compared to the control and other dietary groups. From these outcomes, Bacillus subtilis can be concluded as a potential probiotic for L. rohita culture under a semi-intensive culturing system and can be used for better growth and survival rate of fish in aquaculture practices.

Keywords: Boosting Growth, Labeo rohita, Bacillus subtilis, Hematology, Semi-Intensive Aquaculture.

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Introduction

Aquaculture is one of the fastest-growing animal food production sectors and is a significant contributor to global food security (Jones et al. 2014). The worldwide decline in the global fish population in marine and freshwaters has become an impetus for the rapid increase in aquaculture practices (Naylor et al. 2000). Between 1990 and 2015, global aquaculture doubled in weight and value. In 2015, 106 million tons of live fish were farmed with an expected value of $164 billion (Zhou, 2017). One of the basic needs of humans is to secure food for nutritional use. Many areas of the world lack an adequate food supply. In 2009, 960 million people around the world were undernourished (Johnson, 2009). The increasing population and intensified urbanization are associated with higher and more nutritious food demand (Gjedrem et al. 2012). With all this in mind, the production of food with high protein content must be of interest.

Materials and methods

Experimental setup

At Azhar Baloch fish farm, Adda Balochan, District Khanewal, Punjab, located at latitude and longitude 30°18' and 71°55'0E respectively, with an altitude of 128 metres, a 60-day experiment was devised and run in triplicate in clay ponds under a semi-intensive culture system. The semi-intensive culture system was managed with organic, inorganic, lime, and prepared feed. For the study, nine ponds, each with an area of 0.20 ha, were employed. All ponds were sun-dried, and calcium carbonate 125 kg ha-1 was added for disinfection and pH stabilization. Animal dung 3333.33kgha^{-1} was also added to boost pond productivity. Throughout the experiment, the water level in all of the ponds was maintained at 1.5 meters, and pond productivity was measured using a saccharin disc. One control group (G1) without any probiotic supplementation and two experimental groups, one with 10^3 CFUml^{-1} (G2) and the other 10^6 CFUml^{-1} (G3) of B. subtilis, were made.

Fish collection

About 900 infection-free L. Rohita fingerlings 19.51± 1.91g were cultured in each earthen pond. Before stocking, the total biomass of the experimental fish was measured. For 15 days, the fish has been acclimatized. During the acclimation period, a control diet with a Cp level of 35% was given.

Physiochemical parameters

The water temperature was 27±2 ^{o} C. Total ammonia, DO, and pH were also kept within acceptable limits. All the groups were in a similar environment. The nine ponds were situated next to each other in the same area. As a result, in terms of water quality, there were no notable variations between the three groups, as shown in Table 3.1.

Feeding schedule

After an acclimatization period, D1 was given feed without any probiotic supplementation, while D2 was given feed with 10^3 CFU/g, and D3 with 10^6 CFU/g of B. subtilis.

Growth measurement

The mean weight of fish in all the ponds was calculated before the feeding trial. After that, the gross weight of an equal number of fish from each pond was calculated every week. Then, at the end of the trial, 15 fish from each pond were randomly selected, and their mean weight was calculated. Weight gain was measured by subtracting the final weight from the initial weight. The specific growth rate, percentage weight gain, and feed conversion ratio were calculated according to the following:

Weight gain (WG)

WG (g) of fish was calculated by applying to the average weight of fish of each treatment.

$\text{WG (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$

Weight gain% (WG %)

WG% % of fish was calculated by the following formulae:

$\text{WG\% \%} = weight - Initial\ weight/Initial \text{X } 100$

Survival rate % (SR %)

Survival rate (%) of fish was estimated by the standard formula: $\text{Survival rate (\%)} = fish\ 100$

Hematological parameters

The nine fish from each pond (27 from each group) were taken and anesthetized with clove oil, and then the blood of the fish was drawn with the help of a 1 ml syringe from the caudal vein and gathered in purple top K2 EDTA tubes. Further analysis to find out the value of different hematological indices like WBCs (CBC leukocytes), RBCs (erythrocytes), Hb (hemoglobin), and HCT% % (hematocrit value) of all studied groups was done with the help of a hematology analyzer.

Furthermore, the values of HB, RBC, and HCT% % were further used to calculate the MCH (Mean corpuscular hemoglobin), MCHC (Mean corpuscular hemoglobin concentration), and MCV (Mean corpuscular volume).

$$\text{MCH} = \{(10 \times \text{Hb}) \div \text{RBC}\}$$

$$\text{MCHC} = \{\frac{\text{Hb}}{\text{HCT(\%)}}\}$$

$$\text{MCV} = \{\frac{\text{HCT\%} \times 10}{\text{RBC}}\}$$

Standard absorption × Concentration of standard

Statistical analysis

All results are shown as mean ±SD. Significant differences in hematology and immunological data and Biochemical assays among experimental treatments were evaluated by using one–way ANOVA followed by LSD test at the 5% level of significance using SPSS version 20. GraphPad Prism 5 was used for the graphical representation of the data.

Results

The study was conducted to investigate the effect of dietary supplementation of Bacillus subtilis on the growth performance, hematological, and immune parameters of tilapia fingerlings. The following parameters were discussed during this study.

1. **Growth performance**

Initial weight (g)
Final weight (g)
Absolute weight gain (g)
Weight gain %
Survival rate %
Specific growth rate

2. **Hematological parameters**

WBCs (CBC leukocytes)
RBCs (erythrocytes),
Blood Glucose level
Hb (hemoglobin),
HCT% % (hematocrit value)
MCH (Mean corpuscular hemoglobin),
MCHC (Mean corpuscular hemoglobin concentration)

Growth performance

Initial weight (g) of tilapia fingerlings

The initial weight (g) of rohu fingerling was 18.7-19.57g. The initial weight (g) of the fish was kept constant for all the treatments.

Final weight (g) of rohu fingerlings

The maximum value of weight gain was recorded in the group fed with the highest dietary supplementation of (D3) of the probiotic strain, i.e. 10^6 cfu/g. A significant ($p< 0.05$) difference was recorded in the value of dietary treatments and control. The group fed with the control diet showed the minimum final weight.

AWG (g) of rohu Fingerlings

The data show the mean values of AWG (g) of tilapia fingerlings fed with a probiotic Bacillus subtilis supplemented diet. Groups fed with a probiotic-supplemented diet showed a significant ($p<0.05$) difference in AWG (g) as compared to the control. The highest value was observed in the group fed with diet D3, and the lowest value of average weight gain was observed in the control diet.

Specific growth rate (SGR) (g) of Rohu Fingerlings

As per other growth parameters, the maximum value of specific growth rate was observed in the group fed with a probiotic Bacillus subtilis supplemented diet having 10^5 cfu/g of diet. The minimum value was observed in the control group, which was fed with a basal diet only. There was a statistically significant difference ($p<0.05$) between the group fed with the highest concentration of probiotic and the control group. Supplementary groups were not significantly different from each other.

Total Erythrocyte Count

According to the obtained results, the total number of red blood cells increased in tilapia blood as the concentration of probiotic Bacillus subtilis increased in the diet. The largest number of cells was found in fish fed with diet D3, and the lowest number was found in the control diet. There is a statistically significant difference ($p<0.05$) between the control and D3 group. All groups have significant variations from each other.

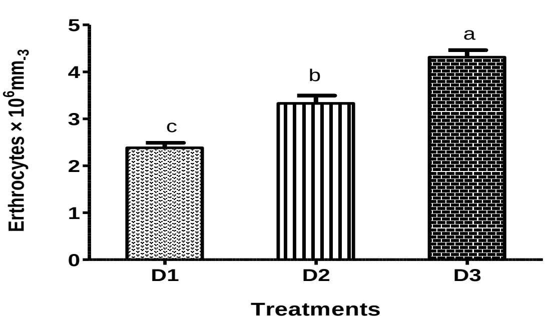

Figure 3.1: Effect of dietary Bacillus subtilis supplementation on total erythrocyte count of rohu fingerlings.

Here, D1 is control = without any probiotic in the feed, D2 = Probiotic (10^3 CFU/g) in the feed, and D3 = Probiotic (10^6 CFU/g) in the feed. One-way ANOVA followed by post hoc LSD Tukey test represents a comparison between the studied groups. Different alphabets on bars indicate a significant difference ($P < 0.001$) between the studied groups. Error bar = ±SD; n = 6.

Blood Glucose Level

From the obtained values, it can be inferred that the blood glucose level of rohu fingerlings decreased as the concentration of probiotic Bacillus subtilis increased in the diet. The highest level of glucose was observed in the control diet, while the lowest level was observed in fingerlings fed with the probiotic-supplemented diet D3. A statistically significant difference ($p<0.05$) was found among all dietary groups.

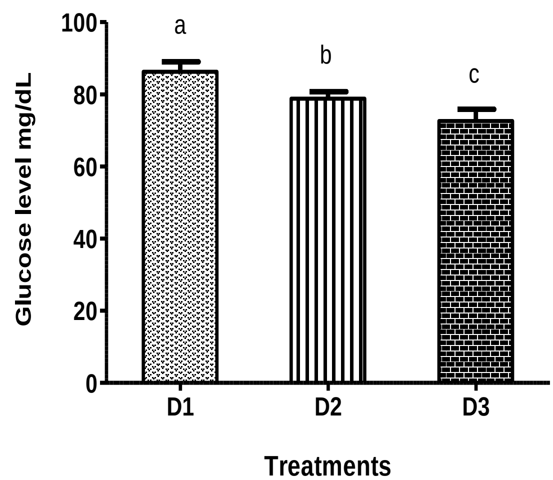

Figure 3.2 Effect of dietary Bacillus subtilis supplementation on the blood glucose level of rohu fingerlings.

Here, D1 is control = without any probiotic in the feed, D2 = Probiotic (10^3 CFU/g) in the feed, and D3 = Probiotic (10^6 CFU/g) in the feed. One-way ANOVA followed by post hoc LSD Tukey test represents a comparison between the studied groups. Different alphabets on bars indicate a significant difference ($P < 0.001$)

between the studied groups. Error bar = ±SD; n = 6.

Hb (hemoglobin)

From the obtained values, it can be inferred that the hemoglobin concentration of rohu fingerlings increased as the concentration of probiotic Bacillus subtilis increased in the diet. The lowest level of hemoglobin was observed in the control diet, while the highest level was observed in fingerlings fed with the probiotic-supplemented diet D3. A statistically significant difference ($p<0.05$) was found among all dietary groups.

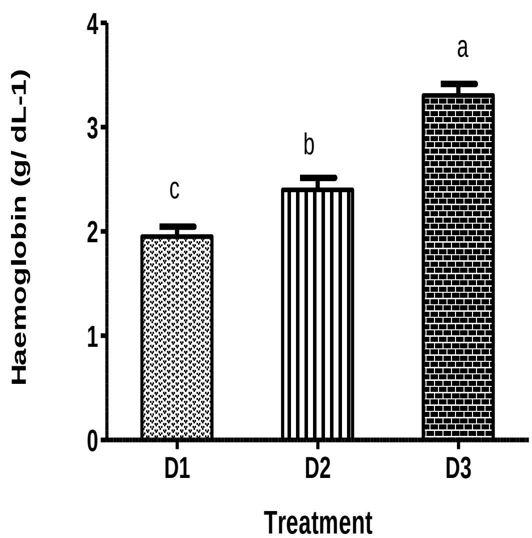

Figure 3.3 Effect of dietary Bacillus subtilis supplementation on the Hemoglobin level of rohu fingerlings

Here, D1 is control = without any probiotic in the feed, D2 = Probiotic (10^3 CFU/g) in the feed, and D3 = Probiotic (10^6 CFU/g) in the feed. One-way ANOVA followed by post hoc LSD Tukey test represents a comparison between the studied groups. Different alphabets on bars indicate a significant difference ($P < 0.001$) between the studied groups. Error bar = ±SD; n = 6.

HCT% % (hematocrit value)

From the obtained values, it can be inferred that the hematocrit value of rohu fingerlings increased as the concentration of probiotic Bacillus subtilis in the diet increased. The highest level of HCT% was observed in fingerlings fed with probiotic-supplemented diet D3, while the lowest level was observed in fingerlings fed with control diet D1. A statistically significant difference ($p<0.05$) was found among all dietary groups.

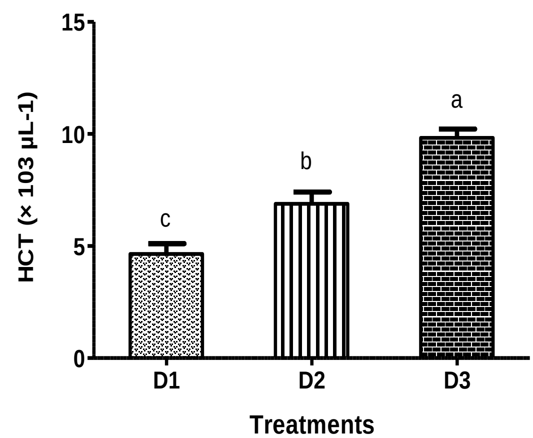

Figure 3.4 Effect of dietary Bacillus subtilis supplementation on the HCT level of rohu fingerlings

Here, D1 is control = without any probiotic in the feed, D2 = Probiotic (10^3 CFU/g) in the feed, and D3 = Probiotic (10^6 CFU/g) in the feed. One-way ANOVA followed by post hoc LSD Tukey test represents a comparison between the studied groups. Different alphabets on bars indicate a significant difference ($P < 0.001$) between the studied groups. Error bar = ±SD; n = 6.

Discussion

In aquaculture, fish are typically exposed to a variety of effects such as improper handling, high intensity, artificial diets, and various stressors (Kumar et al., 2017; Kahlil et al., 2019). Thus, supplying an artificial diet rich in essential nutrients is an important aquaculture technique for maintaining optimal growth performance, physiological status, and resistance to both biotic and abiotic stressors (Kumar et al., 2017; Kahlil et al., 2019; Amir et al., 2019). Aquaculture is one of the most viable and fast-growing systems in the world, in which the availability of an adequate nutritionally balanced diet is one of the most important aspects. Studies about nutrition in freshwater aquaculture have resulted in the development of new feed formulations for carps and other aquaculturally important fish species. Aquaculture production is growing more intense and commercialized, which raises a variety of concerns, including water quality management and disease control. Domesticating and developing brood stock are two further issues (Amir et al., 2019).

In this study, dietary Bacillus subtilis is explored for its possible effects on L. rohita growth rate, feed conversion ratio, intestinal enzyme activities, haematological and immunological parameters. Bacillus subtilis has a crucial role in improving digestibility and raising cellular and enzymatic activities associated with immune response and digestion, which accounts for its beneficial effects. Based on these findings, adding dietary Bacillus subtilis specifically at a level of 10^6 CFU/g to the diet can be considered an efficient, environmentally friendly, and practical method of increasing the production of Fish rohu and other fish species in aquaculture.

Conclusion

Finally, dietary Bacillus subtilis treated formulated feeds (10^6 CFU/g) promoted Labeo rohita growth and digestive enzyme activities, biochemical and immunological parameters, hematological parameters. Our findings suggest that dietary Bacillus subtilis, at a dose of 10^6 CFU/g feed, could be added to a practical diet to boost the growth performance and health status of cultured L. rohita.

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