

Abstract

The increasing concern over antibiotic resistance has driven the search for sustainable alternatives in aquaculture. Among these, probiotics and phytobiotics have shown promising potential to enhance fish health and productivity. This study aimed to evaluate the individual and combined effects of dietary supplementation with the probiotic Bacillus licheniformis and the phytobiotic Cinnamomum verum on growth performance, immune response, gene expression, and disease resistance in Labeo rohita (rohu), a commercially important freshwater fish species. A 12-week feeding trial was conducted using 800 healthy fingerlings randomly assigned to eight groups. The experimental design included one control group (basal diet without additives) and seven treatment groups: three receiving Bacillus licheniformis at graded levels (D1 = 10^5 , D2 = 10^7 , D3 = 10^9 CFU/g), three receiving Cinnamomum verum at increasing concentrations (D4 = 25, D5 = 50, D6 = 75 mg/kg), and one combination group (D7 = B. licheniformis 10^9 CFU/g + C. verum 75 mg/kg). Growth performance was assessed using final body weight, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). Hematological (RBC, WBC, hemoglobin) and immunological parameters (lysozyme activity, respiratory burst, serum protein) were also evaluated. To assess disease resistance, fish from each group were challenged with Aeromonas hydrophila, a common fish pathogen, and survival rates were recorded. Additionally, gene expression analysis of pro-inflammatory cytokines (IL-1 β and TNF- α) was performed in liver and head kidney tissues using quantitative real-time PCR. Results demonstrated that all supplemented groups showed significant improvements compared to the control ($p < 0.05$). The combination group (D7) outperformed all others, exhibiting the highest final weight, SGR, and survival rate, along with the lowest FCR. The D3 group (B. licheniformis 10^9 CFU/g) also showed notable improvements in growth and immune responses, followed closely by D6 (C. verum 75 mg/kg). Enhanced

hematological and immunological responses were observed in these groups. Gene expression analysis revealed a marked upregulation of IL-1 β and TNF- α in the D7 and D3 groups, indicating immune system activation and enhanced disease resistance. These findings highlight the synergistic potential of combining probiotics and phytobiotics in aquafeeds. The co-administration of Bacillus licheniformis and Cinnamomum verum not only improved growth performance and nutrient utilization but also strengthened innate immunity and resistance to bacterial infection in Labeo rohita. This study supports the integration of natural feed additives as effective and environmentally friendly alternatives to antibiotics in sustainable aquaculture practices.

Keywords: Bacillus licheniformis, Cinnamomum verum, Labeo rohita, growth performance, immune response, gene expression, disease resistance, aquaculture sustainability