

2.3 Immune and disease mechanisms

Probiotics enhance fish disease resistance chiefly through innate immune activation, especially by increasing phagocytic activity, lysozyme production, complement activity, respiratory burst, and cytokine expression. In finfish, lactic acid bacteria often increase neutrophil activity, lysozyme secretion, phagocytosis, and pro-inflammatory cytokines such as IL-1 β , IL-6, IL-8, and TNF- α , although some strains instead preferentially induce IL-10, showing that immune effects are strain and host dependent rather than uniform (Ringø et al., 2018). This host- and strain-specificity is a central feature of probiotic immunobiology in aquaculture, and immune outcomes vary with source, dose, and duration of supplementation. Beyond soluble effectors, probiotics can stimulate gut-associated immunity, including increases in Ig-positive cells and acidophilic granulocytes, thereby strengthening mucosal defense at a major portal of pathogen entry.

Experimental studies show that these mechanisms translate into higher survival after pathogen challenge. In fish fed *Lactococcus lactis*, probiotic supplementation increased growth and survival, upregulated anti-inflammatory cytokines, and moderated excessive pro-inflammatory cytokine responses after *Aeromonas hydrophila* infection. In goldfish, *Exiguobacterium acetylicum* elevated respiratory burst, phagocytosis, antimicrobial enzymes, immunoglobulin levels, and cytokine gene expression, increasing post-challenge survival from 33.2% in controls to 73.2% in the high-dose group. In grass carp specifically, *Pseudomonas monteilii* JK-1 reduced *Aeromonas* load while increasing IL-1 β , IL-10, TNF- α , TGF- β , and antioxidant enzymes in gut and head kidney, linking microbial regulation with coordinated immune and oxidative-stress control. *Bacillus*-based products can extend beyond classical probiotics into adjuvant-like platforms, since recombinant *B. subtilis* spores have enhanced survival and specific IgM and IgZ responses in virus-challenged grass carp, underscoring the broader immunobiological value of *Bacillus* in healthy aquaculture (Soltani et al., 2019). In grass carp, probiotics therefore act through three connected mechanisms: functional taxa selection, intestinal barrier stabilization, and immune priming. These mechanisms make *Bacillus*, LAB, and host-derived strains especially relevant for antibiotic-reduced and health-oriented grass carp farming.

3 Effects of Probiotics on Growth Performance of Grass Carp

3.1 Effects of probiotics on feeding behavior and feed utilization efficiency

Probiotics improve feed utilization in grass carp primarily by strengthening digestive capacity rather than by simply increasing feed intake. In a 6-week trial, a duo-strain preparation of *Bacillus subtilis* and *Lactobacillus plantarum* increased protease, amylase, lipase, and trypsin activities while also lowering feed conversion ratio, indicating more efficient digestion and nutrient use (Luo et al., 2022). A broader review of aquaculture nutrition supports the same mechanism, concluding that probiotics enhance intestinal microbial balance, digestive enzyme activity, food absorption, and ultimately feed efficiency when properly applied.

Evidence from grass-carp-specific trials shows that this nutritional effect can occur with or without major changes in voluntary feed intake. Juvenile fish receiving *Cetobacterium somerae* showed significantly higher feed intake together with improved feed conversion ratio and feed efficiency ratio, suggesting that this host-associated strain promoted both appetite and utilization efficiency (Figure 1) (Chen et al., 2025). By contrast, a multi-strain probiotic improved growth and feed utilization most clearly at 0.34-1.68 g/kg, implying that the quality of nutrient conversion, not maximum consumption alone, explains much of the probiotic growth response.

3.2 Growth parameters and productivity

Across studies, probiotic supplementation consistently improves core growth indicators in grass carp, especially weight gain, specific growth rate, and feed conversion ratio. Dietary supplementation with *Bacillus subtilis* Ch9 for 56 days significantly increased specific growth rate and reduced feed conversion ratio relative to the unsupplemented control. Similar benefits were reported in a 2024 study of host-derived *Pseudomonas monteilii* JK-1, which significantly increased weight gain, specific growth rate, and survival rate while reducing pathogen burden, linking better productivity to better overall health status.