

Probiotics appear to influence these metabolic processes partly by remodeling microbiota-linked pathways. In grass carp receiving *B. velezensis* LH023, KEGG analysis showed enrichment of pathways related to carbohydrate, amino acid, lipid, cofactor, and vitamin metabolism, suggesting coordinated improvement of nutrient transformation capacity. A complementary signal comes from *C. somerae*, which increased intestinal acetic acid concentration in juvenile grass carp, consistent with enhanced microbial fermentation and better local energy supply for intestinal development and absorption (Chen et al., 2025). Overall, probiotics regulate the grass carp digestive system through barrier reinforcement, enzyme activation, and metabolic reprogramming. These coordinated effects help explain why probiotic supplementation often improves nutrient utilization, intestinal resilience, and growth performance in healthy farming systems.

## 5 Effects of Probiotics on Immune Function and Disease Resistance of Grass Carp

### 5.1 Regulatory effects of probiotics on non-specific immune responses

Probiotics enhance the non-specific immune system of grass carp by stimulating humoral and cellular defense indicators that respond rapidly to infection. In a 60-day feeding trial, a multi-strain probiotic increased plasma myeloperoxidase activity and complement C3 content at higher inclusion levels, showing that immune enhancement can be dose dependent rather than identical across all supplementation levels. A separate study using autochthonous intestinal bacteria found that probiotic-fed grass carp had higher respiratory burst, phagocytic activity, and lysozyme activity than controls, together with increased complement C3, total serum protein, albumin, and globulin, indicating broad activation of innate defense pathways.

This immune stimulation is also reflected at the gene-expression level in multiple immune organs. Feeding *Pediococcus pentosaceus* SL001 significantly upregulated IgM and C3 expression in the liver, spleen, and head kidney while downregulating IL-8, suggesting stronger baseline defense with lower inflammatory burden (Gong et al., 2019). Similarly, *Bacillus velezensis* B8 increased serum alkaline phosphatase and SOD activity, and after 3-4 weeks it significantly upregulated IgM and TNF- $\alpha$  in the spleen and kidney, showing that probiotic immunoregulation can intensify over time rather than appear only immediately after feeding begins (Wu et al., 2021).

### 5.2 Effects of probiotics on antioxidant capacity and stress responses

Probiotics improve antioxidant capacity in grass carp by increasing key enzymatic defenses and reducing oxidative damage. Under *Aeromonas hydrophila* challenge, dietary *Bacillus subtilis* reduced malondialdehyde while increasing total antioxidant capacity, SOD, CAT, GSH, and the expression of antioxidant enzymes including SOD, CAT, and GPx, indicating protection against infection-induced oxidative stress. Comparable effects were observed with duo-strain supplementation of *B. subtilis* and *Lactobacillus plantarum*, which significantly improved SOD and CAT and lowered MDA during a 6-week feeding period, showing that antioxidant benefits are not limited to single-strain preparations (Luo et al., 2022).

These antioxidant effects appear closely linked to stress resilience and intestinal protection. In juvenile grass carp, dietary probiotics including *Bacillus cereus*, *B. subtilis*, *Paracoccus marcusii*, and *Lactobacillus plantarum* elevated serum C3/C4 or AKP, lowered intestinal MDA, and in the *Bacillus* groups activated the Nrf2 pathway while upregulating CAT and SOD-related genes, which supports a mechanistic role for antioxidant signaling (Xue et al., 2020). In the multi-strain trial, probiotic-fed fish also showed lower liver MDA, higher glutathione peroxidase, and a significantly stronger respiratory burst after hypoxia stress, indicating that probiotic-enhanced redox control can translate into better tolerance of environmental stressors.

### 5.3 Roles of probiotics in enhancing disease resistance and reducing disease incidence

A consistent outcome across studies is that probiotics increase post-challenge survival and reduce disease-related mortality in grass carp. Host-derived *Pseudomonas monteilii* JK-1 significantly increased survival rate and reduced *Aeromonas* pathogen load, while also upregulating IL-1 $\beta$ , IL-10, TNF- $\alpha$ , and TGF- $\beta$  in the gut and head kidney, indicating that improved resistance is coupled to coordinated immune activation rather than pathogen suppression alone. Likewise, dietary supplementation with *Lactobacillus buchneri* L3-9 or its extracellular