

Duration also shapes probiotic outcomes, because physiological gains can appear early and then plateau over longer feeding periods. In the Ch9 trial, digestive enzyme activities rose from days 14 to 56, but the increase was greatest in the shorter term and no longer continued upward by day 56, even though values remained above control fish. A similar pattern of dose optimization was quantified in other studies: broken-line analysis identified about 1.34 g/kg as the optimum level for a multi-strain probiotic, while *C. somerae* produced the best overall growth, intestinal development, and nutrient retention at about $1.27\text{--}1.35 \times 10^9$ cells/kg rather than at the highest inclusion level (Chen et al., 2025). Overall, probiotics improve grass carp growth performance mainly by enhancing digestive efficiency, nutrient retention, and feed conversion, but the magnitude of benefit depends on the probiotic strain, supplementation level, and feeding duration. For grass carp farming, the evidence supports probiotics as effective growth promoters when matched carefully to species-specific and dose-specific conditions.

4 Regulatory Effects of Probiotics on the Digestive System and Nutrient Metabolism of Grass Carp

4.1 Effects of probiotics on intestinal structure integrity and functional improvement

Probiotics improve intestinal structural integrity in grass carp by promoting mucosal development and strengthening epithelial barrier function. In juvenile grass carp, dietary *Cetobacterium somerae* increased intestinal length index, somatic index, and fold height, indicating better intestinal development and a larger absorptive surface. The same study further showed that this probiotic reduced intestinal permeability, preserved tight-junction ultrastructure, and increased tight-junction and adherens-junction biomarkers, supporting a direct role in barrier maintenance rather than only a secondary growth effect (Chen et al., 2025).

Barrier protection is also evident with other host-associated probiotics and under challenge conditions. *Bacillus velezensis* LH023 increased expression of ZO-2, ZO-3, and claudin-12 in the intestine of grass carp, which was interpreted as reduced mucosal permeability and improved protective function (Liu et al., 2025). In infected carp, compound probiotics alleviated villus swelling and prevented the decline of Occludin, Claudin-1, and ZO-1, showing that probiotic barrier support can persist even during pathogen-induced intestinal injury.

4.2 Digestive enzymes and absorption

Probiotics enhance digestive enzyme activity in grass carp across multiple enzyme systems, which helps explain improvements in nutrient digestion and feed use. Feeding *Bacillus subtilis* Ch9 increased protease, amylase, and lipase activities in the intestine and hepatopancreas over 14–56 days, with the best overall response reported at 3×10^9 CFU/kg feed (Wu et al., 2012). Similar species-dependent effects were reported in fingerlings given *Bacillus coagulans*, *Rhodopseudomonas palustris*, or *Lactobacillus acidophilus*, where all probiotics improved growth but *B. coagulans* produced the strongest increase in protease activity.

Improved enzyme activity is closely tied to stronger absorptive function. *C. somerae* enhanced both digestive enzymes and brush-border enzymes in juvenile grass carp, and these changes were accompanied by improved nutrient retention, indicating more effective digestion-to-absorption transfer. This pattern is consistent with broader fish evidence showing that probiotic supplementation improves intestinal microbial balance, digestive enzyme activity, and food absorption, thereby increasing feed efficiency rather than acting only as a passive microbial additive (Assan et al., 2021).

4.3 Metabolism and energy utilization

The metabolic effects of probiotics in grass carp extend beyond digestion to the regulation of lipid deposition, carbohydrate use, and microbial energy metabolism. In fish fed a high-fat diet, *Bacillus subtilis* improved growth and serum biochemical indices while reducing hepatic lipid accumulation, indicating that probiotics can redirect nutrient partitioning away from fatty liver formation (Guo et al., 2022). In parallel, intestinal microbiota studies show that the grass carp gut microbiome itself contributes substantially to nutrition metabolism, with carbohydrate metabolism increasing along the intestine and the hindgut acting as a major site of fiber fermentation that helps the host obtain nutrients and energy from plant material.