

Effects of Dietary *Bacillus Amyloliquefaciens* on Growth Performance, Immunity and Intestinal Health of Juvenile Triploid Rainbow Trout (*Oncorhynchus mykiss*) in Low-Fishmeal Diets

LIU Yangting^{1,2}, WANG Hanya¹, DU Fei¹, WANG Jiahao², LU Shaoxia², HAN Shicheng², WANG Chang'an^{2*}, LIU Hongbai^{2*}

(1. College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China; 2. Key Laboratory of Aquatic Animal Diseases and Immunity of Heilongjiang Province, Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Heilongjiang Harbin 150000, China.)



ABSTRACT

This study evaluates the effects of dietary *Bacillus amyloliquefaciens* on growth performance immunity and intestinal health in juvenile triploid rainbow trout (*Oncorhynchus mykiss*). A total of 345 triploid rainbow trout (7.68 ± 0.20 g) were allotted in 5 groups with 3 tanks each and fed with 5 diets including G1 (control diet), G2(control diet containing 3.34×10^7 colony-forming unit (CFU)/g *B. amyloliquefaciens*), G3(control diet containing 2.7×10^8 CFU/g *B. amyloliquefaciens*), G4(control diet containing 2.7×10^9 CFU/g *B. amyloliquefaciens*) and G5 (control diet containing 10^{10} CFU/g *B. amyloliquefaciens*), respectively. After 8 weeks, addition of *B. amyloliquefaciens* restored growth retardation and intestinal injury induced by soybean meal in rainbow trout. Significant enhancements in growth performance, including improved weight gain rates, protein efficiency rates, specific growth rates and decreased feed conversion ratios in the G3 group, were indicative of enhanced nutrient utilization ($P < 0.05$). With the increase of *B. amyloliquefaciens* supplementation level, there were no significant differences in crude lipid and moisture content ($P > 0.05$). The crude protein content of fish significantly increased first and then decreased ($P < 0.05$). The contents of superoxide dismutase, catalase and lysozyme in serum and liver of the experimental group significantly increased first and then decreased, the contents of malondialdehyde and trace reduced glutathione in liver significantly decreased first and then increased ($P < 0.05$), and the alkaline phosphatase content did not change significantly ($P > 0.05$). Compared with the control group, when supplemented with 10^8 - 10^{10} CFU / g *B. amyloliquefaciens*, the levels of intestinal protease and amylase significantly increased ($P < 0.05$), the intestinal lipase levels were no significant change ($P > 0.05$). when supplemented with 2.7×10^8 CFU / g *B. amyloliquefaciens*, the villi height, villi width and basal layer thickness were significantly improved ($P < 0.05$), the expression of intestinal tight junction protein(Cldn-1, Ocldn) were significantly increased, and the expression of intestinal inflammatory factors(TNF- α , IL-6, IL-8) were significantly decreased($P < 0.05$). Addition of *B. amyloliquefaciens* resulted increased the relative abundance of beneficial intestinal bacteria (*Firmicutes*, *Proteobacteria*, *Lactobacillaceae*) and decreased the relative abundance of pathogenic bacterium (*Nostocaceae*, *Aeromonas*, *Ruminococcaceae*). However, the high level of *B. amyloliquefaciens* supplementation caused intestinal flora imbalance and intestinal barrier damage. In conclusion, dietary 2.7×10^8 *B. amyloliquefaciens* had a positive role on growth performance, immunity and intestinal health of juvenile triploid rainbow trout (*Oncorhynchus mykiss*) in low fishmeal diets.



KEY WORDS

Bacillus amyloliquefaciens, Low-Fishmeal, *Oncorhynchus mykiss*, Growth Performance, Immunity, Intestinal health

RESULTS

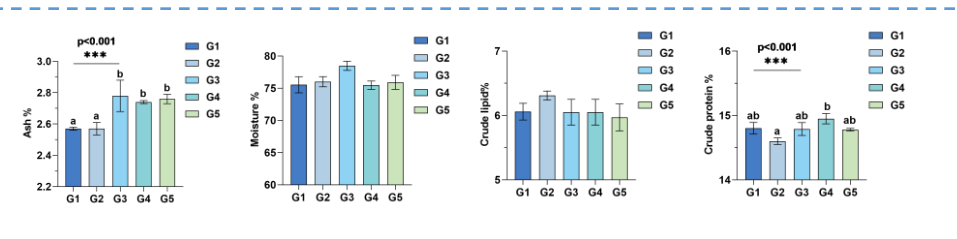


Fig.1 Body composition of triploid *O. mykiss* in five groups

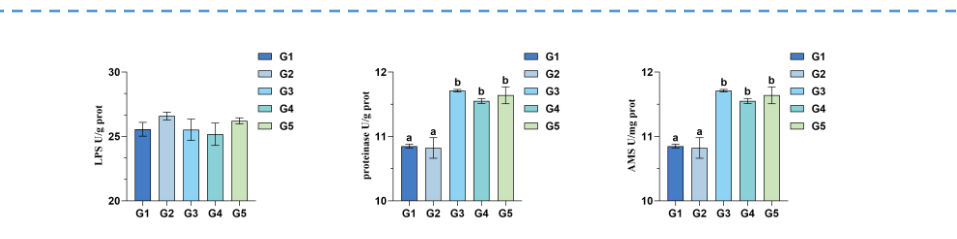


Fig.2 The intestinal digestive enzyme and intestinal morphology in triploid *O. mykiss* in five groups

组别 Index	G1	G2	G3	G4	G5
溶菌酶 LZM (μg/ml)	33.01 ± 6.09 ^a	63.12 ± 6.46 ^b	77.12 ± 0.99 ^b	65.16 ± 2.31 ^b	64.9 ± 2.36 ^b
过氧化氢酶 CAT (U/mg prot)	136 ± 2.37 ^{ab}	149.76 ± 1.68 ^b	181.53 ± 6.62 ^c	136.83 ± 2.49 ^{ab}	132.88 ± 4.77 ^a
超氧化物歧化酶 SOD (U/mg prot)	22.61 ± 0.38 ^c	21.73 ± 0.51 ^{ab}	24.02 ± 0.56 ^c	20.98 ± 0.42 ^a	21.75 ± 0.43 ^{ab}
微量还原型谷胱甘肽 GSH (μmol/g prot)	52.92 ± 3.62 ^b	48.83 ± 3.96 ^{ab}	47.57 ± 1.26 ^{ab}	39.69 ± 0.38 ^a	42.8 ± 0.75 ^a
丙二醛 MDA (nmol/mg prot)	10.55 ± 0.76 ^a	5.90 ± 0.42 ^a	6.47 ± 0.34 ^b	7.04 ± 0.33 ^b	6.13 ± 0.34 ^b
碱性磷酸酶 AKP (金氏单位/gprot)	58.46 ± 3.65	58.49 ± 3.27	62.31 ± 4.68	53.33 ± 4.77	62.82 ± 0.28

Table.1 The Liver antioxidant enzyme activity in triploid *O. mykiss* in five groups

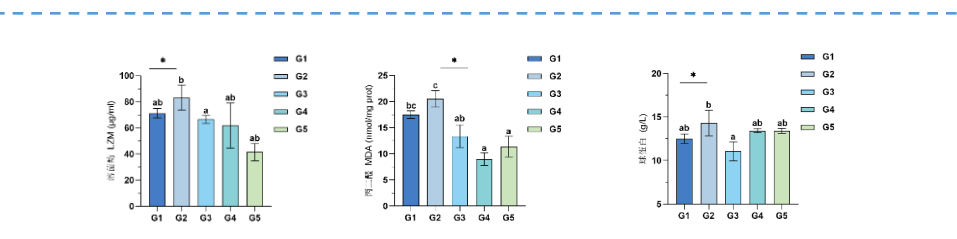


Fig.3 Effects of dietary *B. amyloliquefaciens* supplementation on the antioxidative function of serum in rainbow trout

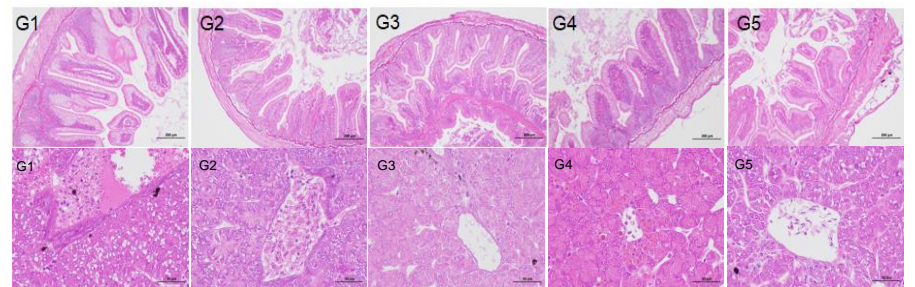


Fig.4 Effects of *B. amyloliquefaciens* supplementation on the Intestines and liver morphology of triploid *O. mykiss*.

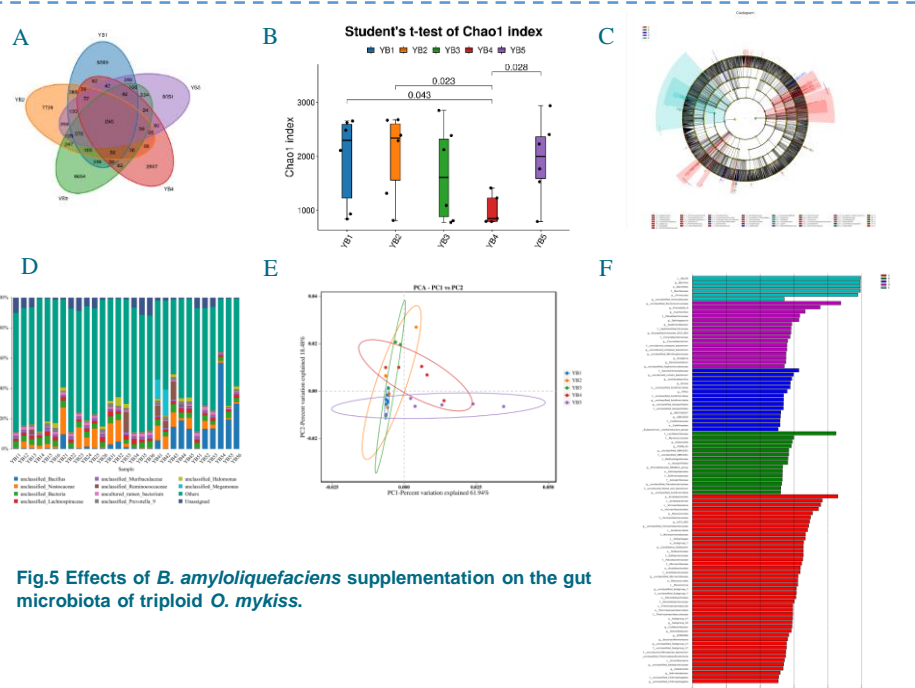


Fig.5 Effects of *B. amyloliquefaciens* supplementation on the gut microbiota of triploid *O. mykiss*.

Conclusions

The low-fishmeal diet (10%) supplemented with an appropriate level of *B. amyloliquefaciens* has positive effects on improving the growth, digestive enzyme activities, intestinal morphological structure development, and body immunity of juvenile triploid *O. mykiss*. Using specific growth rate as evaluation index, the optimal requirement of *B. amyloliquefaciens* for triploid *O. mykiss* was 2.7×10^8 CFU/g. This finding offers a promising strategy for the feed industry to incorporate soybean meal into fish feed formulations.

Acknowledgments

The authors thank the participants who gave their time to the trial. This study was supported by the Central Public-Interest Scientific Institution Basal Research Fund, HRFRI (HSY202202M), the China Agriculture Research System of MOF and MARA (CARS-46), the Natural Science Funds of Heilongjiang (LH2023C057).