Research Article

Effects of Dietary *Bacillus velezensis* LSG2-5 on Growth, Immunity, Antioxidant Capacity, and Disease Resistance of Amur minnow (*Rhynchocypris lagowskii* Dybowski)

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The study was aimed at investigating the effects of *Bacillus velezensis* LSG2-5 isolated from host's intestinal tract on growth performance, immune response, antioxidant capacity, and disease resistance of Amur minnow (*Rhynchocypris lagowskii* Dybowski). 535 fish were divided into 5 groups with 3 replicates per group. Diets containing different doses (0, 10^6 , 10^7 , 10^8 , and 10^9 CFU/g, respectively) of *B. velezensis* LSG2-5 were used to feed fish (9.95 ± 0.05 g) for 56 days. Results showed that compared with control, weight gain rate and specific growth rate of fish were significantly increased by dietary 10^7 – 10^9 CFU/g *B. velezensis* ($p < .05$). While for the feed conversion ratio, all treatment groups were significantly decreased ($p < .05$). In serum, the contents of lysozyme and immunoglobulin M were significantly increased by dietary 10^6 – 10^9 CFU/g *B. velezensis* ($p < .05$); the activities of component 3, component 4, and alkaline phosphatase were significantly increased by dietary 10^8 CFU/g *B. velezensis* ($p < .05$). The activities of catalase, glutathione peroxidase, total antioxidant capacity, and superoxide dismutase in hepatopancreas were significantly increased by dietary 10^8 – 10^9 CFU/g *B. velezensis* ($p < .05$). The activities of catalase, glutathione, glutathione peroxidase, total antioxidant capacity, and superoxide dismutase in the intestinal tract of 10^8 CFU/g group were significantly increased ($p < .05$). However, malondialdehyde levels in the hepatopancreas and intestines were significantly decreased by dietary 10^7 – 10^8 CFU/g *B. velezensis* ($p < .05$). Dietary 10^7 – 10^9 CFU/g *B. velezensis* significantly upregulated catalase, glutathione peroxidase, and copper/zinc superoxide dismutase mRNA levels ($p < .05$). The levels of transforming growth factor β and interleukin-10 mRNA in the hepatopancreas of fish fed with *B. velezensis* of 10^8 CFU/g were significantly increased ($p < .05$), and these in the intestinal tract of 10^6 – 10^9 CFU/g groups were upregulated ($p < .05$). Feeding dietary 10^7 – 10^8 CFU/g *B. velezensis* significantly downregulated interleukin-8, interleukin-1 β , and tumour necrosis factor α mRNA levels in hepatopancreas and intestines ($p < .05$). After 56 days of feeding, 225 fish (23.20 ± 0.59 g) were challenged with *Aeromonas hydrophila* (10^8 CFU/g), and survival rates were recorded for the next 14 days. Meanwhile, the serum immune parameters of fish were determined. Results showed that compared with control, the survival rates of the treatment groups were significantly increased ($p < .05$). Lysozyme, component 3, component 4, immunoglobulin M, acid phosphatase, and alkaline phosphatase levels in serum by dietary 10^6 – 10^9 CFU/g *B. velezensis* showed significant promoting effects ($p < .05$). In conclusion, dietary 10^7 – 10^9 CFU/g *B. velezensis* boosts the growth, immunity, antioxidant capacity, and against *A. hydrophila* infection of Amur minnow. Regression analysis suggested that the optimal dose of *B. velezensis* LSG2-5 in the Amur minnow diet was 5.00×10^8 CFU/g.

1. Introduction

Probiotics have received attention as alternatives to antibiotics and other drugs in aquaculture [1]. *Bacillus* can regulate humoral immunity [2], mucosal immunity [3], or the expressions of immune-related genes [4] to enhance the immunity of fish. Studies have shown that both heterogeneous and indigenous *Bacillus* affect the growth, immunity, antioxidant capacity, and disease resistance of fish [5, 6]. Endogenous *Bacillus* has more excellent potential than exogenous *Bacillus* because of its ability to adapt to the same environment and avoid competitive rejection [7, 8].

Bacillus velezensis is a new species that has been applied to aquaculture in recent years [9]. It mainly acts on the immune system and disease resistance of fish to inhibit the attack of pathogenic bacteria [10]. It is a potential candidate probiotic. A strain of *B. velezensis* isolated from healthy *Macrobrachium nipponense* has been proven to inhibit pathogenic bacteria in vitro and is not pathogenic [11]. The research on the prevention and control of aquatic animal diseases by *B. velezensis* is still limited to the isolation of superior microbial resources in specific habitats and the analysis of biological characteristics of superior strains. For large-scale applications, further research and systematic evaluation are needed. As a probiotic feed additive, *B. velezensis* comes from different sources, either in the gastrointestinal tract of the fish itself or in the environment where the fish live. It was found that *B. velezensis* from the intestinal tract of fish had probiotic effects on the immune system of channel catfish (*Ictalurus punctatus*) [12], Nile tilapia (*Oreochromis niloticus*) [13], crucian carp (*Carassius auratus*) [14, 15], hybrid grouper (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀) [16], and others, such as inhibiting pathogenic bacteria. Nonfish *B. velezensis* also had probiotic and disease-resistant effects on grass carp [10], rainbow trout (*Oncorhynchus mykiss*) [17], juvenile Atlantic salmon (*Salmo salar* L.) [18], and others. We speculate that *B. velezensis* still has probiotic effects on native fish in northern China. However, the adaptability of different doses of *B. velezensis* to different fish in vivo requires further research.

The Amur minnow (*Rhynchocypris lagowskii* Dybowskii) is an omnivorous cold-water fish with fresh and tender meat. It is one of the main economic fish promoted in Northeast China in recent years [19]. Countries have completely banned antibiotics worldwide [1]. Farming of Amur minnow has been facing diseases caused by pathogenic bacteria or environmental factors, which may cause economic losses. The current research on Amur minnow mainly focused on the nutritional requirements, including the effects of dietary carbohydrate sources [20], differential protein levels [21], β -conglycinin [22], glycinin [23], L-carnitine [24], and oxidized fish oil [25, 26]. In our laboratory, a strain LSG2-5 isolated from the intestinal tract of Amur minnow was identified as *B. velezensis* by 16S rDNA sequence analysis, and its probiotic evaluation and safety test were conducted [27]. To verify the positive effects of *B. velezensis* LSG2-5 on the host in vivo, Amur minnow was fed with different doses of dietary *B. velezensis* LSG2-5 in this research. The effects of *B. velezensis*

LSG2-5 on growth performance, serum immunity, antioxidant enzymes, and resistance to *Aeromonas hydrophila* infection of Amur minnow were investigated, and the optimal dose of LSG2-5 was analyzed.

2. Materials and Methods

2.1. Probiotic Preparation. *B. velezensis* isolated and screened from the intestines of Amur minnow was cultured in the past. *B. velezensis* was placed into Luria-Bertani (LB) broth and cultured in a shaker incubator at 37°C for 16 h. *B. velezensis* was coated on LB agar plate and cultured at 37°C for 24 h. A single colony of *B. velezensis* was selected and placed into LB broth, divided into four groups, and cultured in new LB broth. The cells were collected and suspended in sterile phosphate-buffered saline (PBS). LB agar plate counting method was used to count, and the bacterial counts in the 4 groups were determined to be 10^6 CFU/mL, 10^7 CFU/mL, 10^8 CFU/mL, and 10^9 CFU/mL, respectively. Four groups of purified *B. velezensis* with different doses were prepared to feed immediately as needed.

2.2. Experimental Diets. The isonitrogenous and isolipidic basal diets were used in this study. The composition and nutritional composition of the basal diets are shown in Table 1. On an air-dried basis, the crude protein content was 370.0 g/kg and the gross energy level was 168.0 MJ/kg. The ingredients were pulverized and passed through 250 μ m sieves, weighted according to the proportion of the formula, and mixed evenly. An appropriate amount of water was added, and then fed pellets of 1.5 mm diameter were processed by a commercial feed extruder (9KLP-125, Yongfeng Machinery Equipment Co., LTD, Zhengzhou, China). During the production of diets, the temperature was controlled from 55°C to 65°C. The diets were naturally air-dried in the shade and stored at -20°C until used. Four different doses of *B. velezensis* (10^6 CFU/g, 10^7 CFU/g, 10^8 CFU/g, and 10^9 CFU/g) were added to the basal diet, and the control group was not added (0 CFU/g). Spraying bacterial solution was carried out in a sterile environment. *B. velezensis* was suspended in PBS and uniformly sprayed on the surface of the basal diets using a high-temperature sterilized 30 mL spray bottle. The surface of diets was covered entirely with bacterial suspension, at which time 15 mL of bacterial suspension was sprayed for every 100 g of diets. Before the experiment, the diets were dissolved to measure the living bacteria count. To ensure that the actual bacterial dose of each group in diets met the test requirements. All diets supplemented with *B. velezensis* were sprayed and dried at room temperature, sprayed daily, and stored at 4°C for short periods out of light.

2.3. Experimental Fish and Feeding Trial. Amur minnow (*R. lagowskii* Dybowskii) was provided by Nian Nian You Yu Fishery (Jilin, China). The experiment was conducted at the Aquaculture Laboratory of Jilin Agricultural University (Changchun, China). The volume of the aquarium was 120 L, and the experimental units were cycled in

TABLE 1: Composition and proximate analysis of basal diets (g/kg air dry basis).

Ingredients	g/kg	Proximate composition	g/kg
Fish meal	200.0	Crude protein	370.0
Soybean meal	330.0	Crude lipid	65.6
Fish oil	20.0	Moisture	45.9
Corn oil	20.0	Ash	77.5
Wheat bran	60.0	Gross energy (MJ/kg)	168.0
Wheat flour	100.0	Nitrogen-free extract	420.6
Dextrin	70.0		
Corn protein flour	150.0		
Calcium dihydrogen phosphate	30.0		
Compound premix 1% ^a	10.0		
Lysine	2.0		
Methionine	5.0		
Choline chloride 50%	3.0		

^aCompound premix 1%: vitamins and mineral included. Vitamin A (IU/kg) 3600; vitamin D3 (IU/kg) 1200; vitamin E (mg/kg) 20, vitamin K3 (mg/kg) 5; vitamin B1 (mg/kg) 5; vitamin B2 (mg/kg) 7; vitamin B6 (mg/kg) 6; vitamin B12 (ug/kg) 20; calcium pantothenate (mg/kg) 20; nicotinic acid (mg/kg) 30; folic acid (mg/kg) 1.7; biotin (mg/kg) 0.05; VC phosphate (mg/kg) 171.4; inositol (mg/kg) 90; Mg (mg/kg) 150; Fe (mg/kg) 120; Zn (mg/kg) 60; Mn (mg/kg) 30; Cu (mg/kg) 4; Co (mg/kg) 0.5; Se (mg/kg) 0.1; I (mg/kg) 1.

natural light and dark (approx. 13:11 hours light: dark). The fish were fed the basal diets and domesticated for 14 days. A total of 535 fish (initial average weight: 9.95 ± 0.05 g) were randomly assigned to 15 buckets and divided into 5 groups with 3 replicates per group. Fish were starved for 24 h before feeding experiment. The fish were artificially fed to apparent satiation twice a day (8:00 and 17:00) for 56 days during the experiment. The water temperature was $(20 \pm 1)^\circ\text{C}$, the pH was 7.5 ± 0.1 , and the dissolved oxygen was >8.0 mg/L. A third of the water was exchanged every day. Clean the bottom of the tank every other day.

2.4. Sample Collection. After 56 days of rearing, the fish were fasted for 24 h. 6 fish were randomly selected from each replicate for analysis. All fish were anesthetized with eugenol solution (1:10,000). Then, the weight, length, viscera, and hepatopancreas of all fish were measured. Blood was collected from the tail vein for serum immunity analysis, stored at 4°C for 24 h, and centrifuged at $3000 \times g$ for 15 min to separate the serum. The hepatopancreas and intestines (proximal intestine [PI], mid intestine [MI], and distal intestine [DI]) were isolated on ice for analysis of enzyme activities and gene expressions. Muscles were isolated on ice for the expressions of relative genes. All samples were immediately frozen in liquid nitrogen and stored at -80°C until analyzed.

2.5. Related Index Determination

2.5.1. Growth Performance Measurement. Growth factors such as body weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor

(CF) were calculated as follows:

$$\begin{aligned}
 \text{WGR}(\%) &= 100 \times (\text{FW} - \text{IW}) \times \text{IW}^{-1}, \\
 \text{SGR}\left(\% \frac{\text{day}}{\text{day}}\right) &= 100 \times (\ln \text{FW} - \ln \text{IW}) \times \text{day}^{-1}, \\
 \text{FCR} &= \frac{\text{FI}}{\text{FW} - \text{IW}}, \\
 \text{HSI}(\%) &= 100 \times \left(\text{hepatopancreas} \frac{\text{weight}}{\text{FW}} \right), \\
 \text{VSI}(\%) &= 100 \times \left(\text{viscera} \frac{\text{weight}}{\text{FW}} \right), \\
 \text{CF}(\%) &= 100 \times \left[\frac{\text{FW}}{(\text{body length})^3} \right].
 \end{aligned} \tag{1}$$

where FI is the feed intake, IW is the fish initial weight, and FW is the fish final weight.

2.5.2. Analysis of Immune and Antioxidant Capacity. The levels of component 3 (C3), component 4 (C4), immunoglobulin M (IgM), lysozyme (LZM), acid phosphatase (ACP), and alkaline phosphatase (AKP) in serum were measured and analyzed following the kit's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) levels in hepatopancreas and intestines were measured and analyzed by the same methods.

2.5.3. Analysis of Gene Expression. Total RNA was extracted from the hepatopancreas and intestinal tract using a TRIzol kit (Takara, Dalian, China). Fluorescence quantitative real-time PCR was used to detect the expression of genes related to inflammation and antioxidation. Follow the instructions of the One-Step TB Green PrimeScript RT-PCR Kit II (Takara, Dalian, China). Using 20 μ L reaction system, 10 μ L One-Step TB Green RT-PCR Buffer, 0.8 μ L PrimeScript 1 Step Enzyme Mix, 0.8 μ L forward and 0.8 μ L reverse primer, 2 μ L of total RNA template, and 5.6 μ L RNase free dH₂O. Reverse transcriptional reaction conditions: 42°C for 5 min and 95°C for 10 s. Circulation conditions: 95°C for 5 s, 60°C for 30 s, and 40 times. Primers are shown in Table 2. The analysis was conducted according to the $2^{-\Delta\Delta CT}$ methodology of a previous study [19].

2.6. Challenge Test. *A. hydrophila* used was stored in the Aquaculture Laboratory of Jilin Agricultural University (Changchun, China), and its median lethal dose (LD₅₀) was determined by the method described previously [29]. *A. hydrophila* was cultured in LB broth at 28°C for 16 h, and the cells were collected and suspended in sterile PBS. Amur minnow was challenged with 200 μ L of serially diluted *A. hydrophila* suspensions for a final dose of 1.50×10^5 , 1.50×10^6 , 1.50×10^7 , 1.50×10^8 , and 1.50×10^9 CFU/mL. The cumulative mortality was calculated for 14 consecutive days.

At the end of the feeding experiment, 225 fish (average weight: 23.20 ± 0.59 g) of *B. velezensis* treatment groups were each injected with 200 μ L of PBS containing *A. hydrophila*, and the fish in the control group were each injected with 200 μ L of PBS. Fishes were fed and observed for 14 days, and survival rates were recorded. At the end of the challenge experiment, the survival fish serum was collected according to the above methods for the analysis of immune indexes.

2.7. Data Analysis. All data were expressed as means $\pm$ standard error of the mean (means $\pm$ SEM). One-way ANOVA was used to analyze the difference between the treatment and control groups by SPSS22.0. Tukey's test was used to determine the difference between the experimental groups ($p < .05$). The relationships between growth parameters and *B. velezensis* doses were analyzed by regression analysis.

3. Result

3.1. Growth Performance. The effects of dietary *B. velezensis* LSG2-5 on fish growth performance are shown in Table 3. FW, WGR, and SGR of the fish fed with *B. velezensis* LSG2-5 (10^7 - 10^9 CFU/g) were significantly higher than those of the control ($p < .05$). FCR in experimental groups significantly decreased compared to the control ($p < .05$). Only the HSI of the 10^8 CFU/g group was significantly increased than the control group ($p < .05$). Compared with the control group, HSI in 10^7 and 10^9 CFU/g groups showed upward trends, but the differences were insignificant ($p > .05$). There

were no significant differences in VSI and CF among all groups ($p > .05$).

According to the method described previously [30], different doses of *B. velezensis* LSG2-5 (X) in feed and the FW (Y_w), WGR (Y_s), and SGR (Y_f) of Amur minnow were analyzed by parabolic regression analysis. The resulting equations are shown in Table 4. The maximums FW, WGR, and SGR of Amur minnow were obtained. The results combined with ANOVA analysis showed that the optimal dose of *B. velezensis* LSG2-5 was 5.00×10^8 CFU/g.

3.2. Immunological Parameters. Table 5 shows the serum immune parameters of fish fed with different doses of *B. velezensis* LSG2-5. The levels of LZM and IgM were significantly elevated in the four treatment groups relative to the control ($p < .05$). With the increase of *B. velezensis* LSG2-5 doses, the content of C3 and C4 showed a trend of first increasing and then decreasing. Among them, the content of C3 in the 10^8 and 10^9 CFU/g groups was remarkably increased than that in the control ($p < .05$), and only the content of C4 in the 10^8 CFU/g group was significantly higher than that in the control ($p < .05$). The activity of ACP in the 10^7 CFU/g group was significantly elevated than that in the control and other groups ($p < .05$). The activities of AKP in the 10^8 and 10^9 CFU/g groups were significantly higher relative to the control ($p < .05$).

3.3. Antioxidant Capacity. Table 6 lists the antioxidant capacity in the hepatopancreas and intestines of Amur minnow fed with different doses of *B. velezensis* LSG2-5. In hepatopancreas, the activities of CAT in the 10^7 - 10^9 CFU/g groups were significantly higher than in the control ($p < .05$). The content of GSH was significantly increased in the 10^6 and 10^7 CFU/g groups compared with the control ($p < .05$). A significant increase in the activities of GSH-Px and T-AOC in the treatment groups was found compared with the control ($p < .05$). The activities of SOD in the 10^8 and 10^9 CFU/g groups were significantly increased than in the control ($p < .05$). A significant decrease of MDA levels in the 10^6 - 10^8 CFU/g groups was found compared to the control ($p < .05$).

In the proximal intestine, fish fed the 10^8 and 10^9 CFU/g diets demonstrated a significant increase in activity of CAT compared to the control ($p < .05$). The content of GSH in 10^6 and 10^7 CFU/g diets was significantly higher than the control ($p < .05$). The activity of GSH-Px in the 10^7 and 10^8 CFU/g groups was significantly higher than in the control ($p < .05$). Compared to the control group, a significant decrease of MDA levels in the 10^7 and 10^8 CFU/g groups was found ($p < .05$). The SOD levels were significantly elevated in the 10^7 and 10^8 CFU/g groups relative to the control ($p < .05$). However, T-AOC activity was significantly increased in all experimental groups ($p < .05$).

In the mid intestine, CAT activity was significantly increased in the 10^7 and 10^8 CFU/g groups compared to the control ($p < .05$). The contents of GSH in the 10^6 - 10^8 CFU/g groups were significantly higher than the control ($p < .05$). Only the activity of GSH-Px in the 10^8 CFU/g group was significantly higher than the control ($p < .05$). A

TABLE 2: Primer sequences of the gene.

Genes	Sequences (5'-3')	Reference GenBank number
β -Actin	Forward	CGGTATCCATGAGACCACCT
	Reverse	CTTCTGCATCCTGTCAGCAA
TGF- β	Forward	TTGGGACTTGTGCTCTAT
	Reverse	AGTTCTGCTGGGATGTTT
IL-10	Forward	AA TCCCTTTGA TTTTGCC
	Reverse	GTGCCTTATCCTACAGTATGTG
IL-8	Forward	ATGAGTCTTAGAGGTCTGGGTG
	Reverse	ACAGTGAGGGCTAGGAGGG
IL-1 β	Forward	CTGGAGCAATGCAATACAAA
	Reverse	AGGTAGAGGTTGCTGTTGGAA
TNF- α	Forward	CCAGGCTTTCACCTCACG
	Reverse	GCCATAGGAATCGGAGTAG
CAT	Forward	ACTACCAGTCAACTGCCCCGTC
	Reverse	TTTAGCACCTGAGTGAAGAACG
GSH-PX	Forward	ACAGAGGGTGGGCGTTAT
	Reverse	TTCGGGCACCAGAGGA
CuZn-SOD	Forward	GGCCAACCGATAGTGTTAGA
	Reverse	CCAGCGTTGCCAGTTTTTAG

TABLE 3: Effects of *Bacillus velezensis* LSG2-5 on the growth performance of Amur minnow (*Rhynchocypris lagowskii* Dybowski).

Indexes	Addition of <i>Bacillus velezensis</i> LSG2-5 (CFU/g)				
	0	10 ⁶	10 ⁷	10 ⁸	10 ⁹
IW (g)	10.00 $\pm$ 0.23	9.99 $\pm$ 0.36	10.00 $\pm$ 0.55	10.01 $\pm$ 0.24	9.98 $\pm$ 0.49
FW (g)	20.19 $\pm$ 0.58 ^a	21.23 $\pm$ 0.41 ^a	23.87 $\pm$ 0.15 ^b	25.34 $\pm$ 0.41 ^b	25.36 $\pm$ 0.25 ^b
WGR (%)	110.94 $\pm$ 3.90 ^a	114.79 $\pm$ 4.41 ^a	141.31 $\pm$ 3.17 ^b	153.28 $\pm$ 3.57 ^b	154.05 $\pm$ 2.77 ^b
SGR (%/day)	1.25 $\pm$ 0.03 ^a	1.36 $\pm$ 0.04 ^a	1.57 $\pm$ 0.02 ^b	1.66 $\pm$ 0.02 ^b	1.66 $\pm$ 0.02 ^b
FCR	1.42 $\pm$ 0.05 ^c	1.28 $\pm$ 0.04 ^b	1.04 $\pm$ 0.02 ^a	0.97 $\pm$ 0.03 ^a	0.98 $\pm$ 0.02 ^a
HSI (%)	3.04 $\pm$ 0.03 ^a	3.07 $\pm$ 0.03 ^a	3.14 $\pm$ 0.04 ^{ab}	3.26 $\pm$ 0.06 ^b	3.17 $\pm$ 0.03 ^{ab}
VSI (%)	15.31 $\pm$ 0.16	15.52 $\pm$ 0.05	15.66 $\pm$ 0.07	15.49 $\pm$ 0.16	15.80 $\pm$ 0.02
CF (%)	1.42 $\pm$ 0.04	1.49 $\pm$ 0.01	1.56 $\pm$ 0.02	1.54 $\pm$ 0.03	1.49 $\pm$ 0.04

Note: Values followed by different letters are significantly different ($p < .05$).

TABLE 4: Effects of *Bacillus velezensis* LSG2-5 on the growth performance of Amur minnow (*Rhynchocypris lagowskii* Dybowski) (regression equation).

Indexes	Regression equation	X_{opt}	Y_{max}
FW	$y = -4E - 17x^2 + 4E - 08x + 21.534, R^2 = 0.7066$	5.00×10^8	31.53
WGR	$y = -4E - 16x^2 + 4E - 07x + 117.09, R^2 = 0.671$	5.00×10^8	217.09
SGR	$y = -3E - 18x^2 + 3E - 09x + 1.3795, R^2 = 0.6521$	5.00×10^8	2.13

Note: FW means fish final weight; WGR means weight gain rate; SGR means specific growth rate; X_{opt} means the optimal value of X; Y_{max} means the maximum value of Y.

significant decrease of MDA levels in 10⁷ and 10⁸ CFU/g groups was found compared to the control ($p < .05$). The activity of SOD was significantly elevated in 10⁷-10⁹ CFU/g groups relative to the control ($p < .05$). T-AOC activity was significantly increased in the treatment groups ($p < .05$).

In the distal intestine, the activity of CAT in the 10⁸ CFU/g group was significantly higher than in the control ($p < .05$). Amur minnow fed 10⁷-10⁹ CFU/g diets demonstrated a significant increase in the content of GSH relative to the control ($p < .05$). Compared to the control

TABLE 5: Effects of *Bacillus velezensis* LSG2-5 on immunological parameters of Amur minnow (*Rhynchocypris lagowskii* Dybowski) in serum.

Indexes	Addition of <i>Bacillus velezensis</i> LSG2-5 (CFU/g)				
	0	10 ⁶	10 ⁷	10 ⁸	10 ⁹
LZM (µg/ml)	40.79 ± 0.97 ^a	45.20 ± 0.43 ^b	48.69 ± 0.78 ^b	52.90 ± 0.66 ^c	53.29 ± 1.15 ^c
C3 (mg/L)	30.59 ± 0.71 ^a	32.54 ± 0.63 ^{ab}	32.90 ± 1.21 ^{ab}	36.38 ± 0.42 ^c	34.25 ± 0.16 ^{bc}
C4 (mg/L)	51.02 ± 1.08 ^a	53.91 ± 0.83 ^{ab}	53.67 ± 0.92 ^{ab}	57.20 ± 0.90 ^b	54.73 ± 0.39 ^{ab}
IgM (mg/L)	36.52 ± 1.52 ^a	43.24 ± 1.24 ^b	47.58 ± 1.01 ^b	47.08 ± 1.06 ^b	42.55 ± 1.46 ^b
ACP (U/L)	55.68 ± 1.89 ^a	58.11 ± 1.13 ^a	65.01 ± 1.52 ^b	59.20 ± 1.12 ^a	58.33 ± 0.88 ^a
AKP (U/L)	85.22 ± 1.79 ^a	86.54 ± 1.11 ^{ab}	87.66 ± 1.25 ^{ab}	93.68 ± 1.18 ^c	91.61 ± 1.20 ^{bc}

Note: Values followed by different letters are significantly different ($p < .05$).

TABLE 6: Effects of *Bacillus velezensis* LSG2-5 on antioxidant capacity in hepatopancreas and intestines of Amur minnow (*Rhynchocypris lagowskii* Dybowski).

Indexes		Addition of <i>Bacillus velezensis</i> LSG2-5 (CFU/g)				
		0	10 ⁶	10 ⁷	10 ⁸	10 ⁹
CAT (U/mgprot)	H	32.21 ± 0.75 ^a	34.78 ± 0.79 ^a	40.37 ± 0.64 ^b	44.67 ± 1.16 ^c	38.89 ± 1.02 ^b
	PI	36.49 ± 1.16 ^a	37.93 ± 1.26 ^{ab}	38.15 ± 1.17 ^{ab}	44.22 ± 0.90 ^c	42.44 ± 0.79 ^{bc}
	MI	35.12 ± 1.53 ^a	38.47 ± 0.35 ^{ab}	39.75 ± 0.17 ^b	45.22 ± 0.93 ^c	39.18 ± 1.11 ^{ab}
	DI	35.41 ± 0.84 ^a	37.16 ± 1.49 ^a	37.45 ± 1.25 ^a	42.72 ± 0.84 ^b	38.50 ± 1.28 ^{ab}
GSH (µmol/mgprot)	H	2.19 ± 0.14 ^a	3.01 ± 0.13 ^b	3.17 ± 0.13 ^b	2.71 ± 0.19 ^{ab}	2.69 ± 0.11 ^{ab}
	PI	2.26 ± 0.14 ^a	2.87 ± 0.09 ^b	2.83 ± 0.11 ^b	2.65 ± 0.08 ^{ab}	2.44 ± 0.06 ^{ab}
	MI	1.76 ± 0.13 ^a	2.53 ± 0.08 ^b	2.69 ± 0.14 ^b	2.91 ± 0.17 ^b	1.99 ± 0.14 ^a
	DI	1.50 ± 0.09 ^a	1.87 ± 0.07 ^{ab}	1.96 ± 0.14 ^b	2.52 ± 0.07 ^c	2.63 ± 0.07 ^c
GSH-Px (U/mgprot)	H	216.51 ± 13.60 ^a	288.62 ± 15.67 ^b	370.68 ± 11.88 ^c	411.66 ± 14.58 ^c	382.76 ± 6.49 ^c
	PI	186.02 ± 8.71 ^a	225.23 ± 6.95 ^{abc}	244.78 ± 11.20 ^{bc}	273.18 ± 11.54 ^c	187.31 ± 19.82 ^{ab}
	MI	165.94 ± 12.21 ^a	193.92 ± 8.07 ^{ab}	200.58 ± 17.71 ^{ab}	232.04 ± 9.00 ^b	184.84 ± 7.69 ^{ab}
	DI	129.34 ± 10.38 ^a	168.56 ± 8.73 ^b	239.73 ± 8.28 ^c	256.75 ± 4.82 ^c	227.76 ± 5.83 ^c
MDA (nmol/mgprot)	H	3.26 ± 0.10 ^c	2.88 ± 0.08 ^b	2.31 ± 0.05 ^a	2.72 ± 0.10 ^b	3.05 ± 0.05 ^{bc}
	PI	3.25 ± 0.15 ^c	2.94 ± 0.11 ^{bc}	2.42 ± 0.08 ^a	2.74 ± 0.09 ^{ab}	2.93 ± 0.07 ^{bc}
	MI	3.39 ± 0.07 ^c	3.10 ± 0.14 ^{bc}	2.89 ± 0.04 ^{ab}	2.53 ± 0.14 ^a	3.09 ± 0.10 ^{bc}
	DI	3.28 ± 0.08 ^c	2.76 ± 0.07 ^b	2.26 ± 0.10 ^a	2.45 ± 0.08 ^{ab}	2.77 ± 0.05 ^b
SOD (U/mgprot)	H	83.45 ± 2.56 ^a	93.51 ± 4.14 ^{ab}	95.02 ± 2.55 ^{ab}	112.22 ± 2.13 ^c	104.11 ± 2.39 ^{bc}
	PI	79.60 ± 2.03 ^a	85.03 ± 2.12 ^{ab}	100.06 ± 3.64 ^c	93.59 ± 1.38 ^{bc}	88.80 ± 3.26 ^{ab}
	MI	76.23 ± 0.91 ^a	82.62 ± 2.25 ^{ab}	92.59 ± 1.74 ^c	94.17 ± 2.73 ^c	88.94 ± 0.73 ^{bc}
	DI	66.00 ± 2.56 ^a	67.88 ± 1.98 ^a	70.62 ± 4.41 ^a	86.52 ± 3.55 ^b	88.51 ± 1.07 ^b
T-AOC (U/mgprot)	H	2.54 ± 0.05 ^a	3.52 ± 0.16 ^b	3.84 ± 0.11 ^{bc}	4.33 ± 0.12 ^c	4.04 ± 0.09 ^c
	PI	1.86 ± 0.06 ^a	2.24 ± 0.09 ^b	2.53 ± 0.11 ^{bc}	2.88 ± 0.07 ^c	2.79 ± 0.05 ^c
	MI	1.58 ± 0.07 ^a	2.44 ± 0.08 ^b	2.49 ± 0.15 ^b	2.78 ± 0.09 ^b	2.84 ± 0.10 ^b
	DI	1.35 ± 0.04 ^a	1.67 ± 0.13 ^a	1.82 ± 0.06 ^{ab}	2.25 ± 0.19 ^{bc}	2.53 ± 0.13 ^c

Note: Values followed by different letters are significantly different ($p < .05$). H: hepatopancreas; PI: proximal intestines; MI: mid intestine; DI: distal intestine.

group, the activity of GSH-Px in the four experimental groups was significantly increased ($p < .05$). The activities of SOD and T-AOC in the 10⁸ and 10⁹ CFU/g groups were significantly increased ($p < .05$), but the content of MDA in the four experimental groups was significantly decreased ($p < .05$).

3.4. Relative Gene Expression

3.4.1. Relative mRNA Expression of Antioxidant-Related Genes. The effects of *B. velezensis* LSG2-5 on related mRNA levels in hepatopancreas, intestines, and Amur minnow muscles are shown in Figures 1–3. The relative expressions of CAT mRNA in the hepatopancreas of fish treated with *B. velezensis* LSG2-5 were significantly increased compared with the control ($p < .05$, Figure 1). The relative expressions of CAT mRNA in the proximal, mid, and distal intestines of

FIGURE 1: Effects of *Bacillus velezensis* LSG2-5 on the relative expression of CAT mRNA in hepatopancreas, intestine, and muscle of Amur minnow (*Rhynchocypris lagowskii* Dybowski). Different letters indicate significant difference ($p < .05$).

FIGURE 2: Effects of *Bacillus velezensis* LSG2-5 on the relative expression of GSH-Px mRNA in hepatopancreas, intestine, and muscle of Amur minnow (*Rhynchocypris lagowskii* Dybowski). Different letters indicate significant difference ($p < .05$).

the 10⁷-10⁹ CFU/g groups were significantly higher than that of the control ($p < .05$, Figure 1). The relative expressions of CAT mRNA in the 10⁶-10⁸ CFU/g group muscle were significantly higher than that of the control ($p < .05$, Figure 1).

Compared with the control group, Amur minnow fed 10⁷-10⁹ CFU/g *B. velezensis* demonstrated a significant increase in the relative expression of GSH-Px mRNA of the hepatopancreas and distal intestine ($p < .05$, Figure 2).

The relative expressions of GSH-Px mRNA in the proximal and mid intestines of all *B. velezensis* LSG2-5 treatment groups were significantly increased ($p < .05$, Figure 2). The relative expressions of GSH-Px mRNA in the muscle of the 10⁷ and 10⁸ CFU/g groups were significantly increased ($p < .05$, Figure 2).

The relative expressions of copper/zinc superoxide dismutase (CuZn-SOD) mRNA in hepatopancreas and

FIGURE 3: Effects of *Bacillus velezensis* LSG2-5 on the relative expression of CuZn-SOD mRNA in hepatopancreas, intestine, and muscle of Amur minnow (*Rhynchocypris lagowskii* Dybowski). Different letters indicate significant difference ($p < .05$).

proximal and mid intestines were significantly increased by feeding dietary *B. velezensis* LSG2-5 ($p < .05$, Figure 3). In the distal intestine and muscle, the relative expressions of CuZn-SOD mRNA in the 10^7 - 10^9 CFU/g groups were significantly increased relative to the control ($p < .05$, Figure 3).

3.4.2. Relative mRNA Expression of Genes Related to Inflammation. In this study, the relative expressions of mRNA of genes related to inflammation in the hepatopancreas, intestines, and muscles were also examined (Figures 4–8). Compared to the control, adding 10^6 - 10^8 CFU/g of *B. velezensis* LSG2-5 to the diet significantly increased the expressions of transforming growth factor β (TGF- β) mRNA in hepatopancreas ($p < .05$, Figure 4); TGF- β mRNA expressions in mid intestines were significantly increased in the 10^7 - 10^9 CFU/g groups ($p < .05$, Figure 4). The expressions of TGF- β mRNA in proximal, distal intestines, and muscle of the four treatment groups were significantly higher than that of the control ($p < .05$, Figure 4).

The expression of interleukin-10 (IL-10) mRNA in hepatopancreas increased first and then decreased, which was significantly higher in the 10^8 CFU/g group than the control ($p < .05$, Figure 5). The expressions of IL-10 mRNA in proximal and distal intestines of the four experimental groups were significantly higher than that of the control ($p < .05$, Figure 5), and it in the mid intestine of the 10^8 and 10^9 CFU/g groups was significantly increased ($p < .05$, Figure 5). The expressions of IL-10 mRNA in the muscle of the 10^7 and 10^8 CFU/g groups were significantly higher than that in the control ($p < .05$, Figure 5). The expressions of IL-10 mRNA in the muscles of the 10^6 and 10^9 CFU/g groups also showed increasing trends but were not significant ($p > .05$, Figure 5).

The expressions of interleukin-8 (IL-8), interleukin-1 β (IL-1 β), and tumor necrosis factor α (TNF- α) mRNA in hepatopancreas, intestines, and muscles showed opposite trends compared with the TGF- β and IL-10. The relative expressions of IL-8 mRNA in the hepatopancreas and mid intestine of the 10^7 - 10^9 CFU/g groups were significantly lower than that in the control group and 10^6 CFU/g group ($p < .05$, Figure 6). Compared to the control, the relative expressions of IL-8 mRNA in proximal intestines and muscles of the treatment groups were significantly decreased ($p < .05$, Figure 6). The relative expression of IL-8 mRNA in the distal intestine of the 10^8 CFU/g group was significantly decreased ($p < .05$, Figure 6), and there was no significant difference between 10^6 , 10^7 , and 10^9 CFU/g groups and the control group ($p > .05$, Figure 6).

The expressions of IL-1 β mRNA in the hepatopancreas and distal intestines of the 10^7 - 10^9 CFU/g groups were significantly lower than that of the control ($p < .05$, Figure 7). The expressions of IL-1 β mRNA in proximal and mid intestines of the 10^8 and 10^9 CFU/g groups were significantly decreased compared to the control ($p < .05$, Figure 7). The IL-1 β mRNA levels in the muscle of all the treatment groups were significantly decreased than that of the control ($p < .05$, Figure 7).

Compared with the control, the expressions of TNF- α mRNA were significantly decreased in the hepatopancreas and proximal intestines of all the treatment groups ($p < .05$, Figure 8). The expressions of TNF- α mRNA were significantly decreased in mid intestines, distal intestines, and muscles of the 10^7 - 10^9 CFU/g groups ($p < .05$, Figure 8).

3.5. Effect of *A. hydrophila* Challenge on Disease Resistance of Amur minnow (*R. lagowskii* Dybowski). The mortality of challenged fish is shown in Figure 9. The LD₅₀ dose of Amur

FIGURE 4: Effects of *Bacillus velezensis* LSG2-5 on the relative expression of TGF- β mRNA in hepatopancreas, intestine, and muscle of Amur minnow (*Rhynchocypris lagowskii* Dybowski). Different letters indicate significant difference ($p < .05$).

FIGURE 5: Effects of *Bacillus velezensis* LSG2-5 on the relative expression of IL-10 mRNA in hepatopancreas, intestine, and muscle of Amur minnow (*Rhynchocypris lagowskii* Dybowski). Different letters indicate significant difference ($p < .05$).

minnow injected with *A. hydrophila* at 14 d was 10⁸ CFU/mL. The survival rate of Amur minnow challenged by *A. hydrophila* is shown in Figure 10. The survival rates of the *B. velezensis* LSG2-5 treatment groups were significantly increased compared to the control ($p < .05$). There was a significant increase in the 10⁸ CFU/g group compared with the 10⁶ CFU/g group ($p < .05$), and there was no significant difference between the 10⁷-10⁹ CFU/g groups ($p > .05$).

Serum immunity of Amur minnow challenged by *A. hydrophila* is presented in Table 7. The levels of LZM, C3, IgM, ACP, and AKP in the *B. velezensis* LSG2-5 treatment groups were significantly higher than those in the control ($p < .05$). The content of C4 in the 10⁷-10⁹ CFU/g groups was significantly higher than that in the control ($p < .05$), and there was an increasing trend in the 10⁶ CFU/g group but not significant ($p > .05$).

FIGURE 6: Effects of *Bacillus velezensis* LSG2-5 on the relative expression of IL-8 mRNA in hepatopancreas, intestine, and muscle of Amur minnow (*Rhynchocypris lagowskii* Dybowski). Different letters indicate significant difference ($p < .05$).

FIGURE 7: Effects of *Bacillus velezensis* LSG2-5 on the relative expression of IL-1 β mRNA in hepatopancreas, intestine, and muscle of Amur minnow (*Rhynchocypris lagowskii* Dybowski). Different letters indicate significant difference ($p < .05$).

4. Discussion

Probiotics such as yeast, lactic acid bacteria (LAB), and *Bacillus* added to feed were widely used in aquaculture for growth promotion, immune enhancement, and disease prevention. Several studies have shown that probiotics positively affect fish, especially *Bacillus* [13, 31–35]. Due to its rich metabolites and wide distribution in nature, *B. velezensis* has become a new strain used in aquaculture in recent years [9].

The antimicrobial properties of probiotics were considered one of the criteria for application to aquatic animal feed [36]. Secondary metabolites produced by *B. velezensis* include bacilysin, difficidin, macrolactin, acetoin, and others [37]. Among them, bacilysin and difficidin yield intense antibacterial effects. In recent years, there have been more and more studies on the antibacterial and probiotic

FIGURE 8: Effects of *Bacillus velezensis* LSG2-5 on the relative expression of TNF- α mRNA in hepatopancreas, intestine, and muscle of Amur minnow (*Rhynchocypris lagowskii* Dybowski). Different letters indicate significant difference ($p < .05$).

FIGURE 9: Cumulative mortality of Amur minnow (*Rhynchocypris lagowskii* Dybowski) challenged with different dose of *Aeromonas hydrophila* by intraperitoneal injection for LD₅₀ test.

effects of *B. velezensis* in vitro or in vivo, including mandarin fish (*Siniperca cheats*) [38], common carp [39], hybrid snakehead [40], and grass carp [41]. However, there are few studies on the probiotic effects and resistance against pathogen infection of Amur minnow, which has hindered the development of Amur minnow culture. The microflora of fish intestines was complex and diverse and has not been fully exploited [42]. The probiotics isolated from the intestinal tract have proved to be more effective in improving the growth and immune function of the host. [38, 43–45]. Therefore, the purpose of this study was to verify the probi-

FIGURE 10: Cumulative survival rate following a 14-day *Aeromonas hydrophila* challenge. Different letters indicate significant difference ($p < .05$).

otic function and disease resistance of *B. velezensis* and to explore the requirements of host for different doses of *B. velezensis*. This is vital for the systematic evaluation of *B. velezensis* as a probiotic agent for different fish species.

The growth performance indexes are big deals to evaluate the breeding effect of Amur minnow. This study showed that compared with the control group, the FW, WGR, and SGR of Amur minnow were significantly improved with the increase of the dose of *B. velezensis* LSG2-5. Except for FCR, each growth index showed a positive correlation with the dose of *B. velezensis* LSG2-5. When the dose reached to 10⁷ CFU/g, a significantly positive effect on the growth of Amur minnow was shown. It is found that diets supplemented with *Bacillus* in appropriate doses impact positively on growth performance of aquatic animals, such as rockfish

TABLE 7: Effects of *Aeromonas hydrophila* challenge in serum immunity of Amur minnow (*Rhynchocypris lagowskii* Dybowski).

Indexes	Addition of <i>Bacillus velezensis</i> LSG2-5 (CFU/g)				
	0	10 ⁶	10 ⁷	10 ⁸	10 ⁹
LZM (µg/ml)	40.42 ± 0.38 ^a	47.84 ± 0.59 ^b	49.80 ± 0.98 ^b	56.62 ± 1.48 ^c	56.68 ± 1.12 ^c
C3 (mg/L)	32.91 ± 0.91 ^a	38.03 ± 0.14 ^b	41.55 ± 0.77 ^{bc}	43.52 ± 1.49 ^c	45.83 ± 1.14 ^c
C4 (mg/L)	54.27 ± 0.64 ^a	56.07 ± 0.66 ^{ab}	59.12 ± 1.27 ^{bc}	63.72 ± 0.34 ^c	60.12 ± 0.65 ^d
IgM (mg/L)	37.67 ± 0.92 ^a	44.26 ± 1.10 ^b	49.07 ± 0.86 ^b	57.23 ± 1.40 ^c	57.88 ± 1.85 ^c
ACP (U/L)	51.30 ± 0.40 ^a	53.96 ± 0.78 ^b	66.34 ± 1.33 ^c	69.29 ± 0.59 ^c	66.54 ± 0.58 ^c
AKP (U/L)	98.46 ± 0.91 ^a	104.96 ± 1.20 ^b	108.98 ± 0.95 ^b	123.52 ± 1.89 ^c	118.79 ± 1.12 ^c

Note: Values followed by different letters are significantly different ($p < .05$).

(*Sebastes schlegelii*) [46], catfish (*Ictalurus punctatus*) [12], Pacific white shrimp (*Litopenaeus vannamei*) [47], and *Epinephelus fuscoguttatus* [48].

According to Afrilasari & Meryandini [49], adding *B. magaterium* from the intestine of catfish (*Clarias* sp.) to the diet has beneficial effects on endogenous digestive enzymes and intestinal flora of catfish, thus improving the growth performance of catfish. Similarly, the addition of *B. subtilis* and *B. licheniformis* also optimized the performance of Asian sea bass (*Lates calcarifer*) [50]. The mechanism by which probiotics can promote the growth performance of fish is not precise. Reda & Selim [51] believe that probiotics can promote the growth of fish by providing nutrients for fish and affecting the digestive enzyme activity to improve the digestibility or feed utilization rate. The reason may be that probiotics can secrete exogenous enzymes to supplement the endogenous enzymes of the host to some extent. Or it could be that probiotics boost the production of endogenous enzymes in fish. Irianto & Austin [52] believe that *Bacillus* increases fish appetite by producing vitamins and organic acids. *Bacillus* degrades indigestible components in the intestine, improves the shape of the intestine, and promotes the absorption of nutrients to promote fish growth [52]. It is recommended that further investigation and research can be conducted to clarify the mechanism of action of *Bacillus* on the growth performance of fish.

Existing studies have shown that dietary *Bacillus* can modulate immune function in fish because *Bacillus* can regulate different immunological parameters [53–55]. LZM is an important non-specific immune factor in the innate immune system of fish, which can hydrolyze the alkaline enzyme of mucopolysaccharides in pathogenic bacteria. It plays an essential role in resisting the invasion of pathogenic bacteria. Under the conditions of this study, fish fed *B. velezensis* LSG2-5 for 56 days could significantly increase the activity of LZM in serum. In the challenge test, LZM activity was also significantly increased in treatment groups. Suggesting that *B. velezensis* LSG2-5 can positively affect the nonspecific immune system of the host. The results are consistent with previous reports [18, 56]. C3 and C4 are essential components in the complement system of fish, which are the main bridges connecting specific and non-specific immune responses. In this study, feeding *B. velezensis* LSG2-5 (10⁸ CFU/g) increased serum C3 and C4 levels in Amur minnow. When infected with *A. hydrophila*, the C3 and C4 levels in experimental groups were increased, indi-

cating that *B. velezensis* LSG2-5 has a positive effect on the immune function of Amur minnow. Similar to our results, *Bacillus* also has a promoting effect on the content of C3 and C4 in the serum of grass carp (*Ctenopharyngodon idellus*) [57] and Nile tilapia (*Oreochromis niloticus*) [58].

Interestingly, in the study of the effects of *B. pumilus* and *B. clausii* on *Epinephelus coioides*, C3 and C4 levels in serum were significantly affected at 30 days but returned to normal levels at 60 days [59]. The inconsistent results may be related to the different strains of *Bacillus* [59]. IgM is an essential mediator in the specific immune response of fish, which plays a role in both cellular and humoral immunity. IgM level was increased in treatment groups fed with *B. velezensis* LSG2-5. In the challenge test, IgM levels were also significantly increased.

Similarly, dietary supplementation with *B. velezensis* also increased serum IgM levels in Atlantic salmon (*Salmo salar* L.) [18] and *Oreochromis niloticus* [60]. Both ACP and AKP are critical components of fish lysosomal enzymes. In this experiment, *B. velezensis* LSG2-5 can increase the activities of ACP and AKP in the serum of Amur minnow at a suitable dose. In the challenge test, all test groups significantly increased ACP and AKP activities. Past reports have shown similar results on *E. coioides* [61], Tilapia (*O. mossambicus*) [62], and *Psetta maxima* [63] in diets supplemented with *Bacillus*. However, Zhao et al. [64] reported that *B. cereus* had no significant effect on the ACP activity of sea cucumber (*Apostichopus japonicus*), which may be due to the nature of *B. cereus* itself.

The above results all indicate that when the dose of *B. velezensis* LSG2-5 is appropriate, it can promote the immune parameters of Amur minnow. In addition, *B. velezensis* LSG2-5 also affected the expression of inflammation-related genes in Amur minnow. It has been reported that NF-κB signaling pathway is involved in developing classical inflammatory pathways. Activation of NF-κB signaling pathway leads to increased levels of IL-1β mRNA, TNF-α and IL-8 mRNA, and decreased levels of IL-10 and TGF-β mRNA. NF-κB signaling pathway plays a central role in inflammation and wound healing [65–67].

In the present study, *B. velezensis* LSG2-5 at the appropriate dose could promote the expressions of TGF-β and IL-10 in hepatopancreas, intestines, and muscles. At the same time, inhibiting the expressions of IL-8, IL-1β, and TNF-α. These results suggest that *B. velezensis* LSG2-5 can inhibit the inflammatory response of fish by inhibiting the

NF- κ B signaling pathway. Similar results were found in the effect of *B. subtilis* [68] and *B. licheniformis* [69] on the expression of inflammatory genes in *Labeo rohita*. Inconsistent with our results, it was found in the influence of *Bacillus*, *Enterococcus* and *Lactobacillus* on tilapia (*O. niloticus*) [70] intestinal immunity that mixed probiotics showed a promoting effect on the expression of anti-inflammatory factors and proinflammatory factors. In the histological examination of the fish body, no evidence of inflammation was found. The author believes that the induction of pro-inflammatory factors improves the immune preparedness of the host. However, more research is needed to understand better the role of *Bacillus* in the activation of immune mechanisms in fish.

Due to changes in the water environment or stimulating fish, the production and removal of reactive oxygen species in tissues or cells are unbalanced, resulting in the oxidative stress reaction of fish. Major antioxidant enzymes, including SOD, GSH, GSH-Px, and CAT, are the first line of defense in the antioxidant defense system of ROS produced by the body in the process of oxidative stress [18]. MDA reflects the peroxidation reaction of the body. Lipid peroxidation led by increased formation of MDA leads to the cross-linking of protein, nucleic acid molecules, and cytotoxicity [71]. Probiotics can act as antigens to stimulate the secretion of antioxidant enzymes, effectively removing excess free radicals and regulating the body's free radical balance, thereby increasing antioxidant capacity [55, 72]. The activities of these enzymes can indicate the antioxidant status of fish. When the activities of SOD, GSH, GSH-Px, and CAT increase, it indicates that the antioxidant capacity of fish increases. A decrease in MDA usually means increased enzymatic antioxidant levels since MDA is direct evidence of a toxic process caused by free radicals [73]. In this study, SOD, GSH, GSH-Px, CAT, and T-AOC activities increased while MDA activities decreased.

Similar trends have been shown in studies of the effects of *Bacillus* on Atlantic salmon (*S. salar* L.) [18] and common carp [55]. This study found that the diet supplemented with *B. velezensis* LSG2-5 significantly increased the mRNA expression levels of GSH-Px, CAT, and CuZn-SOD in hepatopancreas, intestines, and muscles of Amur minnow. These results were similar to those of the antioxidant enzymes mentioned above, which suggested that *B. velezensis* LSG2-5 could enhance the antioxidant capacity of Amur minnow. However, the primary mechanism between probiotics and antioxidant defense is not well understood and deserves further study.

Bacterial infection is the main cause of disease in fish farming. Many reports have shown that the addition of *Bacillus* spp. to diets can improve the disease resistance of fish to *A. hydrophila* [74], *S. agalactiae* [6], *V. harveyi* [75], *V. parahaemolyticus* [76], *A. salmonicida* [18], *A. veronii* [15], *Edwardsiella* [77], and *Streptococcus iniae* [78]. The antibacterial activity of probiotic *Bacillus* is manifested in the active substance produced since it can have an antibacterial effect on various fish pathogenic microorganisms. Similarly, adding probiotics to the biofloc system can promote the resistance of red hybrid tilapia to *S. agalactiae* [79].

The study verified the probiotic effect of *B. velezensis* LSG2-5 on fish by injecting *A. hydrophila* into fish. The results showed that the survival rates were significantly improved in the treatment groups, and the treatment group showed a significant regulatory effect on serum immune response. In particular, LZM, C3, C4, IgM, ACP, and AKP were significantly regulated. These results indicated that *B. velezensis* LSG2-5 prevented pathogenic bacteria and improved the disease resistance of Amur minnow. However, further studies are needed to investigate the specific ways in which probiotics inhibit pathogenic bacteria in fish and whether probiotics have the same inhibitory effect on viral infection in fish.

5. Conclusion

Bacterial diseases frequently occur in fish farming, and probiotics are needed to prevent disease and replace antibiotics. The multifunctional immune feature of *B. velezensis* LSG2-5 is confirmed in this study, especially for resistance to *A. hydrophila*. Meanwhile, dietary *B. velezensis* supplementation can improve the growth performance of Amur minnow. In conclusion, the supplementation of 10^7 – 10^9 CFU/g of *B. velezensis* LSG2-5 positively affected Amur minnow. Based on comprehensive considerations, we suggest that the optimal supplemental dose of *B. velezensis* LSG2-5 to Amur minnow is 5.00×10^8 CFU/g.

Data Availability

The data that support the findings of this study are openly available.

Ethical Approval

All the experimental animals used in the study were performed following the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Jilin Agricultural University (organization number: 20200919007).

Conflicts of Interest

The authors report no declarations of interest.

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References

- [1] S. H. Hoseinifar, Y. Sun, A. Wang, and Z. Zhou, "Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives," *Frontiers in Microbiology*, vol. 9, p. 2429, 2018.

- [2] J. Cha, S. Rahimnejad, S. Yang, K. Kim, and K. Lee, "Evaluations of *Bacillus* spp. as dietary additives on growth performance, innate immunity and disease resistance of olive flounder (*Paralichthys olivaceus*) against *Streptococcus iniae* and as water additives," *Aquaculture*, vol. 402, pp. 50–57, 2013.
- [3] T. Sangma and D. Kamilya, "Dietary *Bacillus subtilis* FPTB13 and chitin, single or combined, modulate systemic and cutaneous mucosal immunity and resistance of catla, *Catla catla* (Hamilton) against edwardsiellosis," *Comparative Immunology, Microbiology and Infectious Diseases*, vol. 43, pp. 8–15, 2015.
- [4] J. Wang, H. L. Yang, H. Q. Xia et al., "Supplementation of heat-inactivated *Bacillus clausii* DE5 in diets for grouper, *Epinephelus coioides*, improves feed utilization, intestinal and systemic immune responses and not growth performance," *Aquaculture Nutrition*, vol. 24, no. 2, pp. 821–831, 2018.
- [5] K. M. Niu, S. Khosravi, D. Kothari et al., "Potential of indigenous *Bacillus* spp. as probiotic feed supplements in an extruded low-fish-meal diet for juvenile olive flounder, *Paralichthys olivaceus*," *Journal of the World Aquaculture Society*, vol. 52, no. 1, pp. 244–261, 2021.
- [6] P. Srisapoome and N. Areechon, "Efficacy of viable *Bacillus pumilus* isolated from farmed fish on immune responses and increased disease resistance in Nile tilapia (*Oreochromis niloticus*): Laboratory and on-farm trials," *Fish & Shellfish Immunology*, vol. 67, pp. 199–210, 2017.
- [7] R. Laloo, G. Moonsamy, S. Ramchuran, J. Görgens, and N. Gardiner, "Competitive exclusion as a mode of action of a novel *Bacillus cereus* aquaculture biological agent," *Letters in Applied Microbiology*, vol. 50, no. 6, pp. 563–570, 2010.
- [8] G. Yang, M. Chen, Z. Yu, Q. Lu, and S. Zhou, "*Bacillus composti* sp. nov. and *Bacillus thermophilus* sp. nov., two thermophilic, Fe(III)-reducing bacteria isolated from compost," *International Journal of Systematic and Evolutionary Microbiology*, vol. 63, no. Part_8, pp. 3030–3036, 2013.
- [9] F. Khalid, A. Khalid, Y. Fu et al., "Potential of *Bacillus velezensis* as a probiotic in animal feed: a review," *Journal of Microbiology*, vol. 59, no. 7, pp. 627–633, 2021.
- [10] L. Cao, L. Pan, L. Gong et al., "Interaction of a novel *Bacillus velezensis* (BvL03) against *Aeromonas hydrophila* in vitro and in vivo in grass carp," *Applied Microbiology and Biotechnology*, vol. 103, no. 21–22, pp. 8987–8999, 2019.
- [11] X. Li, X. Gao, S. Zhang et al., "Characterization of a *Bacillus velezensis* with antibacterial activity and inhibitory effect on common aquatic pathogens," *Aquaculture*, vol. 523, article 735165, 2020.
- [12] C. M. Thurlow, M. A. Williams, A. Carrias et al., "*Bacillus velezensis* AP193 exerts probiotic effects in channel catfish (*Ictalurus punctatus*) and reduces aquaculture pond eutrophication," *Aquaculture*, vol. 503, pp. 347–356, 2019.
- [13] H. Van Doan, S. H. Hoseinifar, C. Khanongnuch, A. Kanpiengjai, K. Unban, and S. Srichaiyo, "Host-associated probiotics boosted mucosal and serum immunity, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*)," *Aquaculture*, vol. 491, pp. 94–100, 2018.
- [14] Y. Yi, Z. Zhang, F. Zhao et al., "Probiotic potential of *Bacillus velezensis* JW: antimicrobial activity against fish pathogenic bacteria and immune enhancement effects on *Carassius auratus*," *Fish & Shellfish Immunology*, vol. 78, pp. 322–330, 2018.
- [15] D. Zhang, Y. Kang, S. Zhan et al., "Effect of *Bacillus velezensis* on *Aeromonas veronii*-induced intestinal mucosal barrier function damage and inflammation in crucian carp (*Carassius auratus*)," *Frontiers in Microbiology*, vol. 10, p. 2663, 2019.
- [16] J. Li, Z. Wu, Z. Zhang et al., "Effects of potential probiotic *Bacillus velezensis* K2 on growth, immunity and resistance to *Vibrio harveyi* infection of hybrid grouper (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀)," *Fish & Shellfish Immunology*, vol. 93, pp. 1047–1055, 2019.
- [17] X. Gao, Y. Liu, L. Miao et al., "Characterization and mechanism of anti-*Aeromonas salmonicida* activity of a marine probiotic strain, *Bacillus velezensis* V4," *Applied Microbiology and Biotechnology*, vol. 101, no. 9, pp. 3759–3768, 2017.
- [18] C. Wang, Y. Liu, G. Sun, X. Li, and Z. Liu, "Growth, immune response, antioxidant capability, and disease resistance of juvenile Atlantic salmon (*Salmo salar* L.) fed *Bacillus velezensis* V4 and *Rhodotorula mucilaginosa* compound," *Aquaculture*, vol. 500, pp. 65–74, 2019.
- [19] T. Yu, Q. J. Wang, X. M. Chen et al., "Effect of dietary L-carnitine supplementation on growth performance and lipid metabolism in *Rhynchocypris lagowskii* Dybowski fed oxidized fish oil," *Aquaculture Research*, vol. 51, no. 9, pp. 3698–3710, 2020.
- [20] M. Li, Y. D. Kong, L. Li, R. Zhu, and L. F. Wu, "Effects of dietary carbohydrate sources on growth performance, immunity and glycometabolic enzyme activity of *Rhynchocypris lagowskii* Dybowski," *Aquaculture Research*, vol. 52, no. 1, pp. 323–333, 2021.
- [21] Z. Yu, Z. Q. Huang, H. L. Du, H. J. Li, and L. F. Wu, "Influence of differential protein levels of feed on growth, copper-induced immune response and oxidative stress of *Rhynchocypris lagowskii* a biofloc-based system," *Aquaculture Nutrition*, vol. 26, no. 6, pp. 2211–2224, 2020.
- [22] L. Li, R. Zhu, M. Li et al., "Effects of β -conglycinin on growth performance, antioxidant capacity and immunity in *Rhynchocypris lagowskii* Dybowski," *Aquaculture Nutrition*, vol. 26, no. 6, pp. 2059–2073, 2020.
- [23] R. Zhu, L. Li, M. Li et al., "Effects of dietary glycinin on the growth performance, immunity, hepatopancreas and intestinal health of juvenile *Rhynchocypris lagowskii* Dybowski," *Aquaculture*, vol. 544, p. 737030, 2021.
- [24] D. Zhang, Z. Guo, Y. Zhao et al., "L-carnitine regulated Nrf2/Keap1 activation in vitro and in vivo and protected oxidized fish oil-induced inflammation response by inhibiting the NF- κ B signaling pathway in *Rhynchocypris lagowskii* Dybowski," *Fish & Shellfish Immunology*, vol. 93, pp. 1100–1110, 2019.
- [25] X. Chen, Q. Wang, Z. Guo et al., "Effects of dietary oxidized fish oil on growth performance and antioxidant defense mechanism of juvenile *Rhynchocypris lagowskii* Dybowski," *Aquaculture*, vol. 512, article 734368, 2019.
- [26] T. Yu, Y. Chen, X. Chen et al., "The effect of oxidized fish oil on lipid metabolism in *Rhynchocypris lagowskii* Dybowski," *Aquaculture Reports*, vol. 17, article 100388, 2020.
- [27] Z. C. Wu, *Screening and Adhesion Characteristics Analysis of Enzyme Producing Probiotics from Intestine of Rhynchocypris lagowskii*, M. Sc, Jilin Agricultural University, 2020.
- [28] Q. Wang, S. Luo, A. Ghonimy et al., "Effect of dietary L-carnitine on growth performance and antioxidant response in Amur minnow (*Phoxinus lagowskii* Dybowski)," *Aquaculture Nutrition*, vol. 25, no. 4, pp. 749–760, 2019.
- [29] L. J. Reed and H. Muench, "A simple method of estimating fifty per cent ENDPOINTS," *American Journal of Epidemiology*, vol. 27, no. 3, pp. 493–497, 1938.

- [30] A. Boonyapakdee and R. C. Bhujel, "Determining the dose of dietary probiotic (bacillus licheniformis) for the nursing of blue swimming Crablets (*Portunus pelagicus*, L., 1758)," *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 20, no. 12, pp. 889–899, 2020.
- [31] V. Hlordzi, F. K. Kuebutornye, G. Afriyie et al., "The use of *Bacillus* species in maintenance of water quality in aquaculture: a review," *Aquaculture Reports*, vol. 18, article 100503, 2020.
- [32] F. K. Kuebutornye, E. D. Abarike, and Y. Lu, "A review on the application of *Bacillus* as probiotics in aquaculture," *Fish & Shellfish Immunology*, vol. 87, pp. 820–828, 2019.
- [33] S. K. Nayak, "Multifaceted applications of probiotic *Bacillus* species in aquaculture with special reference to *Bacillus subtilis*," *Reviews in Aquaculture*, vol. 13, no. 2, pp. 862–906, 2021.
- [34] E. Ringø, H. Van Doan, S. H. Lee et al., "Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture," *Journal of Applied Microbiology*, vol. 129, no. 1, pp. 116–136, 2020.
- [35] M. Soltani, K. Ghosh, S. H. Hoseinifar et al., "Genusbacillus, promising probiotics in aquaculture: aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish," *Reviews in Fisheries Science & Aquaculture*, vol. 27, no. 3, pp. 331–379, 2019.
- [36] T. Borah, B. Gogoi, A. Khataniar, M. Gogoi, A. Das, and D. Borah, "Probiotic characterization of indigenous *Bacillus velezensis* strain DU14 isolated from Apong, a traditionally fermented rice beer of Assam," *Biocatalysis and Agricultural Biotechnology*, vol. 18, article 101008, 2019.
- [37] M. F. Rabbee, M. D. Ali, J. Choi, B. S. Hwang, S. C. Jeong, and K. Baek, "*Bacillus velezensis*: a valuable member of bioactive molecules within plant microbiomes," *Molecules*, vol. 24, no. 6, p. 1046, 2019.
- [38] J. Wang, D. Zhang, Y. Wang, Z. Liu, L. Liu, and C. Shi, "Probiotic effects of the *Bacillus velezensis* GY65 strain in the mandarin fish, *Siniperca chuatsi*," *Aquaculture Reports*, vol. 21, article 100902, 2021.
- [39] K. Song, F. Luo, W. Chen et al., "Evaluation on the antiviral activity of *Bacillus velezensis* extract against spring viremia of carp virus," *Aquaculture*, vol. 547, article 737477, 2022.
- [40] D. F. Zhang, X. L. Xiong, Y. J. Wang et al., "*Bacillus velezensis* WLYS23 strain possesses antagonistic activity against hybrid snakehead bacterial pathogens," *Journal of Applied Microbiology*, vol. 131, no. 6, pp. 3056–3068, 2021.
- [41] P. Wu, C. Liu, and S. Hu, "Probiotic *Bacillus safensis* NPUST1 administration improves growth performance, gut microbiota, and innate immunity against streptococcus iniae in Nile tilapia (*Oreochromis niloticus*)," *Microorganisms*, vol. 9, no. 12, p. 2494, 2021.
- [42] B. Thankappan, D. Ramesh, S. Ramkumar, K. Natarajaseenivasan, and K. Anbarasu, "Characterization of *Bacillus* spp. from the gastrointestinal tract of *Labeo rohita*—towards to identify novel probiotics against fish pathogens," *Applied Biochemistry and Biotechnology*, vol. 175, no. 1, pp. 340–353, 2015.
- [43] A. A. Adeoye, R. Yomla, A. Jaramillo-Torres, A. Rodiles, D. L. Merrifield, and S. J. Davies, "Combined effects of exogenous enzymes and probiotic on Nile tilapia (*Oreochromis niloticus*) growth, intestinal morphology and microbiome," *Aquaculture*, vol. 463, pp. 61–70, 2016.
- [44] S. S. Giri, V. Sukumaran, S. S. Sen, and P. K. Jena, "Effects of dietary supplementation of potential probiotic *Bacillus subtilis* VSG1 singularly or in combination with *Lactobacillus plantarum* VSG3 or/and *Pseudomonas aeruginosa* VSG2 on the growth, immunity and disease resistance of *Labeo rohita*," *Aquaculture Nutrition*, vol. 20, no. 2, pp. 163–171, 2014.
- [45] F. K. Kuebutornye, J. Tang, J. Cai et al., "In vivo assessment of the probiotic potentials of three host-associated *Bacillus* species on growth performance, health status and disease resistance of *Oreochromis niloticus* against *Streptococcus agalactiae*," *Aquaculture*, vol. 527, article 735440, 2020.
- [46] W. J. Jang, S. Lee, M. Jeon et al., "Characterization of a *Bacillus* sp. KRF-7 isolated from the intestine of rockfish and effects of dietary supplementation with mannan oligosaccharide in rockfish aquaculture," *Fish & Shellfish Immunology*, vol. 119, pp. 182–192, 2021.
- [47] K. Amoah, Q. Huang, B. Tan et al., "Dietary supplementation of probiotic *Bacillus coagulans* ATCC 7050, improves the growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp, *Litopenaeus vannamei*," *Fish & Shellfish Immunology*, vol. 87, pp. 796–808, 2019.
- [48] D. Rachmawati, J. Hutabarat, O. Anne, R. H. Setyobudi, and T. Elfitasari, "Floating net aquaculture engineering of Tiger grouper (*Epinephelus fuscoguttatus*) on *Bacillus subtilis* probiotic supplementation in the diet," *Sarhad journal of agriculture*, vol. 37, no. S1, pp. 55–63, 2021.
- [49] W. Afrilasari and A. Meryandini, "Effect of probiotic *Bacillus megaterium* PTB 1.4 on the population of intestinal microflora, digestive enzyme activity and the growth of catfish (*Clarias* sp.)," *Hayati Journal of Biosciences*, vol. 23, no. 4, pp. 168–172, 2016.
- [50] T. J. Adorian, H. Jamali, H. G. Farsani et al., "Effects of probiotic bacteria *Bacillus* on growth performance, digestive enzyme activity, and hematological parameters of Asian sea bass, *Lateolabrax japonicus* (Bloch)," *Probiotics and Antimicrobial Proteins*, vol. 11, no. 1, pp. 248–255, 2019.
- [51] R. M. Reda and K. M. Selim, "Evaluation of *Bacillus amyloliquefaciens* on the growth performance, intestinal morphology, hematology and body composition of Nile tilapia, *Oreochromis niloticus*," *Aquaculture International*, vol. 23, no. 1, pp. 203–217, 2015.
- [52] A. Irianto and B. Austin, "Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum)," *Journal of Fish Diseases*, vol. 25, no. 6, pp. 333–342, 2002.
- [53] S. Lin, S. Mao, Y. Guan, L. Luo, L. Luo, and Y. Pan, "Effects of dietary chitosan oligosaccharides and *Bacillus coagulans* on the growth, innate immunity and resistance of koi (*Cyprinus carpio* koi)," *Aquaculture*, vol. 342–343, pp. 36–41, 2012.
- [54] A. Mukherjee, G. Chandra, and K. Ghosh, "Single or conjoint application of autochthonous *Bacillus* strains as potential probiotics: Effects on growth, feed utilization, immunity and disease resistance in Rohu, *Labeo rohita* (Hamilton)," *Aquaculture*, vol. 512, article 734302, 2019.
- [55] L. Wang, C. Ge, J. Wang, J. Dai, P. Zhang, and Y. Li, "Effects of different combinations of *Bacillus* on immunity and antioxidant activities in common carp," *Aquaculture International*, vol. 25, no. 6, pp. 2091–2099, 2017.
- [56] H. L. Yang, Z. Y. Liu, J. T. Jian, J. D. Ye, and Y. Z. Sun, "Host-associated *Bacillus siamensis* and *Lactococcus petauri* improved growth performance, innate immunity, antioxidant activity and ammonia tolerance in juvenile Japanese seabass

- (*Lateolabrax japonicus*),” *Aquaculture Nutrition*, vol. 27, no. 6, pp. 2739–2748, 2021.
- [57] L. Weifen, Z. Xiaoping, S. Wenhui et al., “Effects of *Bacillus* preparations on immunity and antioxidant activities in grass carp (*Ctenopharyngodon idellus*),” *Fish Physiology and Biochemistry*, vol. 38, no. 6, pp. 1585–1592, 2012.
- [58] B. Han, W. Long, J. He, Y. Liu, Y. Si, and L. Tian, “Effects of dietary *Bacillus licheniformis* on growth performance, immunological parameters, intestinal morphology and resistance of juvenile Nile tilapia (*Oreochromis niloticus*) to challenge infections,” *Fish & Shellfish Immunology*, vol. 46, no. 2, pp. 225–231, 2015.
- [59] Y. Sun, H. Yang, R. Ma, and W. Lin, “Probiotic applications of two dominant gut *Bacillus* strains with antagonistic activity improved the growth performance and immune responses of grouper *Epinephelus coioides*,” *Fish & Shellfish Immunology*, vol. 29, no. 5, pp. 803–809, 2010.
- [60] F. K. Kuebutornye, Z. Wang, Y. Lu et al., “Effects of three host-associated *Bacillus* species on mucosal immunity and gut health of Nile tilapia, *Oreochromis niloticus* and its resistance against *Aeromonas hydrophila* infection,” *Fish & Shellfish Immunology*, vol. 97, pp. 83–95, 2020.
- [61] Y. Sun, H. Yang, K. Huang, J. Ye, and C. Zhang, “Application of autochthonous *Bacillus* bioencapsulated in copepod to grouper *Epinephelus coioides* larvae,” *Aquaculture*, vol. 392, pp. 44–50, 2013.
- [62] N. Gobi, B. Vaseeharan, J. Chen et al., “Dietary supplementation of probiotic *Bacillus licheniformis* Dab1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against *Aeromonas hydrophila* in tilapia *Oreochromis mossambicus*,” *Fish & Shellfish Immunology*, vol. 74, pp. 501–508, 2018.
- [63] S. Ma, D. Yu, Q. Liu, M. Zhao, C. Xu, and J. Yu, “Relationship between immune performance and the dominant intestinal microflora of turbot fed with different *Bacillus* species,” *Aquaculture*, vol. 549, article 737625, 2022.
- [64] Y. Zhao, L. Yuan, J. Wan, Z. Sun, Y. Wang, and H. Sun, “Effects of potential probiotic *Bacillus cereus* EN25 on growth, immunity and disease resistance of juvenile sea cucumber *Apostichopus japonicus*,” *Fish & Shellfish Immunology*, vol. 49, pp. 237–242, 2016.
- [65] L. Feng, Y. Chen, W. Jiang et al., “Modulation of immune response, physical barrier and related signaling factors in the gills of juvenile grass carp (*Ctenopharyngodon idella*) fed supplemented diet with phospholipids,” *Fish & Shellfish Immunology*, vol. 48, pp. 79–93, 2016.
- [66] A. Kaulmann and T. Bohn, “Carotenoids, inflammation, and oxidative stress-implications of cellular signaling pathways and relation to chronic disease prevention,” *Nutrition Research*, vol. 34, no. 11, pp. 907–929, 2014.
- [67] T. Lawrence, “The nuclear factor NF- κ B pathway in inflammation,” *Cold Spring Harbor Perspectives in Biology*, vol. 1, no. 6, article a1651, 2009.
- [68] S. S. Giri, S. S. Sen, J. W. Jun, V. Sukumaran, and S. C. Park, “Role of *Bacillus subtilis* VSG4-derived biosurfactant in mediating immune responses in *Labeo rohita*,” *Fish & Shellfish Immunology*, vol. 54, pp. 220–229, 2016.
- [69] G. S. Sankar, S. S. Sankar, J. J. Woo, V. Sukumaran, and P. S. Chang, “Role of *Bacillus licheniformis* VS16-derived biosurfactant in mediating immune responses in carp rohu and its application to the food industry,” *Frontiers in Microbiology*, vol. 8, p. 514, 2017.
- [70] B. T. Standen, D. L. Peggs, M. D. Rawling et al., “Dietary administration of a commercial mixed-species probiotic improves growth performance and modulates the intestinal immunity of tilapia, *Oreochromis niloticus*,” *Fish & Shellfish Immunology*, vol. 49, pp. 427–435, 2016.
- [71] Y. Chen, M. Li, L. Yuan et al., “Growth, blood health, antioxidant status and immune response in juvenile yellow catfish *Pelteobagrus fulvidraco* exposed to α -ethinyloestradiol (EE2),” *Fish & Shellfish Immunology*, vol. 69, pp. 1–5, 2017.
- [72] S. V. Hindu, S. Thanigaivel, S. Vijayakumar, N. Chandrasekaran, A. Mukherjee, and J. Thomas, “Effect of microencapsulated probiotic *Bacillus vireti* 01-polysaccharide extract of *Gracilaria folifera* with alginate-chitosan on immunity, antioxidant activity and disease resistance of *Macrobrachium rosenbergii* against *Aeromonas hydrophila* infection,” *Fish & Shellfish Immunology*, vol. 73, pp. 112–120, 2018.
- [73] X. Fan, L. Ming, L. Yuan et al., “Effects of feeding frequency on the enzymes and genes involved in oxidative stress in juvenile yellow catfish *Pelteobagrus fulvidraco* (Richardson) exposed to ammonia,” *Aquaculture Research*, vol. 48, no. 12, pp. 5874–5882, 2017.
- [74] S. Addo, A. A. Carrias, M. A. Williams, M. R. Liles, J. S. Terhune, and D. A. Davis, “Effects of *Bacillus subtilis* strains and the prebiotic Previda® on growth, immune parameters and susceptibility to *Aeromonas hydrophila* infection in Nile tilapia, *Oreochromis niloticus*,” *Aquaculture Research*, vol. 48, no. 9, pp. 4798–4810, 2017.
- [75] A. Bahi, F. A. Guardiola, C. M. Messina et al., “Effects of dietary administration of fenugreek seeds, alone or in combination with probiotics, on growth performance parameters, humoral immune response and gene expression of gilthead seabream (*Sparus aurata* L.),” *Fish & Shellfish Immunology*, vol. 60, pp. 50–58, 2017.
- [76] N. Gobi, B. Malaikozhundan, V. Sekar et al., “GFP tagged *Vibrio parahaemolyticus* Dabv2 infection and the protective effects of the probiotic *Bacillus licheniformis* Dab1 on the growth, immune and antioxidant responses in *Pangasius hypophthalmus*,” *Fish & Shellfish Immunology*, vol. 52, pp. 230–238, 2016.
- [77] R. A. Santos, M. Monteiro, F. Rangel et al., “*Bacillus* spp. inhibit *Edwardsiella tarda* quorum-sensing and fish infection,” *Marine Drugs*, vol. 19, no. 11, p. 602, 2021.
- [78] Z. Wu, X. Qi, S. Qu, F. Ling, and G. Wang, “Dietary supplementation of *Bacillus velezensis* B8 enhances immune response and resistance against *Aeromonas veronii* in grass carp,” *Fish & Shellfish Immunology*, vol. 115, pp. 14–21, 2021.
- [79] A. Zabidi, F. M. Yusoff, S. M. Amin, N. J. M. Yaminudin, P. Puvanasundram, and M. M. A. Karim, “Effects of probiotics on growth, survival, water quality and disease resistance of red hybrid tilapia (*Oreochromis* spp.) fingerlings in a biofloc system,” *Animals*, vol. 11, no. 12, p. 3514, 2021.