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Effects of polysaccharide fermentation with *Bacillus coagulans* on growth, antioxidant and immunity of *Macrobrachium nipponense (riental river prawn)*

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Introduction: In recent years, with the continuous expansion of aquaculture areas worldwide and the outbreak of diseases, the use of antibiotics and chemical drugs is limited. Plant polysaccharides have received widespread attention due to their multiple bioactivities. However, research on the combined use of plant polysaccharides and *Bacillus coagulans* is still insufficient. Therefore, this study focuses on the impact of *B.coagulans*-fermented polysaccharides on *Macrobrachium nipponense*.

Methods: An 8-week feeding trial was conducted with seven groups: the control group (CT) and the *Bacillus coagulans* group (N),*Atractylodes macrocephala* polysaccharides group (NB), *Saposhnikovia divaricata* polysaccharides group (NF), *Mannose* group (NG), *Astragalus* polysaccharides group (NH) and *Yu ping feng* polysaccharides group (NP).

Results and discussion: The research results indicate that compared to the CT, the levels of AST and ALT were reduced in the group of N, NF and NG. The NF showed a significant increase in total antioxidant capacity (T-AOC) and total superoxide dismutase (SASC) levels. The NP had a significant increase in T-AOC and superoxide anion scavenging ability. The levels of total protein (TP) and malondialdehyde (MDA) in the group of NG, NB, and NP were significantly higher than those in the CT and N. Compared to the CT, the expression of *Toll* in the NP group, *Myd88* and *Dorsal* in the NH group, and *IMD* and *Relish* in the NF and NP group were all significantly increased. Conversely, the expression of *IMD* in the NB and NG group and *Relish* in the NG group was significantly decreased. Additionally, the survival rate in the NP group was significantly higher than in

other groups, and the NB group enhanced the weight gain of *M.nipponense* compared to the N. In summary, *B.coagulans* fermented with *Yupingfeng polysaccharides* and *Astragalus polysaccharides* can significantly enhance the antioxidant and immune capabilities of *M.nipponense*.

KEYWORDS

antioxidation, *Bacillus coagulans*, *Macrobrachium nipponense*, polysaccharide, immunity

1 Introduction

Macrobrachium nipponense is a freshwater shrimp with a large breeding area in China and is steadily expanding worldwide (Jiang et al., 2019). The aquaculture industry has suffered significant economic losses in recent years due to recurrent outbreaks of prawn diseases (Hou et al., 2018; Joshi et al., 2014). Traditionally, antibiotics and chemical drugs were used to prevent and treat prawn diseases. However, their use is restricted due to increasing bacteria drug resistance and food safety concerns. While plant extracts offer a promising alternative to antibiotics, their cost-effectiveness remains challenging. Therefore, the search for safe, effective, and economically feasible plant extracts for treating prawn diseases remains a priority (Bulfon et al., 2015). Plant polysaccharides from plant extracts are considered natural alternatives to chemical drugs and antibiotics and are widely used in aquaculture (Tzianabos, 2000).

Prebiotics have been demonstrated to improve host health, which in turn facilitates the growth and development of prawns (Kiernan et al., 2023). Related studies have shown that prebiotics have the characteristics of improving the structure of intestinal flora and stimulating the proliferation of probiotics (Li and Gatlin, 2005; Munir et al., 2016). The main physiological functions of prebiotics include enhancing the body's immunity (Sredkova et al., 2020), promoting the absorption of minerals (Karakan et al., 2021) and reducing inflammatory response (McLoughlin et al., 2017) and other efficacy. Prebiotics are polysaccharides extracted from plants and can be digested by the bacteria to produce short-chain fatty acids, which have anti-inflammatory and immune-enhancing effects (Jenab et al., 2020). Currently, Atractylodes rhizoma (Sun et al., 2023), mannooligosaccharides (Forsatkar et al., 2018), *Yupingfeng* polysaccharide (Su et al., 2020), and other prebiotics have been added to aquafeed. *Atrhizoma* polysaccharide plays a vital role in folk medicine, treating a variety of diseases (Pan et al., 2018) and improving immunity (Sun et al., 2015), which may be due to its ability to alleviate immunosuppression induced by cyclophosphamide (Li et al., 2019a, b). At the same time, *Atractylodes* polysaccharides can induce TLR4 to activate NF-κB, which in turn activates macrophages to synthesize cytokines (Wei et al., 2016). Previous studies have shown that *Atractylodes* polysaccharide also plays an important role in heat stress and immune function improvement (Xu et al., 2017; Xu and Tian, 2015). Adding mannooligosaccharides during fish breeding can promote the growth and reproduction (Forsatkar et al., 2018). *Yupingfeng* polysaccharide is an *Astragalus membranaceus* based ancient Chinese herbal medicine that regulates humoral and cellular immunity and inhibits inflammation (Fabrizio et al., 2013). Related studies have shown that *Yupingfeng* polysaccharide can improve the foregut microbiota and intestinal barrier of weanling rabbits and enhance the immunity of *rabbits* (Sun et al., 2016). Moreover, *Yupingfeng* polysaccharide could effectively improve the immune response, disease resistance and growth performance of *Litopenia vannamen* (Su et al., 2020). *Astragalus* polysaccharide has a variety of biological activities, such as immunomodulatory (Liu et al., 2017), antioxidant (Li et al., 2010), and antibacterial effects (Ma et al., 2017). In aquaculture, *Astragalus* polysaccharide has been shown to promote growth performance, improve physiological and biochemical indicators and increase the expression of genes related to lipid metabolism (Huang et al., 2023).*Parsnip* polysaccharide can modulate immune activity, significantly increase immune cell density and macrophage number, and reduce the expression of *NO, TNF-α, IL-1β* and *IL-6* (Fan et al., 2023).

Bacillus coagulans is a lactic-producing bacillus that can produce short-chain fatty acids such as acetic acid and propionic acid, promote gastrointestinal peristalsis, and regulate the structure of microenvironment flora (Zhu et al., 2019). *B.coagulans* utilize polysaccharides for growth and secrete digestive enzymes and bacteriocins that promote digestion and absorption (Mazkour et al., 2022). *B.coagulans* can significantly improve shrimp growth performance and serum antioxidant capacity in shrimp culture (Sadat Hoseini Madani et al., 2018). *B.coagulans* can also up-regulate the transcription level and related enzyme activities of SOD and CAT genes in *zebrafish*, and protect *zebrafish* larvae against oxidative stress induced by copper sulfate (Ai et al., 2023). In livestock and poultry breeding, *B.coagulans* can improve the growth performance of *C. vermilmilium*, enhance intestinal innate immunity and improve intestinal microbial community (Fu et al., 2019). This study addresses the disease issues faced in the cultivation of *M. nipponense* by exploring the potential of *B.coagulans*-fermented plant polysaccharides as a green additive. Plant polysaccharides have

garnered attention in aquaculture due to their diverse bioactivities, while *B.coagulans* is favored for its ability to promote growth, enhance antioxidant capacity, boost immunity and improve gut microbiota. The study focuses on the combined effects of *B.coagulans* and plant polysaccharides on *M.nipponense*, aiming to provide a new green additive application scheme for shrimp farming, which can help improve the breeding environment and increase breeding efficiency.

2 Materials and methods

2.1 Test material

Parsnip polysaccharide (NF), *Atractylodes* rhizoma polysaccharide (NB), *Astragalus polysaccharide* (NH) and *Yuingfeng* polysaccharide (NP) were obtained from Baoding Jizhong Biological Technology Co., Ltd (Hebei, China).*Mannose* (NG)was obtained from Qingdao Hehai Biotechnology Co., Ltd (Shandong, China). *B.coagulans* (N) was obtained from Jiangsu Su Wei Biological Co., Ltd. and Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences. The crude polysaccharide content was determined according to the industry standard NY/T 1676-2008, and its composition contents are shown in Table 1. The experimental prawns were provided by the Dapu Freshwater prawns experiment station of Freshwater Fisheries Research Center, Chinese Academy of Fisheries Sciences.

2.2 Feed preparation

B.coagulans were inoculated into an LB slant medium and cultured at 37°C for 24 hours to obtain the activated strains. Then 1% *Astragalus* polysaccharide,1% *Yupingfeng* polysaccharide, 1% *Mannose*, 4%*Parsnip* polysaccharide 4%,*Atractylodes rhizoma* polysaccharide were added to *B.coagulans* in an incubator at 37° C and 180 rpm for 24 h. *B.coagulans* with the polysaccharide fermentation broth were adjusted to 10^8 CFU/mL and added to the feed. The experimental feed formula is shown in Table 2. The experimental feed was made in the Fresh Water Center of the Chinese Academy of Fisheries Sciences, weighed and mixed with 60 mesh sieve, and finally mixed with oil and cultivated fermentation broth and water. The feed with a diameter of 1.0 mm was made in a twin-screw extruder, and the feed was dried in the shade and put into a refrigerator at -20°C to feed the prawns.

TABLE 1 Polysaccharide content.

Kinds	Content (%)
Yupingfeng polysaccharide	69.88 ± 0.85
Mannose	99.0 ± 0.71
Astragalus polysaccharide	79.77 ± 2.37
Saposhnikovia divaricata polysaccharide	73.44 ± 1.2
Atractylodes macrocephala polysaccharide	70.38 ± 0.92

TABLE 2 Dietary composition and nutritional level of *M.nipponense* (air-dried basis).

Ingredients	Content (%)	Fermentation liquor	Content (%)
Fish meal	25	N	1
Soybean meal	25	NB	1
Shrimp meal	8	NG	1
Peanut meal	15	NF	1
Squid soluble paste	2	NH	1
α-starch	17.7	NP	1
Fish oil	1.5	Proximate Composition	
Soybean oil	1.5	Crude protein	38.45%
Soya lecithin	0.5	Ether extract	7.44
Calcium dihydrogen phosphate	2	Nitrogen-free-extract	22.26
Multi-mineral	0.5		
Multidimensiona	0.5		
Vitamin C	0.5		
Choline chloride	0.1		
Ecdysone	0.2		
Total	100		

1. The fermentation broth was co-fermented with reconfiguration liquid medium and *B.coagulans*; Liquid medium: LB glucose-free medium and an equal amount of polysaccharide substituted for glycogen were mixed and autoclaving. 2. All nutritional levels were calculated.

2.3 Experimental design and feeding management

The experiment was divided into control group (CT) without adding polysaccharide, *B.coagulans* group (N), *B.coagulans* fermentation with *Atractylodes* polysaccharide group (NB), *B.coagulans* fermentation with *Paratractylodes polysaccharide* group (NF), *B. coagulans* fermentation with mannose group (NG), *B.coagulans* fermentation with *Astragalus polysaccharide* group (NH) and *B.coagulans* fermentation with *Yulingfeng* polysaccharide group (NP). A single factor completely randomized group design was used. A total of 1260 prawns were selected with an initial weight of 0.1-0.2 g and randomly divided into seven groups with three replicates per group (60 prawns per replicate) in a total of 21 circular fiberglass tanks (φ1.5 m, 800 L water per tank). After two weeks of temporary rearing, the prawn seedling was fed with the experimental diet thrice daily. The feeding situation was observed and feces and residual feed were sucked out. Ammonia nitrogen, dissolved oxygen, pH and nitrite content in water were measured regularly and cultured for 8 weeks. All procedures used in this experiment have been approved by the Institutional Animal Care and Use Committee of Southwest University of Science and Technology to ensure the legality and ethics of the experiment.

2.4 Sample collection

At the end of the culture experiment, the total weight of the prawns in each tank was weighed and the total number was counted. Then the hemolymph was extracted from the cardiac chamber and centrifuged (4°C, 4000 r/min, 10 min), and the supernatant was aspirated and placed in a -20°C refrigerator. Alsever's solution was used as the anticoagulant (Taking 13.2 g of trisodium citrate solution, 4.8 g of citric acid, 14.7 g of glucose and fixing the volume to 1 L of double-distilled water) in a ratio of 1:1 with the hemolymph. At the same time, 12 prawns were collected from each group and loaded into 4 frozen storage tubes. Then, all the intestines from each group were loaded into two frozen storage tubes for subsequent indices measurement.

2.5 Challenge test

At the end of the culture experiment, 30 prawns were randomly selected from each group. *Aeromonas hydrophila* NJ-35 was activated with LB medium (glucose 15.80g/L, peptone 15.90 g/L, yeast powder 11 g/L, $MgSO_4$ 4.60 g/L), and the concentration of bacteria solution was adjusted to 1×10^7 CFU/mL for intramuscular injection in the experimental group. The mortality of *M.nipponense* was observed within 12h, 24h, 36h, 48h, 72h and 96h, and the survival rate was calculated.

2.6 Determination of growth indicators

Growth indicators were calculated using the following methods (Zhou et al., 2022):

Survival rate (SR, %)
= 100 × final number of prawns/initial number of prawns;

Weight gain rate (WGR, %)
= 100 × (average final body weight
− average initial body weight)/average initial body weight;

Specific growth rate (SGR, %)
= 100 × [Ln (average weight of the final prawns)
− Ln (average weight of the initial shrimp)]/cultured days;

Feed conversion ratio (FCR) = feed consumption/ prawns weight gain;

2.7 Determination of serum biochemical indicators

Serum total cholesterol (TC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB) and triglyceride (TG) were measured by Mindrai automatic analyzer (BS-400). Serum total protein TP was measured by the Coomassie brilliant blue method.

2.8 Determination of antioxidant indexes

Hepatopancreas total protein (TP), malondialdehyde (MDA), superoxide dismutase (SOD), acid phosphatase (ACP), alkaline phosphatase, total antioxidant capacity (T-AOC) and superoxide anion scavenging capacity (SASC) were measured using kits from Nanjing Jiancheng Bioengineering Institute. The hepatopancreas α-amylase and lipase activities were measured using kits from Beijing Solaibao Biotechnology Co., Ltd.

2.9 Gene expression assays

The expression levels of *Toll, Dorsal, Relish, IMD, Myd88* and *β-actin* were determined by fluorescence quantitative PCR method. All primers were synthesized by Shanghai Jierei Bioengineering Co., Ltd. The primer sequences in Table 3 are from the reference (Liu et al., 2022). Sample RNA was extracted using Trizol method, total DNA concentration and OD value were determined by NanoDrop 2000, and the concentration was adjusted to 500 ng/μL. The Novizan HiScript II Q RT SuperMix for qPCR(+gDNA wiper) kit was used, and the experimental procedures were reverse transcribed into cDNA according to the instructions for use, and the gene expression level was detected by real-time fluorescence quantitative analyzer from the reference (Liu et al., 2022).

2.10 Data analysis

Data were expressed as "mean ± standard error" and analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple comparisons using SPSS 26.0 statistical software. The significance level was $P < 0.05$ and indicated by different lowercase letters superscript.

TABLE 3 Primer sequence of real-time fluorescent quantitative PCR of *Macrobrachium nipponense*.

Primer	Primer sequence (5′-3′)	Length (bp)
β-actin	(F) GTGCCCATCTACGAGGGTTA	20
	(R) CGTCAGGGAGCTCGTAAGAC	20
Dorsal	(F) TACGACCAACGGACAAGAGC	20
	(R) CGCATTGTTGCTGTTTCCCA	20
IMD	(F) GGCACCAAGCCTTCTTTTCAG	21
	(R) ATATCCTTCGGGTCGCATTTC	21
Relish	(F) CGGGAAGTTTGGACGGCATA	20
	(R) TCGTTTAAGGCTGTCTGGCA	20
Toll	(F) CGACCTCCACGACAACAAGA	20
	(R) AAAGTTCCTGCACCAATGCG	20
Myd88	(F) GCTGTTCCACCGCCATTT	20
	(R) GCATCATAGTGCTGTAGT	20

The mRNA sequences of the above genes were obtained from the *M.nipponense* transcriptome sequencing database of Freshwater Fisheries Research Center, Chinese Academy of Fisheries Sciences.

3 Results

3.1 Effect of *A.hydrophila* on the survival rate of *M.nipponense with* polysaccharide fermentation with *B.coagulans*

The experimental design is shown in Figure 1A. Overall, *M.nipponense* mortality increased with infection time and the survival rate of the treatment group was higher than that of the CT (Figure 1B).The survival rates of *M. nipponense* at 96 h were 13.33-66.67% in the different groups (Figure 1B). In addition, the survival rate of NF group was 66.67% at 96 h, which was significantly higher than that of CT ($P < 0.05$, Figure 1C). These results indicated that NF group could effectively inhibit the toxic effect of *A.hydrophila* on *M.nipponense*.

3.2 Effects of polysaccharides fermentation with *B.coagulans* on growth performance of *M.nipponense*

Table 4 shows the effect of polysaccharide fermentation with *B.coagulans* on the growth performance of *M.nipponense*. Compared with the CT, the survival rate of the polysaccharide groups except mannose showed an upward trend, but the difference between the groups was not significant ($P > 0.05$).The weight gain rate of NB group was higher than that of the N ($P < 0.05$), but for the other groups there was no significant difference compared to the control ($P > 0.05$).

3.3 Effect of polysaccharides fermentation with *B.coagulans* on serum biochemistry of *M.nipponense*

Figure 2 shows the effects of polysaccharides and *B.coagulans* added to the diet on serum biochemistry of *M.nipponense*. The experimental design is shown in Figure 2A. Compared with the control CT and N, ALB, TC and TG in NH and NP group were significantly increased ($P < 0.05$) (Figures 2B, E, F).

ALT in NB, NG and NH were significantly lower than those of the CT and N ($P < 0.05$) (Figure 2C). TP in NB was significantly higher than that of the CT ($P < 0.05$) (Figure 2G). AST in the NG group was significantly decreased compared with the control ($P < 0.05$) (Figure 2D). Figure 2 showed that the group of N, NF and NG significantly reduced the content of AST and ALT compared to the CT ($P < 0.05$).

3.4 Effect of polysaccharide fermentation with *B.coagulans* on digestive enzymes of *M.nipponense*

Hepatopancreas lipase levels were significantly different between the group of N and NG or NB compared with the CT ($P < 0.05$) (Figure 3A). Hepatopancreatic α-amylase was significantly higher in the group of N, NG, NB, NF and NH than in the CT ($P < 0.05$) except NP group (Figure 3B).

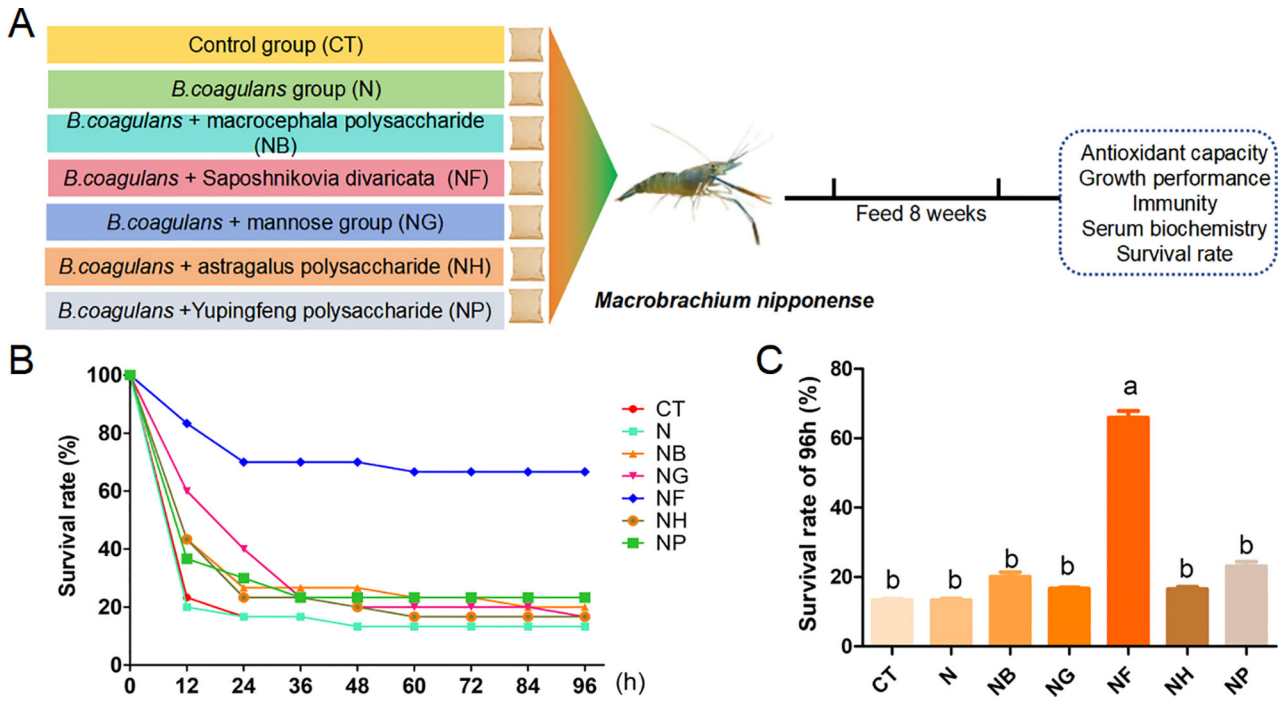

FIGURE 1
Changes in survival rate of *A.hydrophila* to *M.nipponense*. **(A)** Experimental design; **(B)** Change in survival rate of *M.nipponense* within 96 h; **(C)** Survival rate of *M.nipponense*. Means with different lowercase are significantly different ($P < 0.05$, Duncan's multiple comparisons).

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TABLE 4 Effect of polysaccharides fermentation on growth performance of *M. nipponense*.

	CT	N	NB	NG	NF	NH	NP
Initial weight (g)	0.185 ± 0.001	0.179 ± 0.002	0.180 ± 0.004	0.182 ± 0.002	0.176 ± 0.001	0.178 ± 0.002	0.179 ± 0.002
Final weight (g)	0.848 ± 0.001	0.786 ± 0.0283	0.862 ± 0.034	0.868 ± 0.060	0.817 ± 0.060	0.736 ± 0.038	0.756 ± 0.051
Survival rate (%)	67.222 ± 3.380	79.444 ± 3.379	76.111 ± 2.003	62.778 ± 6.550	77.778 ± 2.778	72.778 ± 4.938	75.556 ± 1.111
Weight gain rate (%)	358.392 ± 4.64^{ab}	338.059 ± 9.926^{b}	405.537 ± 5.258^{a}	376.799 ± 35.916^{ab}	386.070 ± 21.100^{ab}	330.663 ± 16.237^{b}	354.169 ± 5.229^{ab}
Specific growth rate (%)	2.537 ± 0.017	2.461 ± 0.038	2.609 ± 0.094	2.594 ± 0.122	2.548 ± 0.110	2.358 ± 0.096	2.395 ± 0.128
Feed rate (%)	3.965 ± 0.325	3.731 ± 0.306	3.561 ± 0.312	4.223 ± 0.292	3.62 ± 0.484	4.166 ± 0.113	3.834 ± 0.395

Means with different lowercase are significantly different (P < 0.05, Duncan's multiple comparisons).

3.5 Effect of polysaccharides fermentation with *B.coagulans* on antioxidant capacity of *M.nipponense*

When polysaccharides and *B.coagulans* were added to the feed, the hepatopancreatic enzyme activities of *M.nipponense* are shown in Table 5. Compared with the group of CT and N, the TP in NG, NB and NP groups significantly increased ($P < 0.05$) and the SOD in NP group significantly decreased ($P < 0.05$). NF group reduced the MDA compared to the N group ($P < 0.05$). ACP in the group of NB and NP, T-AOC and SASC in NP group were significantly increased compared with the CT and N group ($P < 0.05$), but T-AOC and SASC were significantly decreased in NB and NF group ($P < 0.05$).

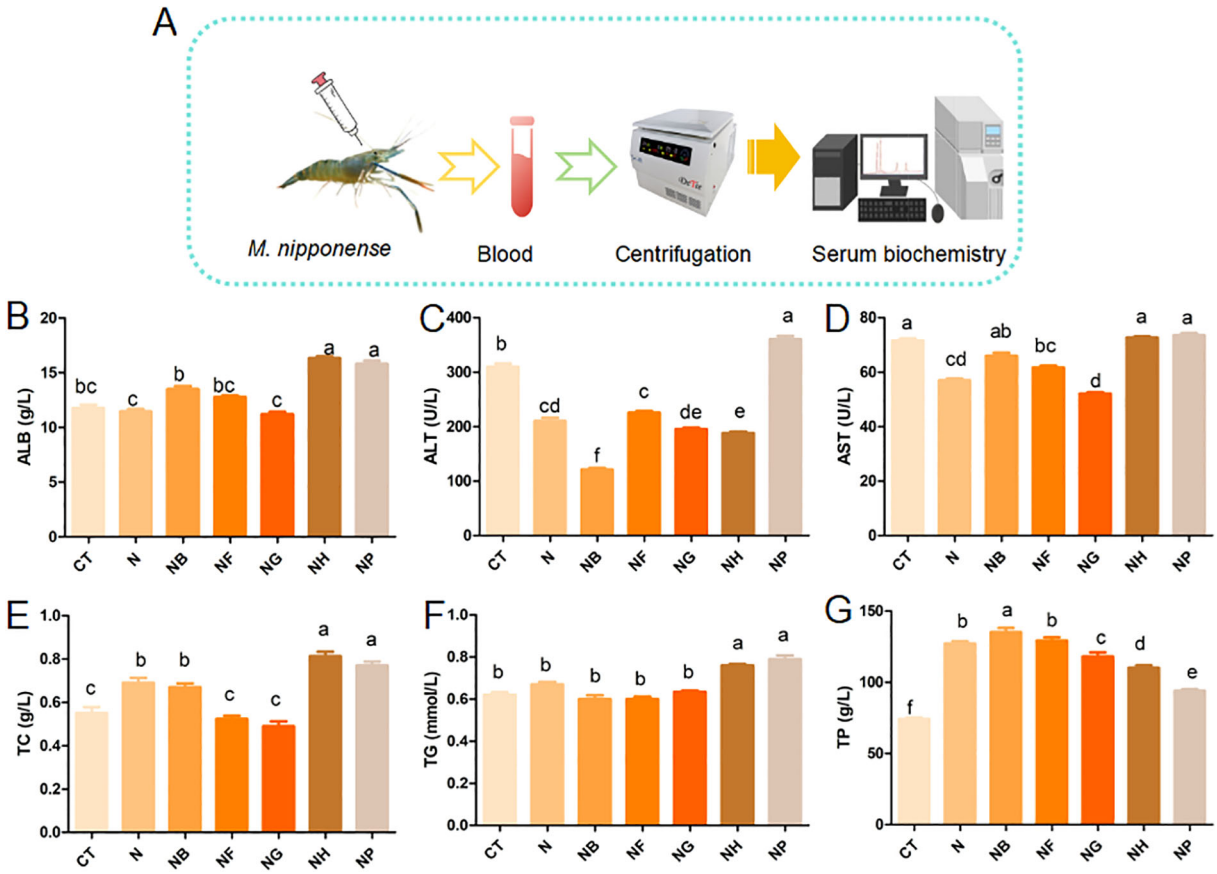

FIGURE 2
Effect of polysaccharide fermentation on serum biochemistry of *M.nipponense* **(A)** Experimental design; **(B)** ALB content; **(C)** ALT content; **(D)** AST content; **(E)** TC content; **(F)** TG content; **(G)** TP content. Means with different lowercase are significantly different ($P < 0.05$, Duncan's multiple comparisons).

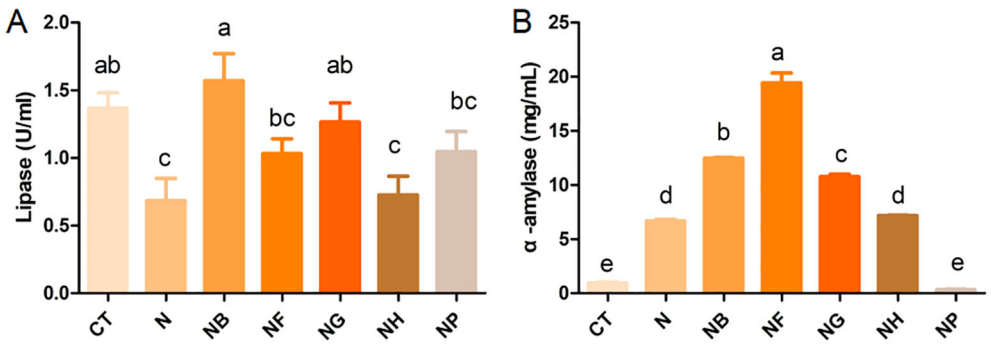

FIGURE 3
Effect of polysaccharide fermentation on digestive enzymes of *M.nipponense* **(A)** Lipase content; **(B)** α-amylase content. Means with different lowercase are significantly different ($P < 0.05$, Duncan's multiple comparisons).

3.6 Effect of polysaccharides fermentation with *B.coagulans* on immune gene expression of *M.nipponense*

The expression levels of *Toll*, *Myd88*, *Dorsal*, *IMD* and *Relish* genes are shown in Figure 4. The expression level of *Toll*, *Myd88*, *Relish* and *Dorsal* in NH group was significantly increased compared with the control group ($P < 0.05$) (Figures 4A–C, E). Compared with the control group, the expression level of Toll in NP was significantly increased and the expression level of *Toll* in NG was reduced considerably ($P < 0.05$) (Figure 4B). The expression of *Myd88* and *Dorsal* in NB, NG and NF group were significantly decreased compared with the CT ($P < 0.05$) (Figures 4A, C).

The expression of *IMD* and *Relish* in NF and NP group increased significantly ($P < 0.05$) (Figures 4D, E), the expression of *IMD* in NB and NG group decreased significantly ($P < 0.05$) (Figure 4D) and the expression of *Relish* in NG group decreased significantly ($P < 0.05$) (Figure 4E).

4 Discussion

Polysaccharides in aquaculture have attracted considerable attention and commercial interest over the past few decades. A continual search for novel, eco-friendly additives remains essential. Therefore, *prebiotic polysaccharides have become a hotspot in research*. *Prebiotics*, which contain polysaccharides and other substances, can promote animal health Plant polysaccharides have been widely studied for their various biological activities. These include antiviral activity, antioxidant activity, anti-fatigue and liver-protective effects (Liu et al., 2015). Five plant polysaccharides (*Atractylodes* polysaccharide, *Paraspinatus* polysaccharide, *Mannose*, *Astragalus* polysaccharide and *Yulingfeng* polysaccharide) can improve growth performance, antioxidant capacity and immune function in animals (Wu, 2020; Su et al., 2020; Cui et al., 2023; Erdenebileg et al., 2023). In addition, *B.coagulans* can promote animal growth, improve antioxidant capacity, enhance immunity and improve intestinal microbial flora (Zhang et al., 2021). Meanwhile, *B.coagulans* can break down plant polysaccharides and promote their uptake in animals (Shinde et al., 2020). However, there are few studies on the use of plant polysaccharides in combination with *B.coagulans*. Therefore, the effect of polysaccharide fermentation with *B.coagulans* for *M.nipponense* was investigated in this experiment.

It was found that the survival rate of the group fed with the polysaccharide *B.coagulans* was the highest. This may be due to the anti-inflammatory, antibacterial and immune-enhancing effects of paraspinals (Javadi and Sahebkar, 2017). Meanwhile, *Paraspinatus* polysaccharides also possessed antioxidant and anti-allergic properties (Guang et al., 2023). In addition, *B.coagulans* was found to fully restore the immunosuppression induced by

TABLE 5 Effect of polysaccharides fermentation on antioxidant capacity of *M.nipponense*.

	CT	N	NB	NF	NG	NH	NP
TP (g/L)	42.24 ± 0.23^{f}	47.19 ± 0.30^{d}	48.13 ± 0.24^{c}	45.09 ± 0.27^{e}	51.98 ± 0.24^{a}	46.40 ± 0.26^{d}	50.10 ± 0.38^{b}
MDA (nmol/mL)	42.24 ± 0.23^{f}	47.19 ± 0.30^{d}	48.13 ± 0.24^{c}	45.09 ± 0.27^{e}	51.98 ± 0.24^{a}	46.40 ± 0.26^{d}	50.10 ± 0.38^{b}
SOD (U/mg prot)	152.42 ± 0.92^{a}	100.91 ± 0.69^{d}	103.23 ± 0.71^{c}	103.74 ± 0.57^{c}	110.69 ± 0.79^{b}	101.78 ± 0.76^{cd}	86.11 ± 0.74^{e}
ACP (King Unite/g prot)	42.66 ± 0.05^{c}	36.15 ± 0.10^{g}	43.86 ± 0.04^{a}	40.35 ± 0.05^{e}	41.47 ± 0.12^{d}	38.33 ± 0.06^{f}	42.90 ± 0.05^{b}
AKP (King Unite/g prot)	178.91 ± 0.15^{d}	192.70 ± 0.17^{a}	189.34 ± 0.19^{c}	191.72 ± 0.34^{b}	189.54 ± 0.14^{c}	188.84 ± 0.18^{c}	189.29 ± 0.33^{c}
T-AOC (U/mg prot)	0.94 ± 0.00^{b}	0.86 ± 0.05^{c}	0.77 ± 0.02^{d}	0.66 ± 0.01^{e}	0.89 ± 0.02^{bc}	0.70 ± 0.02^{de}	1.03 ± 0.01^{a}
SASC (%)	0.94 ± 0.00^{b}	0.86 ± 0.05^{c}	0.77 ± 0.02^{d}	0.66 ± 0.01^{e}	0.89 ± 0.02^{bc}	0.70 ± 0.02^{de}	1.03 ± 0.01^{a}

Means with different lowercase are significantly different ($P < 0.05$, Duncan's multiple comparisons).

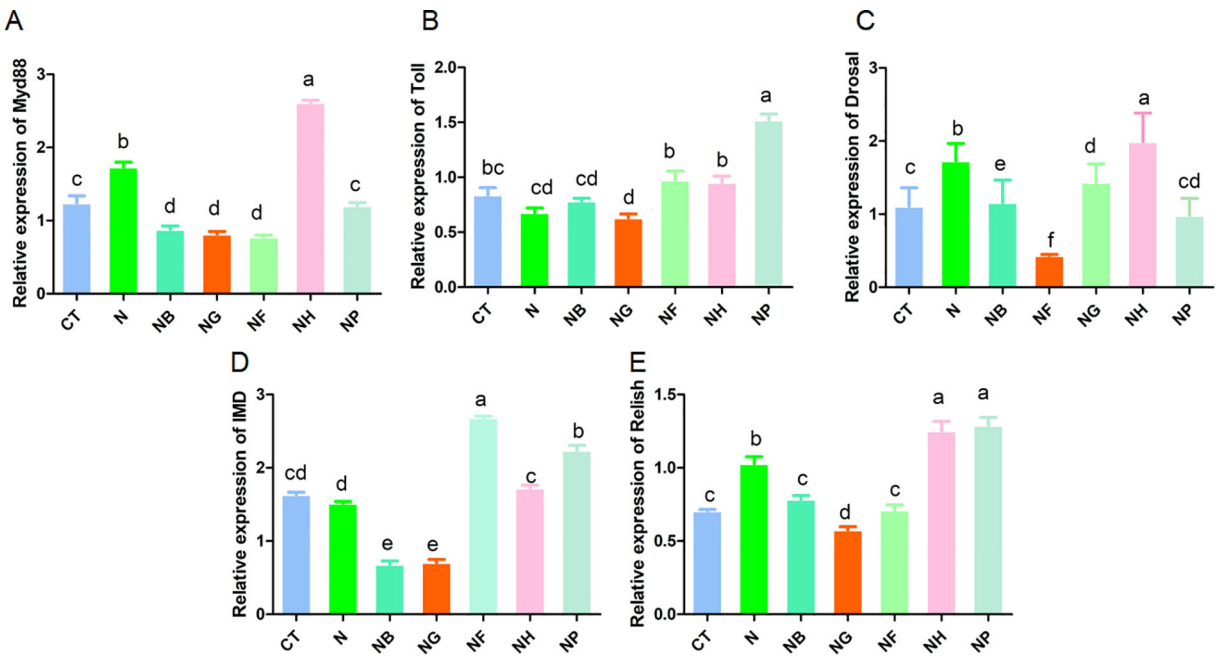

FIGURE 4
Effect of polysaccharide fermentation on gene expression in *M.nipponense* **(A)** *Myd88* expression; **(B)** *Toll* expression; **(C)** *Dorsal* expression; **(D)** *IMD* expression; **(E)** *Relish* expression. Means with different lowercase are significantly different ($P < 0.05$, Duncan's multiple comparisons).

cyclophosphamide, thereby improving its immunity (Bomko et al., 2017), which may also be an essential reason for the improved survival rate. Therefore, it was shown that feeding *M.nipponense* with *Paraspinatus* polysaccharide fermented by *B.coagulans could* improve the survival rate of *A.hydrophila* infection.

B.coagulans can produce enzymes such as protease, lipase and amylase (Gupta et al., 2016), which promote the absorption of nutrients in the intestine of animals. Studies have found that feeding compound bacterial agents such as *Bacillus subtilis* for *Pengze crucian* carassius improved the activities of protease, amylase and lipase (Luo et al., 2021). In addition, studies on *Clostridium butyricum* and *B.coagulans* have found that they can improve the gut microbiota structure and promote the growth of steelhead trout (Fan et al., 2019). The results of this study showed that the hepatopancreatic digestive enzyme activities of *M.nipponense* were increased after *Atractylodes* polysaccharide, mannose and *Paratractylodes* polysaccharide, which was consistent with the results of previous studies. Therefore, adding polysaccharides of *Atractylodes japonicum*, *Paraspinatus*, *Astragalus* and *Yupingfeng* to in the diet can improve the activities of non-digestible enzymes of *M.nipponense*. This result is mainly caused by *B.coagulans*, but there are few studies on the effect of the polysaccharides (*Atractylodes rhizoma* polysaccharide, *Paraspinatus* polysaccharide, *Astragalus* polysaccharide, *Yupingfeng* polysaccharide) on digestive enzymes. The ability was limited to the study of the effects of polysaccharides of *Atractylodes rhizoma*, *Paratractylodes* and *Astragalus* on digestive enzymes of *M.nipponense*. Therefore, the molecular mechanism underlying the effect of polysaccharides on the digestive enzymes of *M.nipponense* could not be determined.

Serum biochemistry reflects homeostatic changes in the body's internal environment. ALT is mainly distributed in hepatocyte plasma and AST is primarily present in hepatocyte mitochondria and plasma. Hepatocyte necrosis causes ALT and AST to enter the blood circulation, thereby increasing the levels of ALT and AST in the body (Iweala et al., 2019). Oxidative stress in the liver can cause lipid peroxidation, thereby changing the permeability of the liver cell membrane and increasing ALP levels *in vivo* (Albasher et al., 2019). At the same time, liver injury can cause the rapid diffusion of fatty acids in the liver, resulting in the increase of TG and TC content in the liver. TG and TC also directly reflect the degree of lipid peroxidation in the liver (Wang et al., 2019b). Reduced liver function can further impair renal function, affecting the synthesis, transport, and release of ALB in the body, thereby reducing the level of ALB in the blood (Dongzhe et al., 2023). In the present study, the N, NF, NG, NH and NB significantly reduced the content of ALT. However, the N, NF and NG significantly reduced AST content. It has been previously found that dietary supplementation of *Bacillus coagulans* significantly reduced serum ALT and AST levels induced by high cholesterol in rats (Aminlari et al., 2018). The elevation of ALT and AST induced by cadmium poisoning was alleviated in rats fed with symbiotics *B.coagulans* with *Lactobacillus plantarum* and inulin (Jafarpour et al., 2017). In other words, *B.coagulans* mixed with polysaccharide can reduce ALT and AST content and protect the liver. *Mannose* and *Atractylodes* polysaccharide can effectively protect the liver injury in mice by significantly decreasing the activities of AST and ALT in serum (Han et al., 2016). Dietary *Astragalus* polysaccharide supplementation can improve the growth performance, alleviate liver dysfunction, and reduce ALT and AST

content in the liver of piglets (Wang et al., 2019a). In addition, relevant studies have shown that dietary *Astragalus* polysaccharide can significantly reduce the content of glucose, triglyceride, cholesterol and nitric oxide in fish serum (Wu et al., 2019). In addition, it was found that replacing fish meal with *Atractylodes Macrocephala Polysaccharide* decreased the albumin and globulin concentrations and increased the feed coefficient and total bile acid activity of catfish (Zhuo et al., 2022). In summary, this is consistent with the present study and confirms the authenticity of the data in this study.

Oxidative stress is a primary biochemical reaction of organisms to resist external environmental stimulation and adapt to the living environment. The oxidative defense system is the basis of resistance to external stimuli, which is a balanced system composed of antioxidant enzymes and oxidative stress products. SOD is an important antioxidant enzyme in the oxidative defense system. SASC and T-AOC reflect the body's overall antioxidant capacity. MDA is critical for oxidative stress and demonstrates the degree of oxidative damage in the body. Oxidative stress can also lead to decreased immunity and damage the immune system. ACP and AKP, as immune enzyme indicators, reflect the body's immune ability. In this study, it was found that diet made from the fermentation broth of *B.coagulans* with *Yupingfeng* polysaccharide significantly increased the levels of ACP, AKP, SASC and T-AOC in *M.nipponense*. These results suggested that the fermentation broth of *B.coagulans* with *Yupingfeng* polysaccharide could increase the antioxidant capacity and immune capacity of *M.nipponense*. This may be due to the improvement of the antioxidant capacity of *M.nipponense* by regulating serum immunity through the fermentation broth of *B.coagulans* of *Yupingping* polysaccharide. Previous studies have shown that *Yupingfeng* polysaccharide could down-regulate the mRNA expression levels of pro-inflammatory cytokines, *NF-kB*, *TLR-4* and *iNOs*, enhancing the antioxidant capacity (Sun et al., 2017). At the same time, *Yupingfeng* polysaccharide could significantly increase the density of immune cells and the number of macrophages and reduce the levels of *NO*, *TNF-α*, *IL-1β* and *IL-6*. Adding 1.6 g/kg *Yupingfeng* polysaccharide to the diet of grass carp can improve the immunity of grass carp (Wang et al., 2016). The above results indicated that *Paratricans* polysaccharide had some immunomodulatory activity (Fan et al., 2023). In addition, it was found that the fermentation broth of *Atractylodes rhizoma* polysaccharide increased the contents of ACP and AKP in *M.nipponense*. *Atractylodes* polysaccharide and *Atractylodes lactone* have been found to have anti-inflammatory and immunomodulatory effects in previous studies (Gu et al., 2019; Tang et al., 2017). However, the defect of this study is that the cause of the high MDA in the fermentation broth of *B.coagulans* with polysaccharide was not explored.

Invertebrates rely on innate immunity to defend against disease invading their bodies (Reboul and Ewbank, 2016) and shrimp are crustaceans and rely mainly on innate immunity (Patnaik et al., 2023). These include physical defense, cellular immunity, and humoral immunity (Riera Romo et al., 2016; Thaiss et al., 2016). In the model organism *Drosophila* antimicrobial peptide gene pathway, Toll and IMD play an important role in mediating G^- and G^+ infections, respectively (Huang et al., 2009) and shrimp immunity (Su et al., 2020). Toll receptor, Myd88 and Dorsal are key signaling molecules in the Toll pathway (Dong et al., 2022) and Myd88 is a receptor protein that connects Toll (Arts et al., 2007). Relish, a downstream transcription factor of the IMD pathway, belongs to the NF-kB family and participates in the body's humoral immune response (Luo et al., 2022). *IMD* immunodeficiency is involved in

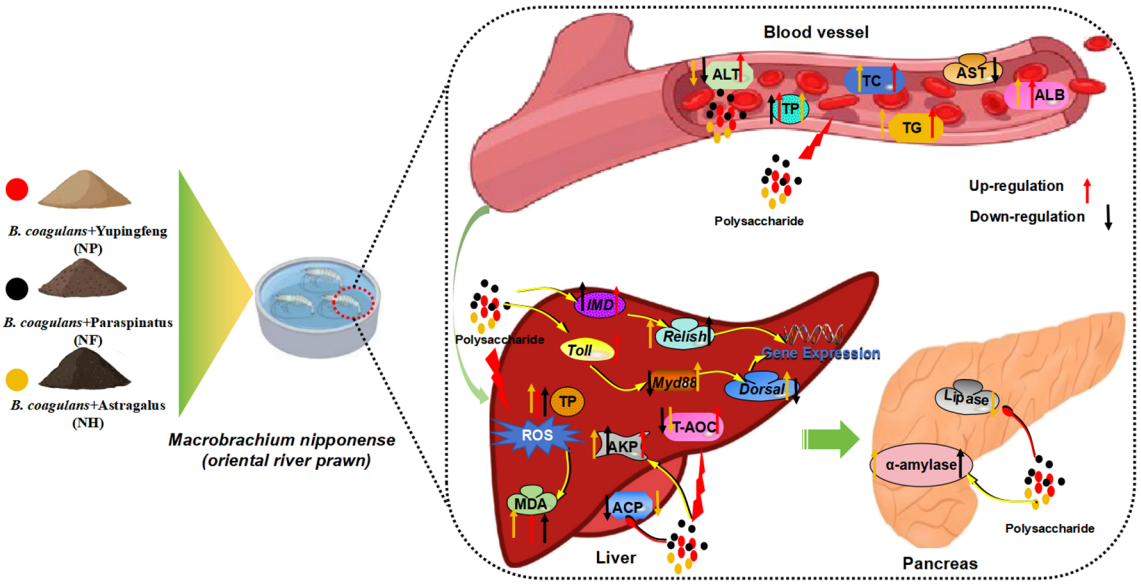

FIGURE 5
The regulatory mechanisms of *Yupingfeng polysaccharide*, *Saposhnikovia divaricata polysaccharides* and *Astragalus polysaccharide* for *M. nipponense*. Toll, Toll-like receptor; Myd88, Myeloid differentiation primary response gene 88; Dorsal, Dorsal-related immunity factor; IMD, Immunodeficiency; Relish, Relish protein; TP, Total protein; MDA, Malondialdehyde; ACP, Acid phosphatase; AKP, Alkaline phosphatase; T-AOC, Total antioxidant capacity.

antimicrobial responses *in vivo*. Previous studies found that *Yupingfeng* polysaccharide could enhance serum immunity and *Toll* gene expression in *Litopenia vannamen* (Su et al., 2020). At the same time, researchers showed that *Yupingfeng* could regulate the release of cytokines in mouse macrophages, mainly due to the enhanced degradation of IkBα, which in turn activated NF-kB, causing pro-inflammatory cytokine-related protein and mRNA expression (Fabrizio et al., 2013). In addition, *Yupingfeng* also inhibited the expression of pro-inflammatory cytokines (Fabrizio et al., 2013). The expression levels of *Toll*, *IMD* and *Relish* in the hepatopancreas of *Litopenaeus vannaeus* can be significantly increased by adding *Marine Rhodotorula* to the diet (Jin et al., 2022). Increased expression of *Relish* was observed in the hepatopancreas after artificial infection with *A. hydrophila* (Liang, 2022). In addition, *Yupingfeng polysaccharide* was found to increase the expression of Toll receptors in the serum of *Litopenia vannamen* and promote the body health (Su et al., 2020). *Astragaloside* has also been found to exert anti-inflammatory effects through the Myd88/NF-kB pathway (Shi et al., 2021). *Astragalus* polysaccharides can improve juvenile crucian carp's growth performance and innate immunity (Wu, 2020). This is consistent with the results of the present study, which showed that the addition of *Astragalus* and *Yupingfeng* polysaccharides to the diet of *M. nipponense* increased the expression of Myd88 and Toll, respectively, and thus increased the expression of related immune genes in *M.nipponense*.

In general, these fermented polysaccharides' primary mechanisms of action involve absorption in the intestines, entry into the bloodstream, and effects on various tissues and organs throughout the body. Specifically, *Yupingfeng polysaccharides* enhance liver function and the liver's antioxidant capacity by increasing the serum levels of TP, ALB, AKP and T-AOC *Saposhnikovia divaricata polysaccharides* can increase the content of TP in the serum, improve liver function, and regulate the liver's antioxidant function by adjusting the content of AKP and TP. Additionally, *Saposhnikovia divaricata polysaccharides* can increase the content of alpha-amylase content, enhancing metabolic function. *Astragalus polysaccharides* can reduce the content of ALT in the serum and increase the content of TP, thereby enhancing liver function. Moreover, *Astragalus* polysaccharides enhance the body's antioxidant function by increasing the content of T-AOC, TP, and AKP, thereby enhancing the body's immune capacity (Figure 5). In summary, among the five types of fermented polysaccharides screened, *Yupingfeng* polysaccharides, *Saposhnikovia divaricata* polysaccharides and *Astragalus* polysaccharides have a significant effect on improving the antioxidant capacity and immunity of *M.nipponense*.

5 Conclusion

M.nipponense was co-influenced by polysaccharides and *B.coagulans* and the adding of *fermented polysaccharides* to the diet increased the weight gain of the prawns. The combined use of polysaccharide fermentation with *B.coagulans* can improve the survival rate of *M.nipponense* infected with *A.hydrophila*; the survival rate of the group with *B.coagulans* and *Saposhnikovia divaricata polysaccharides* (NF) was 66.67% at 96 hours. Adding polysaccharides fermented with *Bacillus coagulans* to the diet significantly impacted the prawns' immune capacity and digestive enzyme activity. The addition of *fermented Astragalus* and *Yupingfeng* polysaccharides with *B. coagulans* to the diet increased the expression of *Myd88* and *Toll*, respectively. The addition of fermented *Atractylodes macrocephala* polysaccharides with *Saposhnikovia divaricata* polysaccharides and *Mannose*, as well as *Yupingfeng* polysaccharides with *B.coagulans*, significantly reduced the expression of *Relish* and promoted the growth of the *M.nipponense*.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

YW: Conceptualization, Investigation, Writing – original draft. YL: Methodology, Project administration, Writing – original draft, Writing – review & editing. JY: Data curation, Methodology, Writing – original draft. ZL: Funding acquisition, Methodology, Writing – review & editing. WW: Conceptualization, Writing – original draft. LJ: Methodology, Supervision, Writing – review & editing. BL: Formal analysis, Funding acquisition, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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