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Optimizing shrimp nutrition and health: ginseng saponins as functional additives in low-fishmeal diets on *Litopenaeus vannamei*

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The research investigated the nutritional physiology effect of ginseng saponins on *Litopenaeus vannamei* (*L. vannamei*) under low-fishmeal diets. In total, five experimental groups were arranged, with 21% fishmeal (high-fishmeal) serving as the positive control (PC), 11% fishmeal (low-fishmeal) serving as the negative control (NC), and 11% fishmeal serving as the addition in all three other groups. Similarly, ginseng saponins (GSP, purity of 2%) were added in the order of 0.1%, 0.3%, and 0.5% (GSP0.1, GSP0.3, and GSP0.5), with an 8-week growth cycle. Both GSP0.1 and GSP0.3 showed significantly higher growth performance (final body weight, FBW; weight gain rate, WGR; specific growth rate, SGR) than the NC group, but significantly lower growth performance than the PC group ($P<0.05$). However, it was found that there was no significant difference in the body composition of the whole shrimp between the experimental groups. Compared to the PC group, the GSP0.3 group exhibited significantly elevated levels of antioxidant enzymes, total antioxidant capacities (T-AOC), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) ($P<0.05$). Additionally, significant differences were observed between the PC and GSP0.3 groups regarding the expression levels of *sod*, *cat*, and *gsh-px* ($P<0.05$). And there was a better morphological organization of shrimp hepatopancreas in the GSP0.3 group than in all other groups. In comparison with the PC group, there was no significant difference in shrimp survival rates after ammonia nitrogen stress with ginseng saponins added ($P>0.05$). Whereas, in terms of the relative expression levels of the corresponding genes, in shrimp of the GSP0.3 group, the relative expression of antioxidant-related genes *sod*, *cat*, and *gsh-px* were significantly higher than that of the PC group ($P<0.05$). *Caspase3* and *p53*, along with *bcl-2* and *bax*, were found to be significantly more expressed in shrimp of the GSP0.3 group than in all other groups ($P<0.05$). These findings imply that in addition to improving growth performance, adding ginseng saponins at a concentration of 11% fishmeal could improve the

antioxidant capacity of *L. vannamei* as well as its resistance to stress. Therefore, ginseng saponins can be utilized as a functional additive to increase *L. vannamei* growth performance, enhance antioxidant capacity, and reduce stress in low-fishmeal diets, 0.3% of ginseng saponins is optimal.

KEYWORDS

***Litopenaeus vannamei*, ginseng saponins, growth performance, antioxidant capacity, ammonia nitrogen stress**

1 Introduction

There are many reasons why fishmeal is recognized as the best source of protein in aquafeeds. These include its high palatability, balanced nutrient profile, easy digestibility, and absorption properties. The current change in global climate, however, that has resulted in a decrease in the catch of capture fisheries, contributing to fishmeal shortages and increased prices (Hodar et al., 2020; Jannathulla et al., 2019). Several studies have been conducted in search of suitable protein sources to replace fishmeal, but a high percentage of fishmeal substitution may negatively affect aquatic animal growth and immunocompetence. As a result of 45% replacement of fishmeal with defatted yellowtail worm, *Larimichthys crocea* exhibits decreased growth performance and immunocompetence (Zhang et al., 2022). It is reported that *Oreochromis niloticus* and *Sarotherodon galilaeus* suffer from poorer growth performance and liver health after 20% and 30% replacements with vegetable meal (Sallam et al., 2021). Enhancing the antioxidant and immune capacities of aquatic animals under low-fishmeal diets is considered as one of the ways to reduce the negative impacts of low-fishmeal diets. Therefore, the aquaculture industry needs feed additives that are cost-effective, environmentally safe, and capable of improving shrimp immune capacity.

An herbaceous perennial plant in the Nansingidae family, Ginseng has a long history of medicinal use and is believed to treat a number of ailments, it regulates the immune system particularly well, and is therefore considered an immunomodulator (Kang and Min, 2012). Among its benefits are the enhancement of immunity, improves blood circulation, antioxidant properties, and anticancer properties (Kim, 2018, 2012; Ratan et al., 2021). A wide range of diseases have been treated with ginseng extracts in aquatic animals. It is found that dietary supplementation of ginseng extract significantly improved the survival rate of juvenile *Oncorhynchus mykiss* (Bulfon et al., 2017). Supplementing *Nile tilapia* diets with ginseng extract improves growth performance, feed utilization, and hematological indices (Goda, 2008). The survival of fish attacked by *Aeromonas hydrophila* increases with increasing levels of ginseng in their diets (Abdel-Tawwab, 2015). The addition of ginseng extract to Nile tilapia diets can be used as a natural alternative to hygromycin as a growth promoter and also as an immunoregulator (Elsayed et al., 2014). The main reason for the medicinal value of ginseng is believed to be its main active ingredient, ginsenoside (Guo et al., 2021; Murthy et al., 2014). A number of studies have demonstrated its immunomodulatory and antioxidant properties, along with its anti-inflammatory and anti-stress effects (Elekofehinti, 2015; Güçlü-Ustündağ and Mazza, 2007). Inflammatory responses may be modulated by ginseng saponins, their metabolites or derivatives, including Rb1, Re, Rg1, Rg3, Rg5, Rh2, and Rp1, which modulates inflammatory signaling pathways (Kim et al., 2017; Riaz et al., 2019).

As one of the most common hazardous substances in the aquaculture environment, ammonia nitrogen (NH_3-N) is derived from feed residues, animal excreta, and microbial metabolism (Abdelfatah, 2022; Edwards et al., 2024; Zhang et al., 2023). Ammonia nitrogen is present mostly as free ammonia (NH_3) and as ammonium ions (NH^{4+}) in water bodies. Free ammonia is less toxic than ammonium ions. Ammonia nitrogen concentrations in the environment cause a wide range of negative effects on aquatic animals, including shrimps (Duan et al., 2024; Lu et al., 2016; Páez-Osuna, 2001). Shrimps will experience physiological stress due to ammonia nitrogen, resulting in an increase in their respiratory rate and blood concentration of ammonia nitrogen, consequently, oxygenation is affected, the acid-base balance and osmotic pressure regulation of the body are affected, and normal metabolism is affected (Valencia-Castañeda et al., 2018). When shrimp are exposed to high concentrations of nitrogen, their growth will be inhibited, resulting in impaired feeding and digestion, slow growth, weight loss, and shortening of their bodies, among other problems (Frías-Espericueta et al., 2000; Lin et al., 1993). Additionally, ammonia nitrogen stress weakens the shrimp's immune system, making it less resistant to pathogenic microorganisms and susceptible to infection with virus-causing diseases such as white spot syndrome virus (WSSV) (Kathyayani et al., 2019; Ma et al., 2023). Ammonia stress poses a serious threat to shrimp survival and health. To reduce the harm caused by ammonia-nitrogen stress, aquaculture managers need to implement various strategies (Emerenciano et al., 2022). To optimize feed management and minimize the production of feed residues, it is essential to avoid overfeeding. Strengthening water quality management through regular water changes is necessary to maintain adequate dissolved oxygen levels. Additionally, it is critical to reasonably control stocking density to prevent water quality

deterioration caused by excessive density. Regular monitoring of key parameters such as ammonia nitrogen concentration, pH, and dissolved oxygen is also imperative, with timely control measures implemented as needed. To protect the health and sustainable development of aquaculture, these methods of scientific management and rational water quality control can effectively reduce ammonia nitrogen stress (Zhang Y. et al., 2020). Current studies indicate that incorporating functional additives into the diet can enhance the stress resistance of aquatic animals under ammonia nitrogen conditions (Jin et al., 2018; Kaleo et al., 2019; Sallam et al., 2020; Yilmaz, 2019).

Ginseng extract has been found to enhance growth and immunity in Nile Tilapia (*Oreochromis niloticus*) and hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) (Ahmed et al., 2022; Riaz et al., 2019; Sun et al., 2018). Due to its fast growth rate, long breeding season, and tasty meat, *L. vannamei* has been introduced and cultivated in many countries (Zhang et al., 2019). However, there is still a lack of information on the application of ginseng saponins in the diet of *L. vannamei*. In this study, we explored the application of ginseng saponins in the diet of *L. vannamei* and investigated the effects of ginseng saponins on growth performance, antioxidant capacity, and ammonia nitrogen stress resistance of *L. vannamei* fed with low-fishmeal diet, which provided a theoretical basis for the development of functional feed additives.

2 Materials and methods

2.1 Diet preparation

According to the diet formula in Table 1, all ingredients were crushed and sieved, weighed and mixed completely, fish oil and soybean oil were added in turn, then water was added and stirred well. An extruder made long strips of the mixed ingredients and a pelletizer made them into feed pellets. Afterwards, the feed was placed in the oven to mature, removed and air-dried, and then packed in sealed plastic bags and stored in the refrigerator at -20°C.

2.2 Culture experiment

For the experiment, shrimp larvae were staged for six weeks prior to being selected and assigned to culture tanks randomly. At the commencement of the study, shrimp of uniform size (approximately 0.45 g in weight) were chosen and randomly assigned to experimental groups. Prior to commencing the formal experiment, the initial sample of 200 shrimp were obtained. Four replicates were established for each treatment, with thirty shrimp allocated to each aquarium (1 m^3). Throughout the experimental duration, the water quality within the culture system was meticulously regulated to ensure optimal conditions for shrimp survival, with a consistent temperature maintained at 28°C. The shrimp were fed three times a day and initially 5% of their body weight was fed to them during the experiment. Following feeding, the feeding rate was increased by 20% if there were no surpluses, while decreased by 10% if there were surpluses.

2.3 Sampling

The feeding was stopped for 24 h at the end of the 8-week culture experiment. Survival rates and the other growth performance were calculated using live shrimp. To analyze the whole shrimp composition, five shrimp were randomly selected from each bucket, stored at -20°C in the refrigerator. In addition, four shrimp were randomly selected for dissection to examine their H&E sections, gene expression, and enzyme activity.

TABLE 1 Ingredients and proximate composition of five experimental diets (g/kg).

Ingredients	PC	NC	GSP0.1	GSP0.3	GSP0.5
Fish meal [1]	21	11	11	11	11
Soybean meal [2]	25	25	25	25	25
Peanut meal [3]	12	12	12	12	12
Tenebrio molitor meal [4]	0	10	10	10	10
Soybean protein isolate [5]	1.7	0	0	0	0
Wheat flour [6]	24.99	24.91	24.81	24.61	24.41
Krill meal [7]	2	2	2	2	2
Beer yeast [8]	4	4	4	4	4
Fish oil [9]	1.25	1.37	1.37	1.37	1.37
Soybean lecithin [10]	1.5	1.5	1.5	1.5	1.5
Soybean oil [11]	1.25	1.37	1.37	1.37	1.37
Vitamin premix [12]	0.5	0.5	0.5	0.5	0.5
Mineral premix [13]	0.5	0.5	0.5	0.5	0.5
Choline [14]	0.5	0.5	0.5	0.5	0.5
$Ca(H_2PO_4)_2$ [15]	1.7	1.7	1.7	1.7	1.7
Vitamin C [16]	0.1	0.1	0.1	0.1	0.1
Sodium alginate [17]	1	1	1	1	1
Y_2O_3 [18]	0.01	0.01	0.01	0.01	0.01
Ginseng saponins [19]	0	0	0.1	0.3	0.5
Methionine [20]	0.24	0.35	0.35	0.35	0.35
Lysine (98%) [21]	0.17	0.47	0.47	0.47	0.47
Threonine [22]	0.29	0.42	0.42	0.42	0.42
Cysteine [23]	0.3	0.35	0.35	0.35	0.35
Arginine [24]	0	0.28	0.28	0.28	0.28
Histidine [25]	0	0.07	0.07	0.07	0.07
Isoleucine [26]	0	0.14	0.14	0.14	0.14
Leucine [27]	0	0.21	0.21	0.21	0.21
Phenylalanine [28]	0	0.15	0.15	0.15	0.15
Valine [29]	0	0.1	0.1	0.1	0.1

(Continued)

TABLE 1 Continued

Ingredients	PC	NC	GSP0.1	GSP0.3	GSP0.5
Total	100	100	100	100	100
Ash	8.45	8.33	8.38	8.39	8.41
Crude lipid	6.71	6.44	6.53	6.50	6.52
Crude protein	36.65	36.21	36.42	36.04	36.41
Amino acid	35.65	35.91	35.75	35.84	35.67

[1] Fish meal: Guangzhou Chengyi Industrial Group Co., Ltd., China. [2] Soybean meal: Yihai Kerry Jinlongyu Grain and Oil Food Co., Ltd., China. [3] Peanut meal: Zhuhai Dehai Biotechnology Co., Ltd., China. [4] Tenebrio molitor meal: Zhuhai Dehai Biotechnology Co., Ltd., China. [5] Soybean protein isolate: Kyorin Industry (Shenzhen) Co., Ltd., China. [6] Wheat flour: Hebei Jinshahe Noodle Industry Group Co., Ltd., China. [7] Krill meal: Qinghai Kunjie environmental protection technology Co., Ltd., China. [8] Beer yeast: Guangzhou Chengyi Industrial Group Co., Ltd., China. [9] Fish oil: Guangzhou Chengyi Industrial Group Co., Ltd., China. [10] Soybean lecithin: Guangzhou Chengyi Industrial Group Co., Ltd., China. [11] Soybean oil: Cofco Co., Ltd., China. [12] Vitamin premix (kg^{-1} of mixture): vitamin A, 250,000 IU; riboflavin, 750 mg; pyridoxine HCL, 500 mg; cyanocobalamin, 1 mg; thiamin, 500 mg; menadione, 250 mg; folic acid, 125 mg; biotin, 10 mg; a-tocopherol, 3750 mg; myo-inositol, 2500 mg; calcium pantothenate, 1250 mg; nicotinic acid, 2000 mg; vitamin D3, 45,000 IU; vitamin C, 7000 mg. Guangzhou Chengyi Company Ltd., China. [13] Mineral premix (kg^{-1} of mixture): Zn, 4000 mg; K, 22,500 mg; I, 200 mg; NaCl, 2.6 g; Cu, 500 mg; Co, 50 mg; FeSO4, 200 mg; Mg, 3000 mg; Se, 10 mg. Guangzhou Chengyi Company Ltd., China. [14] Choline: Guangzhou Chengyi Industrial Group Co., Ltd., China. [15] $Ca(H_2PO_4)_2$: Guangzhou Chengyi Industrial Group Co., Ltd., China. [16] Vitamin C: Guangzhou Chengyi Industrial Group Co., Ltd., China. [17] Sodium alginate: Nanjing Duly Biotechnology Co., Ltd., China. [18] Y_2O_3: Shanghai Haohong scientific Co., Ltd., China. [19] Ginseng saponins: Hunan Micrograss Biotechnology Co., Ltd., China. [20-29] Amino acid: Methionine, Lysine, Threonine, Cysteine, Arginine, Histidine, Isoleucine, Leucine, Phenylalanine, Valine. Shanghai Feeel Technology Development Co., Ltd., China.

2.4 Ammonia nitrogen stress tests

After routine sampling, ammonia nitrogen stress tests were performed according to the experimental method (Liu and Chen, 2004), To determine the amount of ammonium chloride to add to the formal experiments, one culture tank per treatment group was selected for the ammonia nitrogen stress pre-experiment. The concentration of NH_3 in the water was determined to be 4.5 mg/L, at 4-hour intervals, the ammonia concentration in the water was measured to maintain the NH_4Cl concentration, the stress tests kept 12 h. After the ammonia nitrogen stress tests, the survival rate was recorded, four shrimp from each group were randomly selected and sampled for dissection to examine their gene expression, and enzyme activity.

2.5 Proximate composition determination

Moisture, crude protein, crude lipid, and crude ash of whole shrimp were determined by using standard methods (Association of Official Analytical Chemists, AOAC. In order to calculate the moisture content of whole shrimp, the shrimp were dried at 105°C, and their dry weight were measured at constant weight. The dried whole shrimp were ground, and the crude protein and crude lipid contents were determined by using a fully automated Dumas nitrogen tester (N pro (DT Ar/He Basic), Gerhardt GMBH & CO.KG, Germany) and an automated lipid analyzer (Soxtec System HT6, Tecator, Sweden).

2.6 Total RNA extraction and cDNA synthesis

Animal RNA Extraction Kit (Beyotime Biotech Inc, Shanghai, China) was used to extract total RNA from hepatopancreas, and a microspectrophotometer was used to determine its concentration and quality (Thero Scientific, USA). Reverse transcription of cDNA was carried out using the *Evo MMLV* Reverse Transcription Kit II (Accurate Biotechnology, Hunan, China), which was stored in the refrigerator at -20°C for storage.

2.7 Real-time quantitative PCR (qRT-PCR)

All genes were amplified by qRT-PCR using a Roche real-time fluorescence quantitative PCR system (LightCycler 480 II, Roche Diagnostics, Basel, Switzerland). The reagents used were SYBR Green Pro Taq HS premixed qPCR kit (Accurate Biotechnology, Hunan, China), with a reaction system of 10 µL. The PCR reaction conditions consisted of an initial denaturation step at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 34 s, and extension at 95°C for 15 s. A final extension step was performed at 60°C for 60 s, followed by a melt curve analysis. Three biological replicates were performed using the *β-actin* gene as the internal reference gene, and primer sequences are shown in Table 2. Using the $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen, 2001), three replica wells were set up for each reaction, and the relative expression of genes was calculated.

2.8 Enzyme activity assay

A volume of PBS solution was added to approximately 0.5 g of hepatopancreatic sample, and the sample was ground. After centrifugation at 4000 rpm for 15 min at 4°C, the supernatant was extracted. Afterwards, the supernatant was used to detect hepatopancreatic antioxidant enzymes and digestive enzymes. Superoxide dismutase (SOD), total antioxidant capacity (T-AOC), lipid oxidation (MDA), glutathione peroxidase (GSH-Px) were detected using the kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.9 Morphological microscopy of hepatopancreatic tissues

The samples were initially fixed in a 4% paraformaldehyde solution, followed by fixation in a 70% ethanol solution after 24 h. Subsequently, the samples underwent dehydration by using ethanol solutions of varying concentrations, were embedded in paraffin. This experiment used embedded tissue samples cut into 3 µm slices, observing and photographing H&E sections by using a Nikon orthomicroscope (Eclipse Ni-E, Nikon, Japan). The NIS-Elements viewer software (National Institutes of Health, Bethesda, USA) was used to measure and analyze the photographs.

TABLE 2 Real-time quantitative PCR primers for genes of *L. vannamei*.

Gene	Primer sequence(5′ to 3′)	GenBank No.
β-actin F	CGAGGTATCCTCACCCTGA	AF300705.2
β-actin R	CGGAGCTCGTTGTAGAAGG	AF300705.2
sod F	TGCCACCTCTCAAGTATGATTTC	KU958381.1
sod R	TCAACCAACTTCTTCGTAGCG	KU958381.1
cat F	GGGTATTGAGGCTTCCCCTG	AY518322.1
cat R	GGGGCCATCTCTCTGGTAGT	AY518322.1
gsh-px F	AGAAGAGTTCGGCGACAAGC	AY973252.2
gsh-px R	TCGAAGTTGTTCCCAGGACG	AY973252.2
p53 F	TGTCAGTCGGTGGTGTATCAG	KX827274.1
p53 R	ATGTGCCAGAGTAGAGTCAGC	KX827274.1
caspase3 F	CAATGACCAGCAGCGTCTTC	XM_027379995.1
caspase3 R	CACGGAAGGAGGCGTATCAT	XM_027379995.1
bcl-2 F	CCTTGCTTGACACAGTCGGA	XM_027354848.1
bcl-2 R	CAGACAAGGTCGTGAGGTGG	XM_027354848.1
bax F	GCTATGCCCACATGTTCAGC	XM_027376311.1
bax R	TCTTGCCATCGTAGGGTGTG	XM_027376311.1

2.10 Statistical analysis

The formula for calculating the parameters of this test includes the following equations, Initial body weight (IBW, g)=initiaotal wet weight/initial number of tails; Final body weight (FBW, g)=final total wet weight/final number of tails; Weight gain (WG, %)=100×(final body weight-initial body weight)/initial body weight; Specific growth rate (SGR, %/day)=100×(Ln final mean weight-Ln initial mean weight)/number of days; Feed conversion rate (FCR)=dry diet fed/wet weight gain; Survival rate (SR, %)=100×number of terminal surviving tails/number of initial tails.

The results of the experiment were expressed as "mean ± standard error". Data were analyzed using SPSS 26.0, and differences between groups were compared by one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (Tukey HSD) test ($P<0.05$), before running ANOVA, normality tests had been performed.

3 Results

3.1 Growth performance and feed conversion rate

Table 3 showed the growth performance of *L. vannamei* after supplementation with ginseng saponins under low-fishmeal diets. There were significant differences, between the positive control group and the other groups in terms of final body weight, weight gain, specific growth rate and feed conversion rate ($P<0.05$). It was found that the GSP0.1 and GSP0.3 groups had significant improvements in final body weight, weight gain, and specific growth rate when compared with NC group ($P<0.05$). And Shrimp FCR were significantly lower in the GSP0.1, GSP0.3, and GSP0.5 groups compared with the NC group ($P<0.05$).

3.2 Whole shrimp proximate composition

Table 4 showed how ginseng saponins affect the body composition of *L. vannamei* after addition of ginseng saponins to diets in low-fishmeal. The crude lipid of shrimp in the GSP0.3 group increased after ginseng saponins were added, and there was no significant difference between the GSP0.3 group and the PC group. And moisture and crude protein contents were not significantly different between groups.

3.3 Hepatopancreas morphology

Figure 1 showed the hepatopancreas tissue sections of *L. vannamei*. Shrimp in the GSP0.3 group had better hepatopancreatic health than shrimp in any other groups based on the hepatopancreas microsomal compactness, basement membrane integrity, and regularity of stellate duct lume. Hepatopancreatic histomorphology was improved by the addition of ginseng saponins compared to NC group.

3.4 Antioxidant enzyme activities

According to Table 5, ginseng saponins added to low-fishmeal diets increased antioxidant capacity of *L. vannamei*'s hepatopancreas. Compared to NC, GSP0.1, GSP0.3, and GSP0.5 groups had significantly lower MDA levels in the hepatopancreas ($P<0.05$). There was a significant difference between the GSP0.3, GSP0.1, and GSP0.5 groups in terms of hepatopancreatic MDA levels ($P<0.05$). Compared with the other groups, the GSP0.3 group shown significantly higher activities of T-AOC, SOD, and GSH-Px activities ($P<0.05$). With SOD and T-AOC activities in hepatopancreas were significantly higher in GSP0.1 and GSP0.5 than in NC ($P<0.05$).

3.5 Antioxidant gene expression

Figure 2 showed the expression of the hepatopancreatic antioxidant genes in *L. vannamei*, after the addition of ginseng saponins. A significant difference was found between the PC and NC groups in terms of the relative expression level of *sod* gene in the GSP0.3 group ($P<0.05$). Compared to the NC group, the *cat* gene expression level in GSP0.3 was significantly higher ($P<0.05$). It was significant that the relative expression of *gsh-px* gene was higher than that of PC and NC groups ($P<0.05$).

3.6 Survival rate after ammonia nitrogen stress

In Figure 3, compared to the low-fishmeal control group NC, the survival rate of *L. vannamei* after ammonia nitrogen

TABLE 3 Effect of the addition of ginseng saponins at low-fishmeal level on growth performance and feed utilization of *L. vannamei*.

Items	PC	NC	GSP0.1	GSP0.3	GSP0.5
IBW (g)	1.09 ± 0.006	1.08 ± 0.011	1.09 ± 0.005	1.10 ± 0.006	1.08 ± 0.011
FBW (g)	26.47 ± 0.46^{c}	21.28 ± 0.52^{a}	23.87 ± 0.39^{b}	24.99 ± 0.67^{b}	21.90 ± 0.13^{a}
FI (g/shrimp)	31.71 ± 0.34	31.23 ± 0.74	29.21 ± 2.11	29.38 ± 0.32	29.59 ± 0.25
SR (%)	98.33 ± 0.96	97.78 ± 2.22	96.67 ± 1.92	98.89 ± 1.11	95.00 ± 0.96
WG (%)	2340.04 ± 41.16^{c}	1871.44 ± 29.46^{a}	2096.07 ± 31.06^{b}	2173.15 ± 72.28^{b}	1925.48 ± 14.90^{a}
SGR (%/d)	5.70 ± 0.03^{c}	5.32 ± 0.03^{a}	5.52 ± 0.03^{b}	5.58 ± 0.06^{b}	5.37 ± 0.01^{a}
FCR	1.25 ± 0.01^{a}	1.55 ± 0.02^{c}	1.28 ± 0.09^{a}	1.23 ± 0.03^{a}	1.42 ± 0.01^{b}

Values are expressed as the means ± SEM with 4 replicates (n=4). Values in the same row indicate significant differences ($P<0.05$). IBW, initial body weight; FBW, final body weight; FI, food intake; SR, survival rate; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio. Different letters indicate significant differences ($P<0.05$).

stress, which was shown to have been improved significantly with 0.3% of ginseng saponins added ($P<0.05$). In addition, there were no significant differences between the GSP0.1, GSP0.3, and GSP0.5 groups and the PC group in terms of *L. vannamei* survival rate.

3.7 Gene expression after ammonia nitrogen stress

According to Figures 4, 5, the relative expression of antioxidant, and apoptosis genes were altered in *L. vannamei* under ammonia

TABLE 4 Effect of the addition of ginseng saponins at low-fishmeal level on the whole body composition of *L. vannamei* (% dry weight).

Parameters (% dry matter)	PC	NC	GSP0.1	GSP0.3	GSP0.5
Moisture	75.74 ± 0.74	77.01 ± 1.06	77.90 ± 0.54	77.26 ± 0.48	76.82 ± 0.49
Crude protein	14.04 ± 0.75	14.41 ± 1.06	14.05 ± 0.55	13.27 ± 0.28	13.08 ± 0.90
Crude lipid	1.18 ± 0.15^{b}	0.79 ± 0.07^{a}	0.80 ± 0.04^{a}	0.97 ± 0.02^{ab}	0.72 ± 0.08^{a}
Ash	3.23 ± 0.11	3.00 ± 0.05	2.99 ± 0.08	3.15 ± 0.06	3.23 ± 0.08

Values are expressed as the means ± SEM with 4 replicates (n=4). Values in the same row indicate significant differences ($P<0.05$). Different letters indicate significant differences ($P<0.05$).

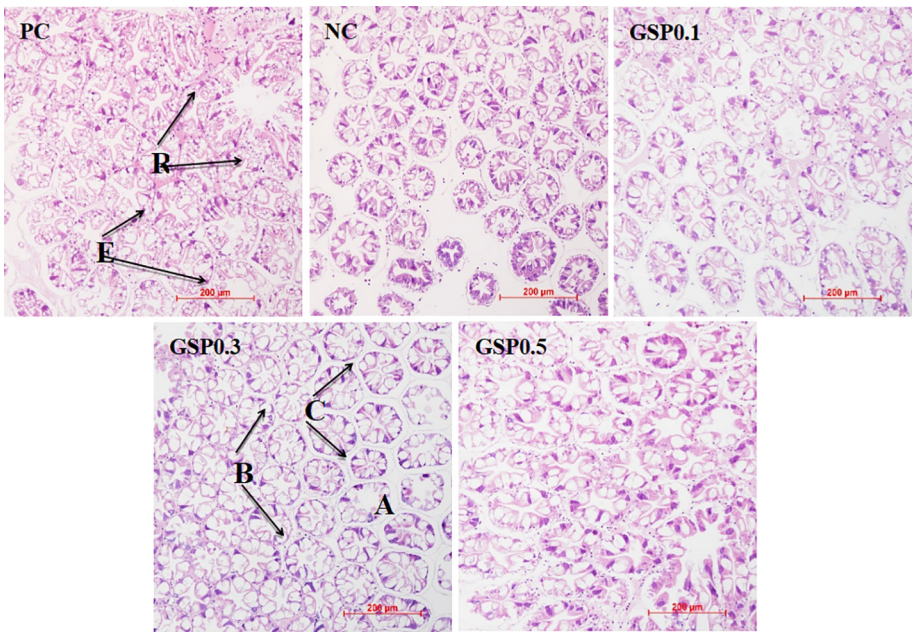

FIGURE 1
Effect of the addition of ginseng saponins at low-fishmeal level on the hepatopancreas morphology of *L. vannamei*. Scale bar: 200 μm. Magnification: 20×.

TABLE 5 Effect of the addition of ginseng saponins at low-fishmeal level on the hepatopancreatic antioxidant enzyme activities of *L. vannamei*.

Items	PC	NC	GSP0.1	GSP0.3	GSP0.5
MDA (nmol·mgprot^{-1})	0.75 ± 0.03^{a}	1.83 ± 0.06^{d}	1.48 ± 0.07^{c}	0.69 ± 0.05^{a}	0.97 ± 0.13^{b}
T-AOC (mmol·gprot^{-1})	0.53 ± 0.06^{ab}	0.34 ± 0.01^{a}	0.74 ± 0.08^{b}	1.51 ± 0.11^{c}	0.66 ± 0.06^{b}
SOD (U·mgprot^{-1})	1.61 ± 0.13^{b}	0.98 ± 0.09^{a}	1.71 ± 0.04^{b}	2.70 ± 0.16^{c}	1.50 ± 0.09^{b}
GSH-Px (U·mgprot^{-1})	635.13 ± 4.28^{b}	544.03 ± 9.16^{a}	596.67 ± 22.3^{ab}	731.81 ± 28.8^{c}	566.76 ± 11.57^{a}

Values are expressed as the means ± SEM with 4 replicates (n=4). Values in the same row indicate significant differences (P<0.05). MDA, malondialdehyde; T-AOC, total antioxidant capacity; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase. Different letters indicate significant differences (P<0.05).

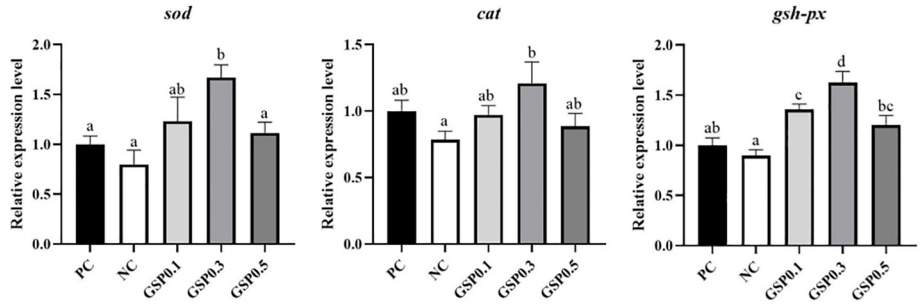

FIGURE 2
Effect of the addition of ginseng saponins at low-fishmeal level on the expression of antioxidant genes in the hepatopancreas of *L. vannamei*. Data represent the means ± SEM (n = 4). Different letters indicate significant differences (P<0.05).

nitrogen stress. The relative expression levels of *sod*, *cat*, and *gsh-px* genes were significantly higher in the GSP0. 3 group than in the other groups ($P<0.05$). After the addition of ginseng saponins, there was a significant increase in *p53* gene relative expression in the GSP0.3 group compared to other groups ($P<0.05$), and in the GSP0.3 group, *caspase3*, *bcl-2*, and *bax* genes shown significantly higher expression levels than those in other groups ($P<0.05$).

4 Discussion

As far as growth performance is concerned, *L. vannamei* in the 0.1% and 0.3% ginseng saponins-supplemented groups outperformed the low-fishmeal negative control. Additionally, the growth performance of *L. vannamei* in the 0.3% ginseng saponins-supplemented groups was highly similar to that of the high-fishmeal positive control. *L. vannamei* increased growth performance by adding 0.1% to 0.3% ginseng saponins to low-fishmeal diets, which was close to the PC group. Ginseng saponins may be one of the most important ingredients in shrimp growth. In the similar studies, that triterpenoid, *Quillaja* saponins has a favorable effect on the growth performance of *Cyprinus carpio* and *Oreochromis niloticus* (Francis et al., 2002, 2001). Furthermore, no significant differences in whole shrimp proximate composition were found between the PC group and the low-fishmeal diets supplemented with ginseng saponins.

There are many different kinds of organisms that produce SOD, an enzyme that scavenges superoxide radicals in their cytosol (Chen et al., 2013; Wang et al., 2015). In shrimp hepatopancreas, the MDA content was not significantly different between the 0.3% ginseng saponins-added diet and the 20% fishmeal-added diet, and was significantly lower than the other three treatment groups, and a significantly higher level of enzyme activity was observed in the SOD, T-AOC, and GSH-Px than in the PC group. These results showed that addition of the 0.3% ginseng saponins to a diet low in

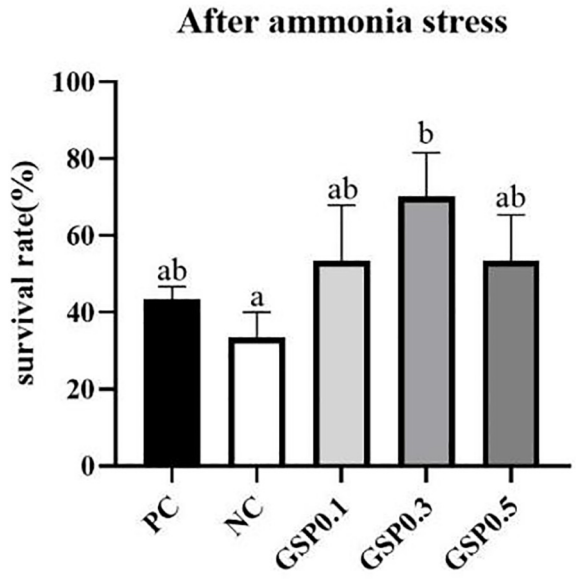

FIGURE 3
Effect of the addition of ginseng saponins at low-fishmeal level on the survival rate of *L. vannamei* under ammonia nitrogen stress. Data represent the means ± SEM (n = 4). Different letters indicate significant differences (P<0.05).

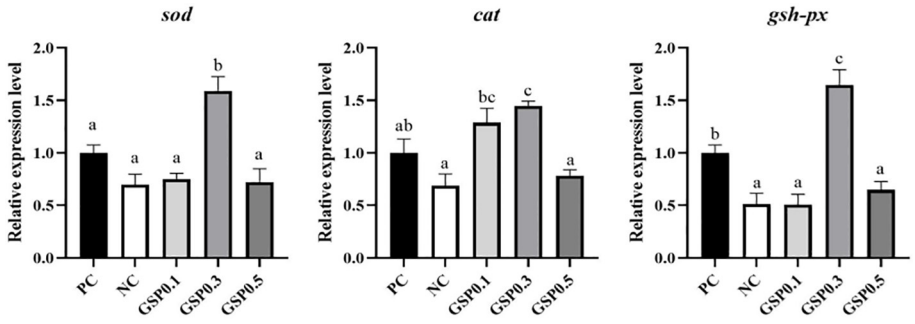

FIGURE 4
Effect of the addition of ginseng saponins at low-fishmeal level on the expression of antioxidant genes in the hepatopancreas of *L. vannamei* under ammonia nitrogen stress. Data represent the means ± SEM (n = 4). Different letters indicate significant differences (P<0.05).

fishmeal improved the antioxidant capacity of *L. vanname*. In related studies, ginseng polysaccharide complex has been shown to improve SOD activity in the hepatopancreas of *L. vannamei*, and to enhance antioxidant activity (Liu et al., 2011). Using the polysaccharide extract from *S. fusiforme*, *Vibrio harveyi* was inhibited in shrimp, *Fenneropenaeus chinensis*, and SOD activity in the shrimp's muscle increased (Huang et al., 2006). A significant increase in SOD activity and cyt-SOD mRNA expression has been observed in tiger shrimp, *Penaeus monodon*, after the addition of sodium alginate (Liu et al., 2006). On the other hand, the relative expression of *sod*, *gsh-px*, and *cat* in shrimp of the 0.3% ginseng saponins group was significantly higher than that of shrimp from the PC group in terms of relative expression, that indicated that adding ginseng saponins to low-fishmeal diets could enhance the antioxidant capacity of shrimp from *L. vannamei*. The study has found that the hepatopancreas mRNA level of *sod*, *cat*, and *gsh-px* can be improved by addition of 0.04% ginseng polysaccharide in diet of *L. vannamei* (Liu et al., 2011). Furthermore, ginseng

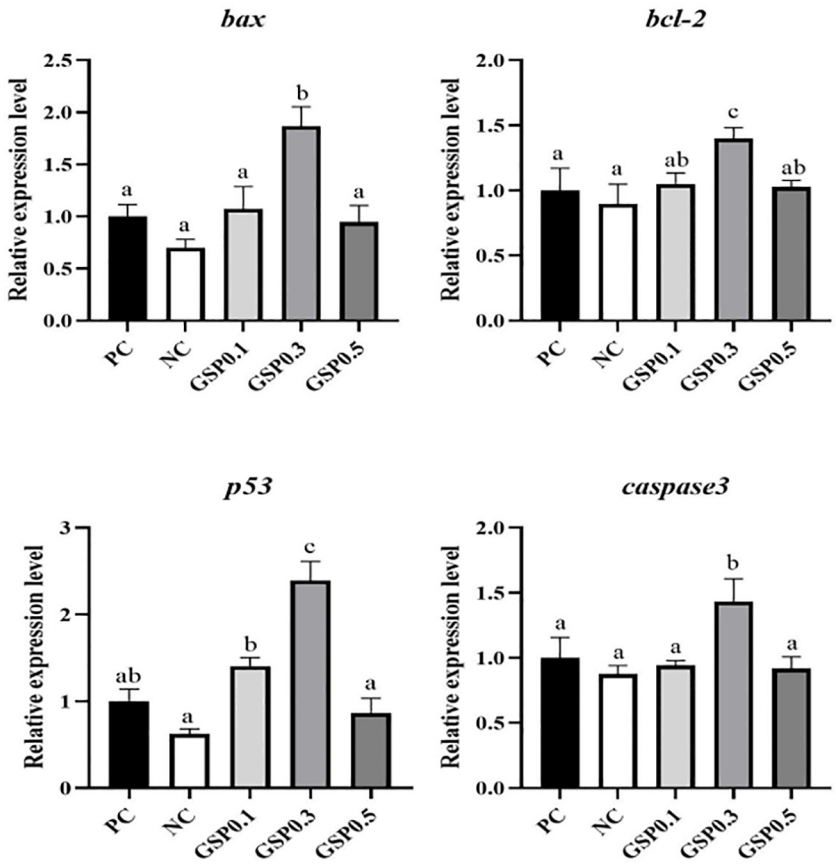

FIGURE 5
Effect of the addition of ginseng saponins at low-fishmeal level on the expression of apoptotic genes in the hepatopancreas of *L. vannamei* under ammonia nitrogen stress. Data represent the means ± SEM (n = 4). Different letters indicate significant differences (P<0.05).

saponins added to low fish meal diets improved the morphology of hepatopancreatic tissue of *L. vannamei*, with 0.3% ginseng saponins being more effective. It was found that under immune stimulation, ginseng saponins increase antioxidant genes expression in the liver of broilers, alleviate liver histology changes, inhibit inflammatory responses, and promote antioxidant activity (Hu et al., 2023). And the protodiol saponin ginsenoside Rk1, isolated from ginseng, has been suggested to have significant antitumor properties (Wu et al., 2024). These studies were consistent with the results of the present experiment. In shrimp, ginseng saponins were found to be beneficial in alleviating the effects of lipid peroxidation damage and improving their antioxidant capacity, especially when 0.3% ginseng saponins were added.

Shrimp is highly susceptible to ammonia stress. Not only does it damage their health, but it may also cause economic losses for aquaculture as well (Chen et al., 2019; Li et al., 2023). Aquatic animals' survival rate decreased as ammonia concentration and exposure time increased, but different species of aquatic animals tolerated ammonia differently (Frances et al., 2000; Kır et al., 2004; Wang and Walsh, 2000). An increase in the resistance of shrimp to ammonia stress was observed in those treated with immune potentiating agents, such as gracilaria tenuistipitata extract, astaxanthin and mannan oligosaccharide (Fu et al., 2007; Pan et al., 2003; Zhang et al., 2012). In terms of stress resistance, following ammonia-nitrogen stress, shrimp supplemented with 0.3% ginseng saponins survived just as well as shrimp in the high-fishmeal control group, as well as significantly more than shrimp in the low-fishmeal control group. A variety of environmental factors, including pH, nitrites, stress, and temperature, can increase oxidative stress and activate antioxidant genes expression (Guo et al., 2013; Wang et al., 2009; Zhou et al., 2008). In the present study, as with survival rate, the relative expression levels of antioxidant genes *sod*, *cat*, and *gsh-px* were significantly higher in shrimp from the low-fishmeal diet supplemented with 0.3% ginseng saponins than in shrimp from the high-fishmeal diet. A key component of the apoptosis process, *caspase3* cleaves a wide range of structural and regulatory proteins through its proteolytic activity (Chang et al., 2009). It is known that oxidative stress activates downstream target genes and induces apoptosis using *p53* as a tumor suppressor (Sun et al., 2016). Nitrite exposure has been shown to increase *Scylla paramamosain* and *Pelteobagrus fulvidraco caspase3* and *p53* expression in previous studies (Cheng et al., 2020; Zhang M. et al., 2020). Previous study had shown that sensitive to apoptosis genes loss increased pro-apoptotic *bax* and *bcl-2* imbalances in mitochondria. *Caspase9* and *caspase3* were activated, thereby promoting cell death (Chang and Ding, 2014). As for apoptosis-related genes, shrimp from the 0.3% ginseng saponins supplemented group showed significantly higher expression levels of *p53*, *caspase3*, *bcl-2*, and *bax* than shrimp from the PC group. As a result of these findings, shrimp stress resistance may be improved by adding 0.3% ginseng saponins to low-fishmeal diets. Ginsenoside Rk1 was found to promote autophagy-dependent apoptosis (Wu et al., 2024). The process of apoptosis is a kind of programmed cell death that is managed by genes. Upregulation of apoptosis may signal that the cells in an organism are still within control. This suggested that shrimp were becoming more stress resistant due to the upregulation of apoptosis genes.

5 Conclusion

In this experiment, different amounts of ginseng saponins were added to high percentage fishmeal replacement diets of *L. vannamei* to investigate the effects of ginseng saponins on the nutritional physiology of *L. vannamei* fed with low fish meal diets. Overall, ginseng saponins, as a natural additive, has a number of positive effects on aquatic animal nutrition, including promoting growth performance, improving antioxidant capacity, and improving anti-stress ability. Ginseng saponins has an extensive application potential in the aquatic feed industry because of its unique characteristics.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and approved by Experimental Animal Ethics Committee of Sun Yat-sen University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SL: Writing – original draft, Visualization, Formal analysis, Conceptualization. RY: Writing – review & editing, Investigation, Formal analysis. XC: Writing – review & editing, Investigation. YG: Writing – review & editing, Investigation. DH: Writing – review & editing, Resources, Conceptualization. BZ: Writing – review & editing, Resources, Conceptualization. ZZ: Writing – review & editing, Resources, Conceptualization. XH: Writing – review & editing, Resources, Conceptualization. ZL: Writing – review & editing, Resources, Conceptualization. BT: Writing – review & editing, Supervision, Project administration. JN: Writing – review & editing, Supervision, Project administration.

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Conflict of interest

Authors DH, BZ, and ZZ were employed by Hunan Micrograss Biotechnology Co., Ltd.

The remaining 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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