Effect of Different Dietary Selenium Sources on Growth Performance, Antioxidant Capacity, Gut Microbiota, and Molecular Responses in Pacific White Shrimp Litopenaeus vannamei

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This study investigated the effect of different dietary selenium (Se) sources on the growth performance, antioxidant capacity, gut microbiota, and molecular responses of the Pacific white shrimp Litopenaeus vannamei. Four Se sources (sodium selenite, L-selenomethionine, selenium yeast, or Se nanoparticles) were added to purified diets to 0.4 mg Se/kg diet for shrimp (1:60 ± 0:14 g). Each treatment was randomly assigned to 3 replicated tanks and 30 shrimp in each tank (500 L). After 8 weeks of breeding, L-selenomethionine and selenium yeast significantly increased weight gain compared with sodium selenite treatment, while sodium selenite significantly decreased the shrimp hepatosomatic index compared with the other groups. The L-selenomethionine significantly increased the superoxide dismutase and glutathione peroxidase activities in the hepatopancreas compared with the shrimp fed sodium selenite and decreased catalase activity and malondialdehyde content compared with other groups. The composition and β-diversity of gut microbiota were markedly changed in each group. The abundances of Rubrobacter and Rubritalea, Winogradskyella and Motilimonas, and Photobacterium in the gut microbiota were specially altered by L-selenomethionine, Se yeast, and Se nanoparticles, respectively. The sodium selenite group showed lower complexity of gut interspecies interactions. RNA-seq analysis showed that “arachidonic acid metabolism”-related genes were significantly enriched in the L-selenomethionine and Se yeast groups; “peroxisome” and “drug metabolism—other enzymes”-related genes were enriched in the Se nanoparticle group. Vibrio, Motilimonas, and Photobacterium were associated with amino acid and lipid metabolism. Pseudoalteromonas, Silicimonas, Roseovarius, and Halomonas inhibited the expression of glutathione peroxidase genes. These results suggested that organic Se, especially selenomethionine, is an effective feed supplement to promote growth and antioxidant capacity, maintain the health of gut microbiota, and promote the utilization of fatty acid and glutathione peroxidase genes in shrimp fed a 0.4 mg Se/kg diet.

1. Introduction

Selenium (Se) is a micronutrient that maintains physiological activities in animals. Several selenoproteins and selenoenzymes are biosynthesized through selenocysteine to protect cells and cellular components from oxidative damage [1–3]. In nature, aquatic animals usually obtain Se from the environment (algae, substrate, and water), while in intensive aquaculture systems, animals depend on Se dietary intake [4]. The optimum selenium content range for aquatic animals is 0.2–1.8 mg Se/kg diet [5, 6]. Se deficiency or excessive supply can reduce antioxidant ability and growth
that the optimal Se levels for farmed around the world. At present, it has been reported stress in microbiota were used to explore the physiological regulation, and hepatopancreas transcriptomics and gut mechanism of Se sources in metabolism has little informa-

Higher dietary levels of Se can inhibit cell protein syn-

L. vannamei performance and weaken the immunity of 0.4 mg Se/kg [6]. Dietary Se de-

Yeasts at 1.0 mg Se/kg [18]. The existing results on the function composition on the breeding of the Chinese mitten crab (Erio-

ff) was similar to that in feed supplemented with Se yeast at 1.0 mg Se/kg [18]. The existing results on the function vary between Se sources. Therefore, there is a need to identify the most suitable Se source for different species.

Pacific white shrimp (Litopenaeus vannamei) are widely farmed around the world. At present, it has been reported that the optimal Se levels for L. vannamei growth are 0.2–0.4 mg Se/kg [6]. Dietary Se deficiency can reduce growth performance and weaken the immunity of L. vannamei [19]. Higher dietary levels of Se can inhibit cell protein synthesis, induce cell damage, and reduce resistance [20, 21]. Appropriate Se supplementation promotes growth, antioxidant performance, tissue repair, and amino acid utilization and reduces the adverse effect on endoplasmic reticulum stress in L. vannamei [22]. However, the physiological mechanism of Se sources in metabolism has little information. In this study, four Se sources were used in diet formulation, and hepatopancreas transcriptomics and gut microbiota were used to explore the physiological regulation and biomarkers of the Se source in L. vannamei.

2. Materials and Methods

2.1. Diet Preparation. Isonitrogenous and isolipidic purified diets (containing approximately 423.2 g/kg crude protein and 74.4 g/kg crude lipid) were formulated. Casein and gelatin were used as the sources of protein, fish oil, soybean oil, soybean lecithin, and cholesterol as the sources of lipids. The basal diet was formulated with a Se-free mineral premix (Table 1). Four Se sources, such as sodium selenite (Na2SeO3, Nanjing Reagent), L-selenomethionine (SeMet, Rhawn), selenium yeast (Se yeast, 2000 mg Se/kg, Angel yeast Co., Ltd.), and Se nanoparticles (SeNPs, Bowei Nano Technology Co., Ltd.), were accurately weighed and thoroughly mixed to homogeneity with 100 mL distilled water and then added to the basal diet at 0.40 mg Se/kg, which was found to be optimal for the growth of L. vannamei [6, 22]. The Se content of feeds was determined by hydride atomic fluorescence spectrometry (GB/T 13883-2008, National Standard of the People’s Republic of China). After each feed was mixed evenly, 100 g was randomly taken and repeated twice by using an atomic fluorescence photometer (AFS-9230, Titan Co, Beijing, China). The average of the two results is the actual Se content of this feed. The actual Se levels in the diet supplemented with sodium selenite, L-selenomethionine, selenium yeast, and Se nanoparticles were 0.42, 0.37, 0.45, and 0.45 mg/kg diet, respectively. All dry ingredients were crushed, weighed, blended, sifted through 60 mesh screens, mixed with oils and deionized water accurately (200 mL/kg diet), and extruded into pellets (2 mm

Table 1: Composition and nutrient levels of the basal diet.

| Ingredients       | Content (%) |
|-------------------|-------------|
| Crude protein     | 42.32       |
| Total lipid       | 7.44        |
| Ash               | 1.25        |
| Moisture          | 9.81        |
| Gross energy (kJ/g) | 16.09 |

*Casein: crude protein ≥ 92%, crude lipid ≤ 2%, Hua Ling Dairy Corporation, Lanzhou, China. **Vitamin premix (per kg of diet): vitamin A: 4800 IU; L-ascorbyl-2-polyphosphate 35% active C: 35.71 g; folic acid: 0.18 g; biotin: 0.05 g; riboflavin 3 g; DL Ca-pantothenate L: 5 g; pyrodoxine HCl B6: 1 g; vitamin B12: 0.002 g; thiamin HCl: 0.5 g; menadione K3: 2 g; DL-alpha-tocopheryl acetate: 20 IU; inositol: 5 g; nicotinamide: 5 g; vitamin D: 8000 IU. **Mineral premix (per kg of diet): ZnSO4·H2O: 20.585 g; Ca(IO3)2: 0.117 g; CuSO4·5H2O: 0.625 g; MnSO4·H2O: 1.625 g; MgSO4·H2O: 59.86 g; CoCl2·6H2O: 0.01 g; FeSO4·H2O: 11.179 g; CaHPO4·2H2O: 166.442 g. **Gross energy was calculated using the following factors: protein, 23 kJ/g; lipid, 35 kJ/g; carbohydrates, 15 kJ/g [67].
diameter) using a pelleting machine. The pellets were air-dried in a cool place to 10% moisture and then stored in a −20°C refrigerator.

2.2. Feeding Trail and Sample Collection. The shrimp larvae were obtained from a shrimp hatchery in Wenchang, Hai-nan, China. In the acclimatization period, 600 shrimp were bred in 200 L seawater in cylindrical fiberglass tanks (500 L). Half of the seawater (100 L, 31% salinity) was refreshed at 10:00 daily. The water environment was 28 ± 2 °C, pH 7.5–8.0, and total ammonia < 0.05 mg/L. Commercial feed was used during the acclimation period. Impurities in the water were discharged with seawater. After 2 weeks of acclimatization, healthy and similarly sized shrimp (1.60 ± 0.14 g) were randomly divided into 4 groups (3 replicates stocked with 30 shrimp per tank). Dietary 4% of the body weight was fed evenly at four times daily (08:00, 12:00, 18:00, and 22:00) by feeding experimental feed. Other breeding regimens were the same as the acclimatization period.

On the last day in eight weeks, all shrimp were fasted. All shrimp body and hepatopancreas weights were measured in each tank. All shrimp were anesthetized in an ice slurry immediately, and then, hepatopancreas and midgut samples were aseptically extracted, placed into 1.5 mL sterile centrifuge tubes, and stored at −80°C.

The growth parameters were obtained using the following equations:

\[
\text{Survival} (\%) = 100\% \times \text{final shrimp number}/\text{initial shrimp number.}
\]

\[
\text{Weight gain} (\%) = \left( \frac{\text{final body weight (g)} - \text{initial body weight (g)}}{\text{initial body weight (g)}} \right) \times 100\%.
\]

\[
\text{Hepatosomatic index} (\%) = 100\% \times \frac{\text{hepatopancreas weight}}{\text{final body weight}}.
\]

\[
\text{Condition factor} (\%) = 100\% \times \frac{\text{final weight (g)}}{(\text{body length (cm)})^3}.
\]

2.3. Feed and Body Composition Analysis. Standard methods [23] were used to detect the moisture, crude protein, total lipid, and ash contents of the 12 diets and 12 shrimp bodies from three random replicates per treatment. These shrimp were dried to a constant weight at 105°C (WFO-520, EYELA, Tokyo, Japan) and stored in a desiccator. The crude protein content was determined using a Dumas nitrogen analyzer (Elementar Rapid N Excess, Frankfurt, Germany). Total lipid content was obtained using the Soxhlet method. The ash content was determined after burning at 550°C from 10 h in a muffle furnace.

2.4. Antioxidant Enzyme Analysis. Hepatopancreases (total number = 24) from 6 random replicates per group were weighed and homogenized at a frequency of 60 Hz at 4°C for 60 s (Tissuelyser-24, Shanghai Jingxin Technology, Shanghai, China) in 0.86% saline solution and centrifuged at 2000 rpm at 4°C for 5 min (3-18 KS, Sigma, Germany). The supernatant was obtained to determine the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA) content, and total protein concentration in 6 hepatopancreases per treatment. All biochemical parameters were determined using assay kits (Jian-cheng Bioengineering Institute, Ltd., Nanjing, China). The testing methods were carried out in accordance with the instructions provided by the manufacturer. Specific experiment method referred to Yu et al., [22].

2.5. Gut Microbiota Analysis

2.5.1. DNA Extraction and Illumina MiSeq Sequencing. Microbial DNA was extracted from gut content samples from three random replicates per treatment using the E.Z.N.A.™ soil DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.) according to the manufacturer’s protocols. The DNA concentration, quality, PCR program, and purified DNA were determined according to previous procedures [24]. Sequencing was performed on an Illumina MiSeq platform (Illumina, San Diego, USA). The sequences obtained are available in the GenBank database (SRP310044).

2.5.2. Comparative Analysis of Bacterial Composition and Differences. Alpha diversity indexes were detected by using Mothur version 1.30. Beta diversity indexes were calculated using QIIME software. Nonmetric multidimensional scaling (NMDS) and analysis of molecular variance (AMOVA) were performed to evaluate the overall differences in bacterial community structure among groups (beta diversity) based on the Bray–Curtis distance. A Venn diagram was constructed to identify the shared and unique OTUs. Linear discriminant analysis (LDA) with LEfSe was applied to identify statistically significant biomarkers among groups using the nonparametric factorial Kruskal–Wallis (KW) sum-rank test. All-against-all strategies were used to compare significant differences among groups.

2.5.3. Function and Correlation Analysis. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) normalized the Operational Taxonomic Units (OTU) table. The Kyoto Encyclopedia of Genes and Genomes Ortholog (KO) information corresponding to OTUs was obtained through the greengene id corresponding to each OTU. According to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, the abundance of three levels of metabolic pathway information was obtained. The Spearman correlation coefficients between the top 30 most abundant genera were calculated by Mothur software. The interspecies interaction network was constructed with the absolute value of correlation coefficient ≥ 0.99 and P < 0.05. Gephi software was used to create visual networks.

2.6. Transcriptome Analysis

2.6.1. Total RNA Extraction and Sequencing. Total RNA isolation and sequencing were performed according to a previous procedure [22]. RNA from the hepatopancreas of 3 shrimp per group (total n = 12) was extracted. The cDNA library was sequenced by an Illumina HiSeq™ 4000 instrument. The raw sequencing data were deposited in the National Center for Biotechnology Information (NCBI) search database (SRP312261).
2.6.2 Differentially Expressed Genes (DEGs) and Functional Enrichment. To identify the DEGs among the three groups, the Na₂SeO₃ group was used as the control group. The expression of unigenes was calculated using the fragments per kilobase of transcripts per million fragments mapped (FRKM). RSEM [25] was conducted using the R statistical package software edgeR. A false discovery rate (FDR) < 0.05 was used as the threshold to judge the significant difference among gene expression levels. The P value was adjusted using Benjamini and Hochberg’s approach (BH) to control the false discovery rate [26]. The false discovery rate < 0.01 and fold change > 2 were considered significantly different. The KEGG pathway software KOBAS 3.0 was used for the analysis. Fisher’s exact test was used to identify the significant KEGG pathways (P ≤ 0.05).

2.7 Statistical Analysis. All data were calculated as the means ± standard errors of the mean (SEM). After using box plot analysis to filter out the outliers of each group of data, all the filtered data were tested to confirm the normal distribution and the homogeneity of variance. One-way analysis of variance (ANOVA) was used to test the main effects of different diets, using Tukey’s honestly significant difference (HSD) test as a post hoc test by using SPSS 22.0 software (SPSS Inc., Chicago, USA). Spearman correlation analysis was used to reveal the association between gut microbiota and metabolism in the hepatopancreas. Spearman correlation analysis was employed using the Cytoscape software coNet plug-in to reveal the correlation between gut bacteria and the DEGs of the hepatopancreas of shrimp. * P < 0.05, ** P < 0.01, and *** P < 0.001 were regarded as statistically significant, very significant, and extremely significant, respectively.

3. Results

3.1 Growth Performance and Body Composition. The weight gain, condition factor, and hepatosomatic index of shrimp
fed the four diets are shown in Figure 1. The SeMet and Se yeast groups showed significantly higher weight gain than the Na$_2$SeO$_3$ group ($P < 0.05$), while the Na$_2$SeO$_3$ group displayed a significantly lower hepatosomatic index than the other groups. The condition factors among the four groups were not significantly different ($P > 0.05$).

Composition of shrimp body is shown in Table 2. There were no significant differences in each item among all groups ($P > 0.05$).

3.2. Antioxidant Enzyme Activity. The activities of SOD and GPx in the SeMet group were higher ($P < 0.05$) than those in the Na$_2$SeO$_3$ group (Figures 2(a) and 2(c)). The CAT activity in the SeMet group was significantly lower ($P < 0.05$) than that in the other groups (Figure 2(b)). The MDA content in the SeMet group was significantly lower ($P < 0.05$) than that in the Na$_2$SeO$_3$ and SeNP groups (Figure 2(d)).

3.3. Gut Microbiota Analysis

3.3.1. Richness and Diversity. A total of 692,237 clean reads were determined from all experimental groups. Alpha diversity analysis showed that no significant change ($P > 0.05$) was found in the Ace, Chao, Shannon, and Simpson indexes (Table 3). The Venn diagram showed that 89 OTUs were shared by all samples, and 8, 16, 7, and 18 OTUs existed in the Na$_2$SeO$_3$, SeMet, Se yeast, and SeNP groups, respectively (Figure 3(a)). Nonmetric multidimensional scaling (NMDS) analysis determined that the gut microbiota in the 4 groups was clearly separated (Figure 3(b)). The farthest distance between the SeNP group and the Se yeast group demonstrated that the gut microbiota of shrimp in these 2 groups was clearly separated.

3.3.2. Gut Microbiota Composition. At the phylum level, the highest relative abundance of Proteobacteria was found in the SeNP group, and the highest relative abundance of Actinobacteria was found in the Se yeast group (Figure 3(c)). At the genus level, Demequina and Photobacterium were the dominant bacteria. The relative abundance of Photobacterium in the SeMet and SeNP groups was significantly higher ($P < 0.05$) than that in the Na$_2$SeO$_3$ and Se yeast groups. In the top 50 genera, Filomicrobium, Winogradskyella, family Rhodobacteraceae, and Sva0996 marine group were significantly changed ($P < 0.05$) in the different groups (Figure 3(d)).

3.3.3. LEfSe, KEGG Functional Prediction, and Correlation Analysis. The LEfSe results showed that bacterial taxa at the genus level (Rubrobacter and Rubritalea,
Figure 3: Continued.
of clean reads was 88.07% and 85,484,798,897 clean bases produced. The mapping rate was 87,579,994,564 raw bases, with 574,854,768 total clean reads. Generated 579,999,964 total raw reads and increased compared with the Na$_2$SeO$_3$ group. The analysis of the 4 treatments (total n = 3). Hepatopancreas Transcriptome Analysis. RNA-seq analysis of shrimp hepatopancreases generated 579,999,964 total raw reads and 87,579,994,564 raw bases, with 574,854,768 total clean reads and 85,484,798,897 clean bases produced. The mapping rate of clean reads was 88.07%–89.48% (Table 4).

Compared with the Na$_2$SeO$_3$ group, a total of 1,397 DEGs were identified in the hepatopancreases in the other three groups. Specifically, the SeMet group contained 257 upregulated and 316 downregulated genes, the Se yeast group contained 189 upregulated and 156 downregulated genes, and the SeNP group contained 481 upregulated and 302 downregulated genes. The dominant KEGG pathways of the SeMet, Se yeast, and SeNP groups are shown in Figure 5. More enriched pathways of SeMet were "arachidonic acid metabolism," "linoleic acid metabolism," "cholesterol metabolism," and "phosphonate and phosphinate metabolism;" those of the Se yeast group were "protein digestion and absorption," "folate biosynthesis," and "arachidonic acid metabolism;" and those of the SeNP group were "peroxisome," "drug metabolism–other enzymes," and "choline metabolism in cancer."

3.5. Correlation between the Gut Microbiota and Metabolism-Related DEGs. In bacteria that were significantly correlated with lipid metabolism-related DEGs (Figure 6(a)), Vibrio was negatively correlated with changes in the cyp2d, pcyt1, ggt1_5, gpcpd1, cyp2j, bdh, lypla3, and enpp6 genes, and Photobacteria was negatively correlated with changes in the scd, ugt-2b19, hadha, ugt, sc5dl, and ugt-2c1 genes. Motilimonas was positively correlated with changes in the ugt-2c1, ugt-2b18, ugt-2b19, and sc5dl genes. The correlations between gut microbiota and amino acid and glutathione metabolism-related DEGs are shown in Figures 6(b) and 6(c). The descriptions of DEGs related to amino acid and lipid metabolism are shown in Table 5.

4. Discussion

4.1. Growth Performance. Dietary Se additives significantly affect the weight gain of aquatic animals [27, 28]. In general, the bioavailability of organic Se was higher than that of inorganic forms [29, 30]. Similar results were also observed in human studies [31]. Indeed, in this study, organic Se (L-selenomethionine and Se yeast) significantly increased weight gain compared to the inorganic source sodium selenite. In the case of L-selenomethionine, it can be used to substitute methionine, if limited or catabolized [32]. Thus, Se bioavailability depends not only on its absorption by the gut but also on its conversion to a biologically active form. This may be
Figure 4: Network analysis of the effect of different dietary selenium sources on gut microbiota in *L. vannamei*. Each node represents a genus. Node colors indicate genera affiliated with different major phyla. The size of each node represents the abundance of the genus. The red edge indicates a positive interaction, whereas the green edge indicates a negative interaction between two individual nodes. (a) Na$_2$SeO$_3$ group; (b) SeMet group; (c) Se yeast group; (d) SeNP group; (e) interaction types, amount, and the ratio of negative interactions in the ecological network; (f) microbial metabolism prediction based on KEGG pathway analysis. *Indicates a significant difference ($P < 0.05$) among groups.
4.3. Gut Microbiota in Response to Different Se Sources. The Se can affect barrier function and immune responses in the gut [39]. Previous studies have mainly focused on the role of Se itself or its metabolites [40], while the influence of the Se intake-related microbiota on gut health has not been thoroughly studied. In this study, the Se source altered the abundances of dominant phyla (Proteobacteria and Actinobacteria) and genera (Rubrobacter, Rubritalea, Winogradskyella, Motilimonas, and Photobacterium) in the gut. Rubritalea can produce antioxidative Glyco-C30-carotenoids and show potent antioxidative activity [41], and its abundance in the sea cucumber (Apostichopus japonicus) gut at 26°C is higher than that at 10°C [42]. Motilimonas abundance decreases when marine medaka (Oryzias melastigma) is exposed to polystyrene microplastics [43]. Most Photobacterium species act as mutualistic bacteria to assist chitin digestion in the host gut [44], but some species (P. damselae subsp. damselae and P. damselae subsp. piscicida) produce neuraminidases [45]. The Photobacterium has been identified in the fish gut microbiota that aids digestion [46]. Therefore, Se may reshape gut digestion by affecting the abundance of these bacteria, thus improving the digestion and absorption of nutrients in the gut. However, their contribution to the growth performance or gut health of shrimp remains unclear.

The gut microbiota is a complex ecological network due to its diversity and richness of bacterial species. Various interactions, such as cooperation, competition, and predation, occur in this network [47], and the positive and negative links present cooperative and competitive interactions [48]. The complexity of the gut microbiota is compromised in unhealthy shrimp [49]. In this study, the SeMet group had the most complex network, and the SeNP group had the maximal negative rate, but the SeNP group had a minimal number of links. Link and negative rates are considered signs of complexity of the gut microbiota [50]. A reduction in complexity is prone to invasion by external strains [51]. The networks in the SeMet and SeNP groups were more complex than those in the Na2SeO3 and Se yeast groups, but the relationship between gut ecological networks and the health of shrimp still needs more research.

4.4. Hepatopancreas Transcriptome in Response to Different Se Sources. In this study, L-selenomethionine affected phosphonate and phosphinate metabolism, linoleic acid metabolism, and arachidonic acid metabolism pathways. Phosphonates and phosphinate are natural products that contain carbon-phosphorus bonds. The function of phosphate-containing macromolecules has not been well established in any organism, but they play an essential role in the organism that produces these molecules. It has been speculated that the nonhydrolyzable C-P bond can enhance the stability of lipids [52]. Linoleic acid is the raw material for the biosynthesis of arachidonic acid metabolism, and arachidonic acid alleviates osmotic shock in gilthead seabream Sparus aurata L. [53]. Biochemical and transcriptomic endpoints in rainbow trout following dietary Se exposure were determined, and molecular networks for lipid metabolism-related pathways were upregulated in the liver of fish fed 40 mg/kg Se [54]. Similarly, arachidonic acid metabolism was enriched in the Se yeast group, and gene enrichment analysis of the nutritional Se on energy metabolism in rainbow trout showed that many DEGs were associated with lipid metabolism pathways [55]. Therefore,
organic Se is involved in polyunsaturated fatty acid metabolism to regulate lipid metabolism.

The peroxisome is an organelle with a single-layer membrane structure that exists in eukaryotic cells. It plays a key role in fatty acid oxidation, phospholipid synthesis, and oxidation [56]. The Se can promote the peroxisome pathway to increase \(\beta\)-oxidation and nutrition regulation to protect the body from damage [57, 58]. The peroxisome pathway was significantly changed when black tiger shrimp *Penaeus monodon* were under ammonia stress [59]. The peroxisome pathway in the SeNP group was enriched in combination with the antioxidant capacity of the hepatopancreas, indicating that Se nanoparticles can cause a stress response in the hepatopancreas.

**Figure 5:** Bubble diagram of the top 20 enriched KEGG pathways of differentially expressed genes in the hepatopancreas of *L. vannamei*: (a) SeMet group; (b) Se yeast group; (c) SeNP group. The vertical axis indicates KEGG pathways, and the horizontal axis represents the rich factor. The enrichment degree was stronger with a larger rich factor. The size of dots indicates the number of genes in the KEGG pathways.
4.5. Correlation between Gut Microbiota and Metabolism.
The composition of nutrients, such as dietary Se, can affect the composition and colonization of gut microbiota [60]. In this study, Vibrio was correlated with lipid metabolism-related DEGs (cyp2d, pcyt1, ggt1_5, gpcpd1, cyp2j, bdh, lypla3, and enpp6) and amino acid metabolism-related DEGs (phnx, cad, aldh18a1, pcyt1, glna, ggt1_5, dot1_1, and tat). Vibrio is a major pathogen of aquatic animals that can affect the normal growth and health of shrimp [61]. The cyp2j (LOC113807693) and ggt1_5 (LOC113815919) genes were involved in arachidonic acid metabolism. Arachidonic acid has a positive effect on aquatic animals and can alleviate osmotic shock [53]. The genes pcyt1 (LOC113800913), lypla3 (LOC113805268), and gpcpd1 (LOC113808219) were involved in glycerophospholipid metabolism. Glycerophospholipids are the main component of the biological membrane [62]. Regulating arachidonic acid metabolism and glycerophospholipid metabolism can alleviate acute ulcerative colitis in mice [63]. In addition, phnx (LOC113815269) and pcyt1 were involved in phosphonate and phosphinate metabolism. The relatively low abundance of Vibrio in the SeMet group reveals that selenomethionine might decrease the abundance of Vibrio to maintain the structural integrity of the cell membrane and promote lipid metabolism. Motilimonas was positively correlated with the alanine, aspartate, and glutamate metabolism genes.

Figure 6: The correlation of the top 20 gut bacteria and differentially expressed genes. (a) The correlation between gut bacteria and amino acid metabolism-related differentially expressed genes; (b) the correlation between gut bacteria and lipid metabolism-related differentially expressed genes; (c) the correlation between gut bacteria and GSH metabolism-related differentially expressed genes represents significantly positive or negative correlations. *P < 0.05; **P < 0.01; ***P < 0.001.
Table 5: Descriptions of differentially expressed genes related to amino acid, lipid, and glutathione metabolism.

| Gene name | Gene ID | Description |
|-----------|---------|-------------|
| aadat     | LOC113823750 | Kynurenine/alpha-aminoadipate aminotransferase, mitochondrial-like |
| ache      | LOC113802319 | Carboxylesterase 4A-like |
| acox1     | LOC113829127 | Peroxisomal acyl-coenzyme A oxidase 1-like, transcript variant X1 |
| akr1b     | LOC113821670 | Aldose reductase-like |
| aldha18a1 | LOC113806982 | Delta-1-pyrroline-5-carboxylate synthase-like |
| aldhsa1   | LOC113826632 | Succinate-semialdehyde dehydrogenase, mitochondrial-like |
| amnep     | LOC113817626 | Aminopeptidase N-like |
| bbox1     | LOC113828035 | Gamma-butyrobetaine dioxygenase-like |
| bdh       | LOC113824573 | D-Beta-hydroxybutyrate dehydrogenase, mitochondrial-like |
| cad       | LOC113813772 | CAD protein-like |
| chat      | LOC113820314 | Choline O-acetyltransferase-like |
| chk       | LOC113813041 | Choline/ethanolamine kinase-like |
| csad      | LOC113825529 | Cysteine sulfenic acid decarboxylase-like |
| cyp2d     | LOC113807691 | Cytochrome P450 2L1-like, transcript variant X2 |
| cyp2j     | LOC113807693 | Cytochrome P450 2L1-like |
| ddc       | LOC113819272 | Aromatic-L-amino-acid decarboxylase-like |
| ddo       | LOC113799947 | D-aspartate oxidase-like |
| dmdgh     | LOC113817447 | Dimethylglycine dehydrogenase, mitochondrial-like |
| dot1      | LOC113817809 | Histone-lysine N-methyltransferase, H3 lysine-79 specific-like, transcript variant X1 |
| ehmnt     | LOC113814977 | Kinase D-interacting substrate of 220 kDa B-like |
| enpp6     | LOC113805716 | Ectonucleotide pyrophosphatase/phosphodiesterase family member 6-like |
| gadb      | LOC113810978 | Cysteine sulfenic acid decarboxylase-like |
| galst3    | LOC113806716 | Galactosylceramide sulfotransferase-like, transcript variant X1 |
| gcat      | LOC113802340 | 2-Amino-3-ketobutyrate coenzyme A ligase, mitochondrial-like |
| gftp      | LOC113808285 | Glutamine–fructose-6-phosphate aminotransferase [isomerizing] 2-like, transcript variant X1 |
| ggt1_5    | LOC113815919 | Glutathione hydrolase 1 proenzyme-like, transcript variant X1 |
| glna      | LOC113823145 | Glutamine synthetase-like |
| glpk      | LOC113822613 | Glycerol kinase-like |
| glsa      | LOC113818992 | Glutaminase liver isoform, mitochondrial-like |
| gpat3_4   | LOC113816633 | Glycerol-3-phosphate acyltransferase 4-like |
| gpcpd1    | LOC113808219 | Glycerophosphocholine phosphodiesterase GPCPD1-like, transcript variant X1 |
| gst       | LOC113817634 | Glutathione S-transferase T2-like |
| ilve      | LOC113821444 | Branched-chain-amino-acid aminotransferase, cytosolic-like |
| ldh       | LOC113809991 | L-lactate dehydrogenase-like, transcript variant X2 |
| lipa      | LOC113821164 | Lipase 3-like |
| lypla3    | LOC113805268 | Group XV phospholipase A2-like |
| metk      | LOC113800268 | S-Adenosylmethionine synthase-like |
| nmt       | LOC113819960 | Phosphomethylthanolamine N-methyltransferase-like, transcript variant X1 |
| ogdh      | LOC113812483 | 2-Oxoglutarate dehydrogenase, mitochondrial-like, transcript variant X1 |
| pcca      | LOC113807294 | Propionyl-CoA carboxylase alpha chain, mitochondrial-like |
| pcyt1     | LOC113800913 | Choline-phosphate cytidylyltransferase B-like, transcript variant X1 |
| phnx      | LOC113815269 | Phosphonoacetaldehyde hydrolase-like |
| pla2g     | LOC113820851 | Group 3 secretory phospholipase A2-like |
| pld3_4    | LOC113825199 | Phospholipase D3-like, transcript variant X1 |
| plpp1_2_3 | LOC113830003 | Phospholipid phosphatase 2-like |
| pnlip     | LOC113800560 | Pancreatic lipase-related protein 2-like |
| pnliprp3  | LOC113807506 | Inactive pancreatic lipase-related protein 1-like |
(aldh5a1, gst, ddo, and aadat). The expression of aldh5a1 (LOC113826632) reveals that Motilimonas may take advantage of Se yeast in the gut and affect amino acid metabolism in the hepatopancreas. Photobacterium was negatively correlated with DEGs (scd, ugt-2b19, hadha, scd, and ugt-2c1). Glucuronidation associated with uridine diphosphate-glucuronosyltransferase (ugt) enzymes is a major metabolic pathway that facilitates the elimination of a large variety of molecules into urine and bile by increasing their water solubility. The ugt2c1 gene expression is significantly downregulated when Gobiocypris rarus is exposed to tributylin [64]. Therefore, the above-downregulated genes in the SeNP group revealed that the toxicity level of Se nanoparticles to shrimp was 0.4 mg Se/kg.

In addition, the changes in Staphylococcus, Pseudoalteromonas, Silicimonas, Roseovarius, and Halomonas were negatively correlated with changes in selenoproteome GPx expression. These results suggest that Staphylococcus, Pseudoalteromonas, Silicimonas, Roseovarius, and Halomonas might inhibit the biosynthesis of GPx. Se-vitamin E injections can boost GPx activity and kill Staphylococcus aureus [65]. These changes in genera may not be conducive to the biosynthesis of the selenoproteome, but it may also be the result of Se regulating the body antioxidant capacity [66]. The mechanisms need to be explored.

5. Conclusion

In conclusion, organic Se is more beneficial to the growth and gut health of L. vannamei by improving antioxidant capacity, decreasing the abundance of potential pathogens, and regulating lipid and amino acid metabolism. In addition, Se nanoparticles can freely diffuse in the body, increase the intercellular free Se concentration, and finally damage the antioxidant system of shrimp. The interaction between gut microbiota and Se can synthesize GPx and promote the metabolism of lipids and amino acids. Organic Se is a healthy Se source that is more beneficial to shrimp growth and health.

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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