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Effects of Partial Substitution of Fish Meal with Soybean Products and Chicken Meal on Growth, Antioxidant Capacity and Intestinal Microbiota of *Penaeus monodon*

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Abstract: The aim of this experiment was to investigate the effects of the partial substitution of fish meal with soybean products and chicken meal on the growth performance, antioxidant capacity and intestinal microbiota of *Penaeus monodon*. A total of 450 healthy, consistent shrimp were randomly divided into five groups, with three replicates per group and 30 shrimp per replicate. The proportion of fish meal substituted with soybean products and chicken meal in the five feed groups was 0% (FM), 40% (40SC), 60% (60SC), 80% (80SC) and 100% (100SC). The experiment lasted for 8 weeks. The results showed that, compared to the FM group, the 40SC and 60SC groups had a decrease in WG and SR, but there was no significant difference ($p > 0.05$). In contrast, compared to the FM group, the FCR in the 100SC group was significantly increased ($p < 0.05$), while there was no significant difference among the FM and 40SC, 60SC and 80SC groups ($p > 0.05$). Compared to the FM group, the ACP in the 80SC and 100SC groups significantly increased ($p < 0.05$), while the 40SC and 60SC groups had no significant difference ($p > 0.05$). The AKP in the 100SC group was significantly higher than that in the FM group ($p < 0.05$), while there were no significant differences among the other four groups ($p > 0.05$). There were no significant differences in T-AOC and T-SOD among all the treatment groups ($p > 0.05$). The next-generation sequencing of the intestinal microbiota showed that Proteobacteria was the most abundant phylum in the five groups, accounting for 37.67%, 66%, 40%, 40% and 43.33%, respectively. Compared to the FM group, the Fusobacteriota in the other four groups decreased significantly ($p < 0.05$). The functional prediction of FAPROTAX indicated that no functional components were observed which are harmful to the body. Considering the effects on growth performance, antioxidant capacity and intestinal microbiota, it is feasible to use soybean products and chicken meal to replace 60% of fish meal in the feed of *P. monodon*.

Keywords: *Penaeus monodon*; growth performance; antioxidant capacity; intestinal microbiota

Key Contribution: A. An appropriate substitution ratio did not affect the growth performance and antioxidant capacity of *P. monodon*, and the intestinal microbiota of *P. monodon* remained stable and did not cause damage to the organism. B. A high substitution ratio can affect the growth and immunoenzyme activity of *P. monodon*.

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1. Introduction

Penaeus monodon, also known as grass shrimp and black tiger shrimp, is a genus of shrimp belonging to the genus Penaeus and family Penaeus. *P. monodon* is the largest individual in the genus Penaeus, with a body mass of 500 g and an average body length of 300–350 mm from maturity. The average body mass is about 350–400 g [1]. *P. monodon* is a valuable edible shrimp with a large body, a delicious taste and strong vitality. So, *P. monodon* is one of the most important marine shrimp breeding varieties in China.

With the rapid development of the aquaculture industry, the demand for aquatic feed is increasing. In aquaculture, feed costs can be as high as 50% [2]. Fish meal has good palatability and has always been an indispensable source of high-quality protein in aquatic feed [3]. It occupies a very high proportion in feed formulas, usually accounting for 20% to 60% of aquatic feed [4]. Fish meal is an unsustainable protein resource. In China, the source of fish meal is mainly imported, resulting in its high cost. The cost of buying fish meal accounts for half of the total breeding cost, so finding new and efficient materials that can replace fish meal protein has become a research hotspot in the aquatic feed industry. At present, many protein sources have been used to replace fish meal in order to achieve the purpose of reducing the use of fish meal in feed, such as poultry by-product meal [5], meat and bone meal [6], soybean meal [7], blood meal [8], cottonseed meal [9], peanut meal [10], rapeseed meal [11], soybean protein concentrate [12] and so on. Soybean meal has been proven to be one of the plant protein sources with a good effect on replacing fish meal. Some studies have found that using a certain amount of soybean meal as a substitute for part of fish meal had no significant effect on the growth performance of *Litopenaeus vannamei* [13]. According to a report by the FAO, currently, poultry meat is ranked in the top category of animal protein in the world. Chicken meal made from the waste, residual mince and offal generated during meat processing has attracted much attention from the animal protein source production market due to its rich nutritional value, high digestibility, amino acid balance and high biological conversion rate [14]. Studies have shown that chicken meal is a high-quality animal protein source for farmed fish [15]. In the process of breeding, differences in feed nutrient composition and raw materials will affect the antioxidant capacity and intestinal microbiota of aquatic animals. Studies have shown that using chicken meal as a substitute for 40% of fish meal could have negative effects on the liver and intestines of *Micropterus salmoides* [16]. The substitution of fish meal with chicken meal can reduce the antioxidant activity of *Trachinotus ovatus* [17]. And the intestinal microbiota of *Procambarus clarkii* can be changed when the additive amount of soybean meal is higher than 40% [18]. There have been many reports which have tested only one of them, while the effect of combining soybean products and chicken mea is unknown.

This study was conducted to study the effects of partially and completely substituting fish meal with soybean products and chicken meal on the growth performance, digestive enzymes, antioxidant capacity of the hepatopancreas, and the intestinal microbiota of *P. monodon*. The results from the current study will enhance our understanding of the effect of soybean products and chicken meal on *P. monodon* and provide basic data and a reference for the formulation and optimization of feed for *P. monodon*.

2. Materials and Methods

2.1. Experiment Materials

The experiment was conducted at the Shenzhen Base of the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The shrimp used in the experiment were of a new variety selected by the research team. The shrimp were taken out of the breeding pond and temporarily raised in an 8 m^3 tank for three days; commercial feed (Guangdong Dongteng Feed Co., Ltd., Guangzhou, China) was fed during this period, and feeding was stopped the day before the experiment began. The body mass of the shrimp was 3.44 $\pm$ 0.03 g.

2.2. Experiment Feeds

According to the nutritional requirements of *P. monodon*, five kinds of iso-nitrogen and iso-lipid diets were designed, and the substitution ratios of the fish meal were 0% (FM), 40% (40SC), 60% (60SC), 80% (80SC) and 100% (100SC), respectively. Additional crystal amino acids were added to meet the amino acid requirements of the shrimp. Methionine and cystine were hydrolyzed using oxidative acid hydrolysis, and the rest of the amino acids were hydrolyzed via acid hydrolysis. The chicken meal, soybean meal and other materials were purchased through the company Guangdong Kingkey Smart Agri Technology Co., Ltd., in Guangzhou, China. All the ingredients were ground into a powder, sieved through an 80-hole mesh and thoroughly mixed with oil and water. The 1.5 mm-diameter doughs were extruded using a twin screw extruder (F-26, South China University of Technology, Guangzhou, China), cut into pelletized feeds using a pelletizer (G-500, South China University of Technology, Guangzhou, China), steamed in a 90 °C electric oven for 2 h, dried in an air-conditioned room and then stored at −20 °C in a refrigerator until used. The feed formulations and the contents of various nutrients are shown in Table 1.

Table 1. Formulation and nutrient levels of the experimental diets (% dry matter).

	FM	**40SC**	**60SC**	**80SC**	**100SC**
Fish meal	25.00	15.00	10.00	5.00	0.00
Soybean meal	23.00	23.50	23.50	24.50	24.50
Chicken meal	0.00	4.50	6.50	8.50	11.00
Peanut hull	14.00	14.00	14.00	14.00	14.00
Wheatmeal	23.20	22.30	22.19	21.36	21.11
Beer yeast	3.00	3.00	3.00	3.00	3.00
Shrimp med	3.00	3.00	4.00	4.00	4.00
Soy protein concentrate	0.00	4.50	6.00	8.00	10.00
Soybean lecithin	1.00	1.00	1.00	1.00	1.00
Fish oil	1.55	1.65	1.70	1.80	1.85
Soybean oil	1.55	1.65	1.70	1.80	1.85
Vitamin C polyphosphate	0.10	0.10	0.10	0.10	0.10
Cholesterol	0.50	0.50	0.50	0.50	0.50
Vitamin premix (Prawn)	1.00	1.00	1.00	1.00	1.00
Mineral premix (Prawn)	1.00	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	1.00	1.75	2.05	2.45	2.85
Lysine hydrochloride (78%)	0.00	0.21	0.31	0.41	0.53
Methionine (99%)	0.00	0.10	0.14	0.19	0.24
Threonine (98%)	0.00	0.09	0.13	0.18	0.23
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00
Taurine (99%)	0.10	0.15	0.18	0.21	0.24
Total	100.00	100.00	100.00	100.00	100.00
Crude protein	39.92	40.00	39.91	39.93	39.89
Crude lipid	7.26	7.22	7.19	7.25	7.26
Methionine	0.83	0.83	0.83	0.83	0.83
Lysine	2.58	2.58	2.58	2.58	2.58
Threonine	1.63	1.63	1.63	1.63	1.63
Taurine	0.27	0.26	0.26	0.26	0.26
Total phosphorus	1.31	1.31	1.29	1.29	1.30

Note: vitamin premix: VA, 18 mg/kg; VD_3, 5 mg/kg; VE, 150 mg/kg; VC, 500 mg/kg; VB_1, 16 mg/kg; VB_6, 20 mg/kg; VB_{12}, 6 mg/kg; VK_3, 18 mg/kg; riboflavin, 40 mg/kg; inositol, 320 mg/kg; calcium-D-pantothenate, 60 mg/kg; niacinamide, 80 mg/kg; folic acid, 5 mg/kg; biotin, 2 mg/kg; ethoxyquin, 100 mg/kg; b. mineral premix: Na, 30 mg/kg; K, 50 mg/kg; Mg, 100 mg/kg; Cu, 4 mg/kg; Fe, 25 mg/kg; Zn, 35 mg/kg; Mn, 12 mg/kg; I, 1.6 mg/kg; Se, 0.2 mg/kg; Co, 0.8 mg/kg.

2.3. Feeding Management

Four hundred and fifty shrimp with a uniform size, normal body color and healthy body mass were randomly selected and divided into breeding barrels (500 L). Each group was set up with 3 replicates and 30 shrimp in each replicate. The shrimp were fed three times daily at 8:00, 15:00 and 22:00. The uneaten pellets and feces were removed by a siphon

method, and the exuviae and dead shrimp were removed with a dredge. The feed surplus in the pellet tray was observed 1 h and 2 h after feeding, respectively. The feeding amount was increased or decreased according to the condition of the remaining pellets, and the daily feeding amount was 2% to 5% of the body mass of the shrimp. The water was treated by sand filtering. During the feeding experiment, the water temperature was maintained at 27–32 °C, with a salinity of 28–32 ppt, a pH of 7.5–8.0, an ammonia nitrogen concentration of 0–0.2 mg/L and a dissolved oxygen concentration of 6–7 mg/L. The feeding experiment lasted for 8 weeks.

2.4. Sample Collection and Index Measurement

After the test, the shrimp were starved for 24 h, and the surface moisture of the shrimp was dried with a towel. The number of shrimp in each glass fiber bucket was calculated, and the total weight (accurate to 0.01 g, Puchun JE2002) was taken to calculate the survival rate, the weight gain rate and the feed conversion rate of shrimp in each feed group. The calculation formula is as follows:

$$\text{Survival rate } (\%) = 100 \times \frac{\text{initial number}}{\text{final number}}$$

$$\text{Weight gain rate } (\%) = 100 \times \frac{\text{final average mass(g)} - \text{initial average mass(g)}}{\text{initial average mass(g)}}$$

$$\text{FCR} = \frac{\text{feed amount(g)}}{\text{final average mass(g)} - \text{initial average mass(g)}}$$

2.5. Composition Analysis of Whole Shrimp

After the experiment, five shrimp were taken from each breeding barrel and stored at −20 °C for a later assessment of their whole body composition. The moisture content was measured after the shrimp were oven-dried at 105 °C to a constant weight. And the ash content was measured by burning them in a muffle furnace for at least 5 h at 550 °C. The crude protein content (N × 6.25) was measured by the Kjeldahl method (Kjeltec™8400; FOSS, Hilleroed, Denmark). The crude lipid content was measured by soxhlet extraction using the soxhlet system HT (Soxtec System HT6, Tacator, Sweden) [19].

2.6. Determination of Antioxidant Enzymes in Hepatopancreas of Shrimp

After the experiment, five shrimp were taken from each breeding barrel. Hepatopancreas tissue was taken. After freezing treatment with liquid nitrogen, the tissue was stored at −80 °C for the determination of antioxidant enzymes. Acid phosphatase (ACP), alkaline phosphatase (AKP), total superoxide dismutase (T-SOD) and total antioxidant activity (T-AOC) were tested using a commercial test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.7. Determination of Intestinal Microbiota in Shrimp

After the experiment, three shrimp were taken from each breeding barrel. The intestines were taken, frozen with liquid nitrogen and then stored at −80 °C. By extracting the total genomic DNA of a sample using the universal primers 16SrRNA or ITS (Internal Transcribed Spacer) for PCR amplification and then sequencing the highly variable region and identifying the strain, the microbial diversity in the sample can be analyzed through sequencing. We obtained classification tables for species annotation by comparing the current ASV sequences with those in the green genes (16 S rRNA) database. Based on the results of a recent study, the mi-biome diversity of five groups of experimental animals was analyzed by evaluating α-diversity indices as well as β-diversity metrics (using the principal coordinate analysis method). FAPROTAX [20] is a database that was manually constructed by Louca et al. It is more suitable for functional annotation in the biochemical processes of marine environments and lakes (sulfur, nitrogen, hydrogen and carbon cycles).

2.8. Data Statistics and Analysis

The statistical analysis was performed using SPSS 21.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA) for Windows. The effect was tested by a one-way ANOVA. When there were significant differences ($p < 0.05$), the groups' means were further compared by Duncan's multiple range test. The results were presented as the means ± SD ($n = 3$).

3. Results

3.1. Growth Performance and Feed Utilization of P. monodon

The effects of the substitution ratio on the growth performance of *P. monodon* are shown in Table 2. The nutritional composition of *P. monodon* is shown in Table 3. There was no significant difference in the nutritional composition among all the groups ($p > 0.05$). With an increase in the substitution ratio, the WGs in all the treatment groups decreased. Compared to the FM group, the WGs in the 80SC and 100SC groups significantly decreased ($p < 0.05$), while those of the 40SC and 60SC groups had a tendency to decrease, but there was no significant difference ($p > 0.05$). Similarly, the SRs in the 80SC and 100SC groups significantly decreased compared with the FM group ($p < 0.05$), while there was no significant difference in the survival rate among the FM, 40SC and 60SC groups ($p > 0.05$). Compared to the FM group, the FCR in the 100SC group was significantly increased ($p < 0.05$), while there was no significant difference among the FM and 40SC, 60SC and 80SC groups ($p > 0.05$).

Table 2. Effects of different substitution ratios on the growth performance of *P. monodon*.

Items	Diets				
	FM	**40SC**	**60SC**	**80SC**	**100SC**
IW (g)	3.41 ± 0.01	3.44 ± 0.01	3.47 ± 0.02	3.42 ± 0.03	3.46 ± 0.03
FW (g)	13.70 ± 0.45 ^{c}	13.22 ± 0.85 ^{bc}	13.38 ± 1.23 ^{c}	11.52 ± 0.74 ^{ab}	10.44 ± 0.08 ^{a}
WG (%)	302.26 ± 12.02 ^{c}	283.69 ± 25.01 ^{bc}	285.98 ± 35.84 ^{bc}	236.65 ± 24.64 ^{ab}	201.90 ± 1.95 ^{a}
SR (%)	82.67 ± 0.02 ^{c}	80.67 ± 0.01 ^{bc}	80 ± 0.02 ^{bc}	76.67 ± 0.01 ^{b}	71.33 ± 0.01 ^{a}
FCR	1.33 ± 0.08 ^{a}	1.42 ± 0.15 ^{ab}	1.48 ± 0.29 ^{ab}	1.51 ± 0.09 ^{ab}	1.78 ± 0.14 ^{b}

Note: IW: initial body weight; FW: final body weight; SR: survival rate; WG: weight gain rate; FCR: feed coefficient ratio. Data are expressed as mean ± SD ($n = 3$). Means with different superscripts are significantly different ($p < 0.05$).

Table 3. Nutritional composition of *P. monodon*.

Items	Diets				
	FM	**40SC**	**60SC**	**80SC**	**100SC**
Moisture	74.00 ± 0.31	74.42 ± 0.71	74.81 ± 0.55	74.28 ± 0.71	72.32 ± 0.47
Crude protein	73.42 ± 1.06	73.58 ± 0.48	74.04 ± 0.16	73.29 ± 0.77	72.83 ± 0.47
Crude fat	7.13 ± 0.18	6.95 ± 0.20	5.97 ± 0.56	6.16 ± 0.74	6.69 ± 0.09
Crude ash	16.20 ± 0.28	15.91 ± 0.71	16.60 ± 0.10	16.65 ± 0.56	14.50 ± 0.47

Note: unit: %.

3.2. Hepatopancreas Antioxidant Capacity of P. monodon

The activities of ACP, AKP, T-AOC and T-SOD in *P. monodon* are shown in Figure 1. Compared to the FM group, the ACP activity (Figure 1a) in the 80SC and 100SC groups significantly increased ($p < 0.05$), while that of the 40SC and 60SC groups had a tendency to increase, but there was no significant difference ($p > 0.05$). The AKP activity (Figure 1b) in the 100SC group was significantly high ($p < 0.05$), while there were no significant differences among the other four groups ($p > 0.05$). There were no significant differences in the T-AOC (Figure 1c) and T-SOD (Figure 1d) activities among all the treatment groups ($p > 0.05$).

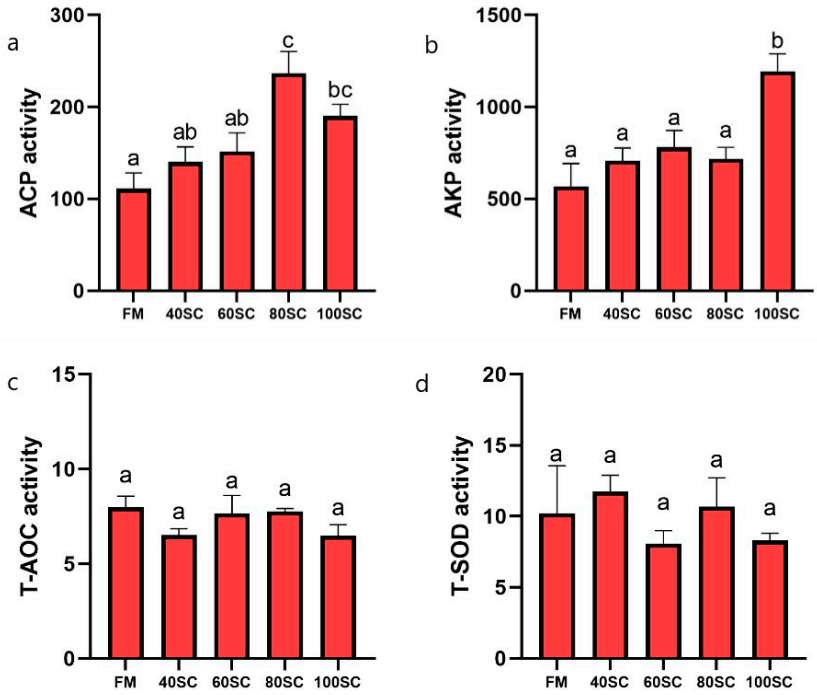

Figure 1. Hepatopancreas antioxidant capacity of *P. monodon*. (**a**): ACP activity; (**b**): AKP activity; (**c**): T-AOC activity; (**d**): T-SOD activity. Means with different letters are significantly different ($p < 0.05$).

3.3. Composition and Rationality Analysis of Intestinal Microbial ASVs in P. monodon

As shown in Figure 2a, a total of 354 ASVs were obtained by 16SrDNA high-throughput sequencing. According to the analysis of the Wayne diagram, 42 ASVs were present in the five test groups, no unique ASV was found in the 60SC group and one unique ASV was found in all the other groups. The Shannon–Wiener curve (Figure 2b) [21] considers both species' evenness and richness. When the curve tends to be flat, it indicates that the amount of sequencing data is large enough. A species accumulation curve can show whether a species increased along with an increase in sample size, so it is an effective tool to determine whether a sample size is sufficient. The curve in Figure 2c gradually inclines and then flattens, proving that the species in the samples did not increase with the increase in the sample size or the number of ASVs in the data analysis. The results indicated that the microbial diversity was sufficient to be fully detected, and the sequencing data were reasonable.

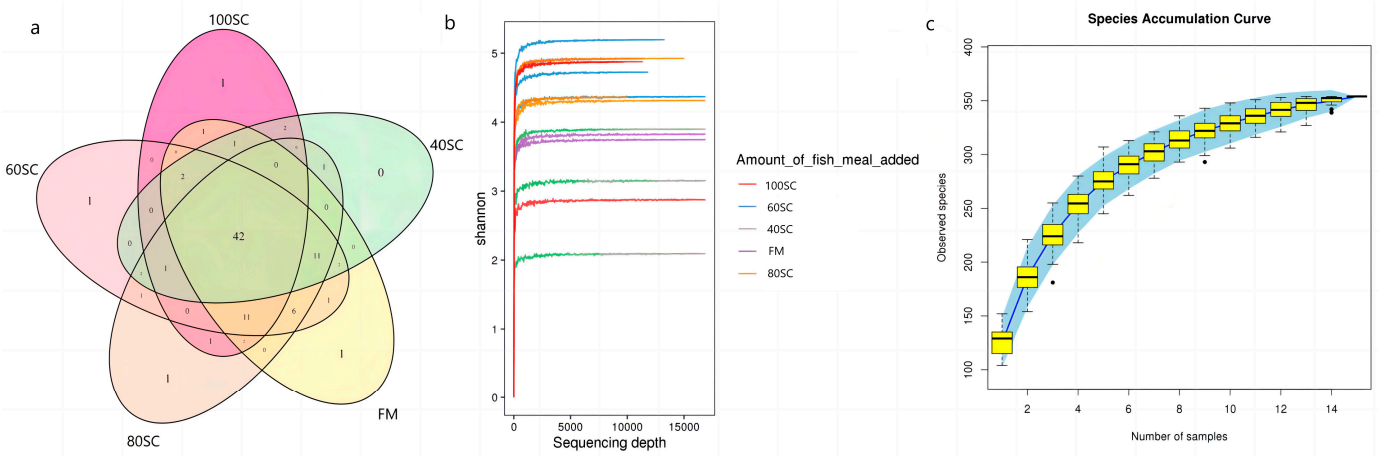

Figure 2. Composition and rationality analysis of intestinal microbial ASVs in *P. monodon*. (**a**) Venn diagram; (**b**) Shannon index; (**c**) species accumulation box diagram.

3.4. The Richness and Diversity of Intestinal Microbiota in P. monodon

The inter-group difference analysis of the α diversity index was evaluated. A box-type diagram of the inter-group difference analysis is shown in Figure 3a. The Shannon indexes of the 100SC, 80SC and 60SC groups were close to that of the FM group. Based on the Jaccard, bray Curtis, unweighted unifrac and weighted unifrac distances, a PCoA analysis was performed (Figure 3b). The sample distances in the FM and 60SC groups were relatively close. The sample distances of the 100SC and 40SC groups were far away. This proved that the species composition structure in the 60SC group was similar to that of the FM group.

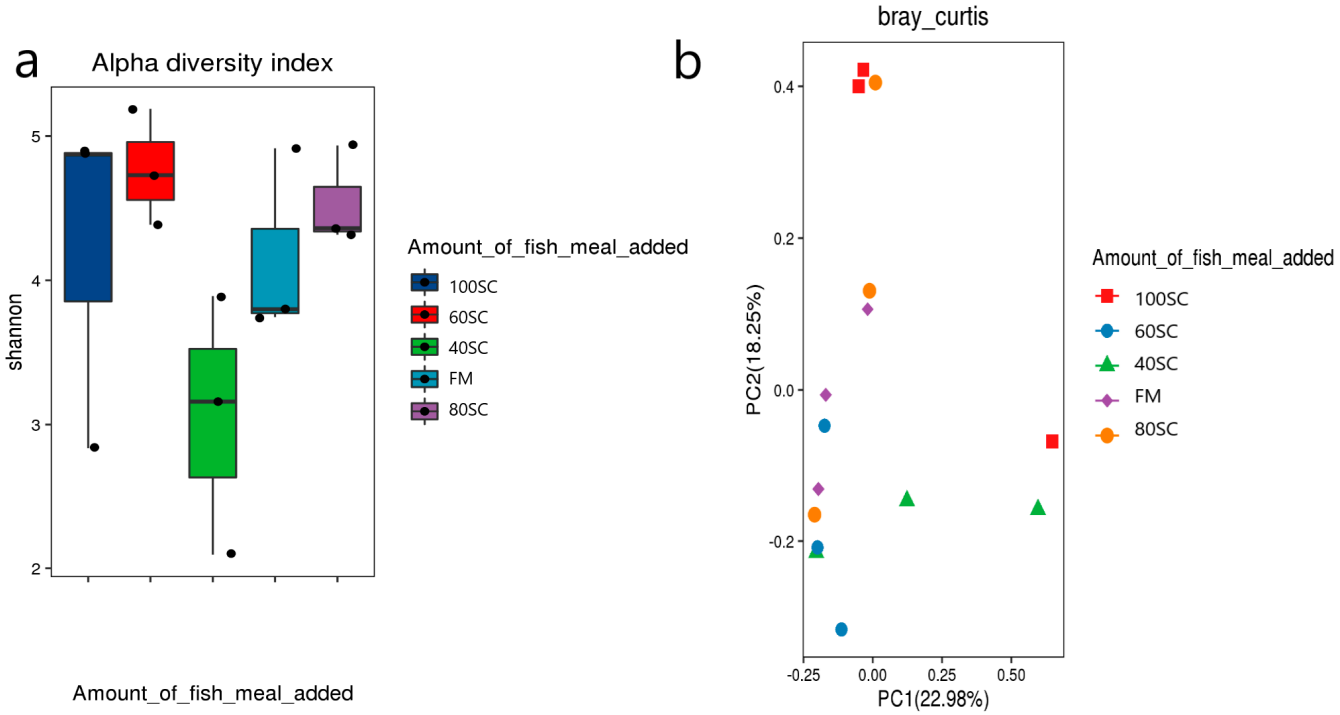

Figure 3. The richness and diversity of intestinal microorganisms in *P. monodon*. (**a**) Index group-difference box chart; (**b**) PCoA analysis chart.

3.5. Intestinal Microbiota Composition of P. monodon

The analysis of the intestinal contents of *P. monodon* encompassed multiple taxonomic levels, specifically the phylum, order, family and genus. The phylum was chosen as the representative taxonomic level for this study. As shown in Figure 4a,b, at the phylum level, Proteobacteria, Bacteroidota and Actinobacteriota formed the core microbiota. Proteobacteria was the most abundant phyla in the five groups, accounting for 37.67%, 66%, 40%, 40% and 43.33%, respectively (Table 4). Compared to the FM group, the Fusobacteriota in the other four groups decreased significantly ($p < 0.05$). There were no significant differences in the other bacteria among the five groups ($p > 0.05$).

3.6. Functional Gene Prediction Analysis of Intestinal Microbiota and Functional Prediction of FAPROTAX

The PCA analysis is presented in Figure 5, which was performed based on the functional abundance table of metabolic pathways predicted by PICRUSt2. The pictures from left to right are the pathway level, protein level and enzyme level. We found that among the three levels, the 100SC and 40SC groups were far apart on the PCA diagram, while on the contrary, the 80SC, FM and 60SC groups were closer together on the PCA diagram, indicating that their functional compositions were similar.

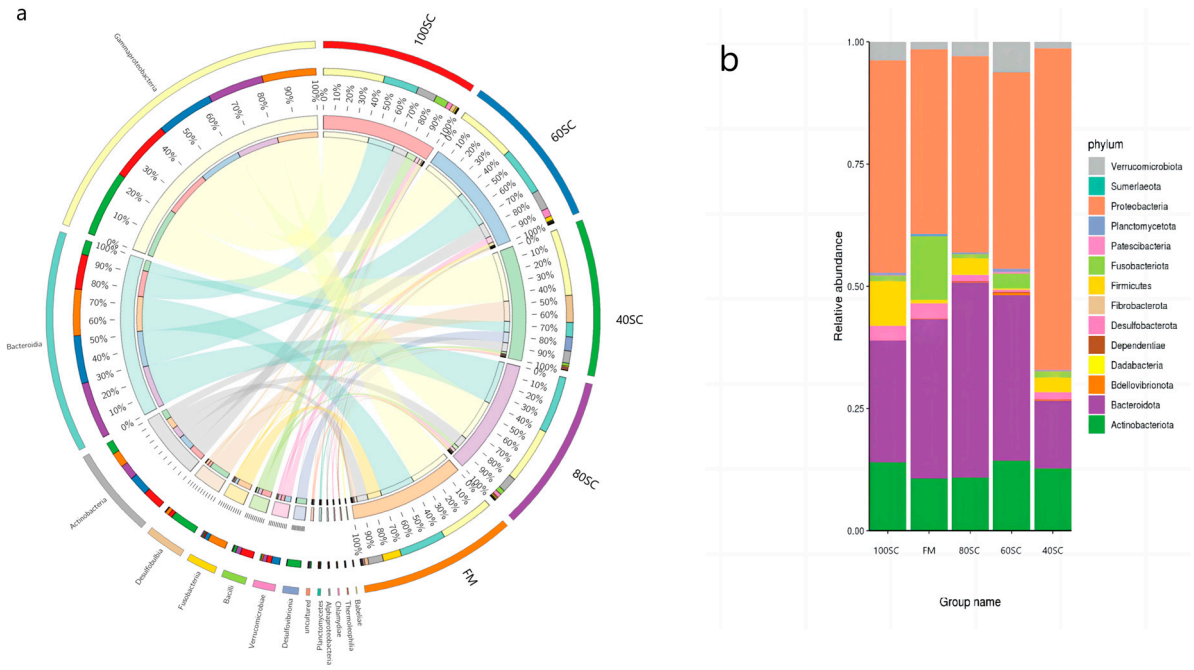

Figure 4. Intestinal microbiota composition of *P. monodon*. (**a**) Circos plot; (**b**) histogram of relative abundance of species.

Table 4. Distribution of the top 10 microbial phylum levels in the intestinal contents of *P. monodon* in different treatment groups.

Phylum	Group				
	FM	40SC	60SC	80SC	100SC
Proteobacteria	37.67 ± 19.03	66.00 ± 9.00	40 ± 21.70	40 ± 15.10	43.33 ± 19.29
Bacteroidota	33.00 ± 18.36	14.00 ± 3.00	34.00 ± 7.81	40.00 ± 25.06	24.67 ± 16.65
Actinobacteriota	10.67 ± 7.37	12.33 ± 11.50	14.33 ± 7.23	10.67 ± 4.04	13.67 ± 10.12
Firmicutes	0.80 ± 1.04	3.02 ± 2.98	0.30 ± 0.10	3.53 ± 5.60	9.06 ± 13.83
Verrucomicrobiota	1.43 ± 1.40	1.10 ± 0.85	6.23 ± 6.24	2.67 ± 1.52	3.50 ± 2.78
Desulfobacterota	3.08 ± 5.12	1.53 ± 1.46	0.40 ± 0.53	1.17 ± 0.72	3.13 ± 2.42
Fusobacteriota	13.33 ± 10.40 ^{a}	1.13 ± 0.81 ^{b}	3.03 ± 3.56 ^{b}	0.84 ± 10.3 ^{b}	10.6 ± 0.90 ^{b}
Planctomycetota	0.38 ± 0.54	0.16 ± 0.14	0.63 ± 0.58	0.30 ± 0.30	0.38 ± 0.53
Patescibacteria	0.37 ± 0.11	0.15 ± 0.05	0.37 ± 0.54	0.05 ± 0.04	0.17 ± 0.21
Dependentiae	0.01 ± 0.11	0.15 ± 0.15	0.27 ± 0.30	0.08 ± 0.10	0.06 ± 0.06

Note: data are expressed as mean ± SD (n = 3). Means with different superscripts are significantly different ($p < 0.05$).

FAPROTAX is more complete for analyzing the classification and function of marine microorganisms. The results are shown in Figure 6. In all the groups, the abundances of chemoheterophya and aerobic chemoheterotrophy were high, while the fermentation and nitrate reduction rates were relatively low. Intracellular parasites, aromatic compound degradation and human-associated factors were not found.

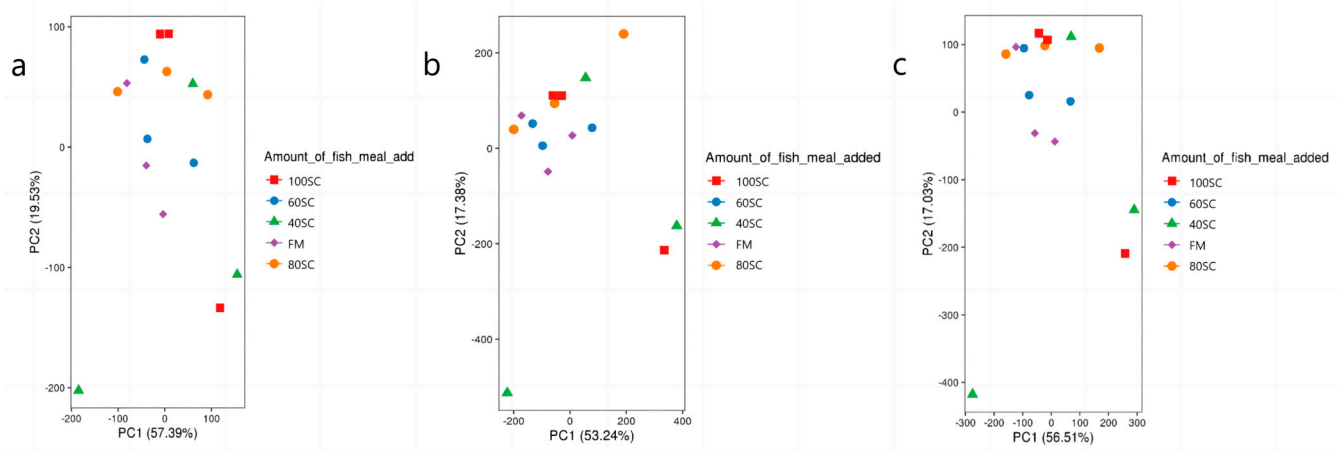

Figure 5. PCA dimensionality reduction analysis diagram. (**a**) PCA analysis at pathway level; (**b**) PCA analysis at protein level; (**c**) PCA analysis at enzyme level.

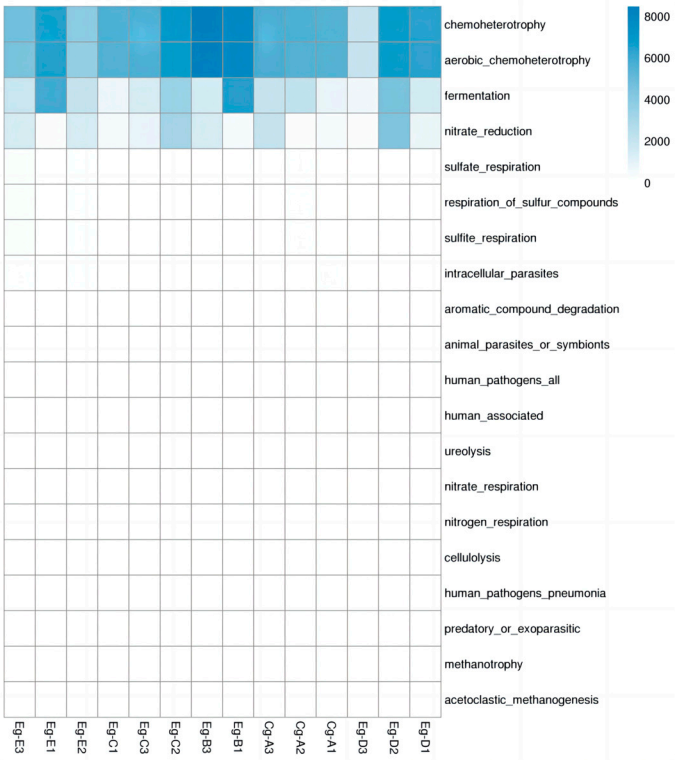

Figure 6. FAPROTAX functional abundance heat map.

4. Discussion

The growth performance of aquatic animals is generally expressed by the WG, SR, FCR, etc. [22]. Yan et al. [23] found that replacing 50% of fish meal protein with soybean meal did not have a negative impact on the growth of *L. vannamei*, but a higher substitution level would lead to a significant decrease in the WGR and SGR. Zhang et al. also found that the proportional replacement of 20% of fish meal with SPC and peanut bran had no significant effect on the growth performance of *P. monodon* [24]. Studies have shown that a high proportion of plant protein replacing fish meal may inhibit the growth and feed utilization of aquatic animals [25]. In a study by Daniela et al., using soybean meal as a substitute for part of fish meal did not inhibit the growth performance of *Centropomus viridis* [26]. A study by Wu et al. also showed that replacing an appropriate amount of fish meal with chicken meal did not inhibit the growth of *Micropterus salmoides* [27]. In a study by Yu et al., replacing part of fish meal with soy protein concentrate also had no significant effect on

the growth performance of *L. vannamei* [28]. Similar conclusions were found in studies by Chen [29] and Wang et al. [30]. In this study, we used chicken meal and soybean products as substitutes for part of fish meal, and with an increase in substitution ratio, the WGs and SRs in all the treatment groups were decreased. The WGs and SRs in the 80SC and 100SC groups significantly decreased compared to those in the FM group, but there was no significant difference, although those in the 40SC and 60SC groups had a slight decrease. The FCR showed a decreasing trend; the FCR in the 100SC group significantly increased ($p < 0.05$), while there was no significant difference between the FM group and the 40SC, 60SC and 80SC groups ($p > 0.05$). These results are similar to those of other studies and prove that it is feasible to partially replace fish meal with soybean products and chicken meal.

Oxidative stress is one of the response mechanisms of animal organisms to environmental stress. Changes in the diet of aquatic animals may affect their antioxidant capacity and immunoenzyme activity. Under environmental stress, many reactive oxygen species will be produced in the body, resulting in organism damage [31]. *P. monodon* has a relatively complete antioxidant system, which can maintain an organism's homeostasis [32]. SOD plays a crucial role in the balance between oxidation and antioxidants in the body and is an important antioxidant oxidase in maintaining a normal metabolism [33]. ACP is an important enzyme in macrophages and plays an important role in the immune response of shrimp [34]. Jiang et al. found that using concentrated dephenolized cottonseed protein (CDCP) as a partial substitute for fish meal did not have a significant effect on the SOD and malondialdehyde (MDA) contents of *P. monodon* [35]. This is consistent with the conclusion of Cui et al. that using soybean meal, fermented soybean meal and cottonseed meal as a substitute for 50% of fish meal did not have a negative impact on the growth performance and antioxidant capacity of juvenile *Eriocheir sinensis* [36]. The study by Wu et al. showed that replacing an appropriate amount of fish meal with chicken meal can improve the antioxidant capacity and immunity of *Micropterus salmoides* [27]. An experiment which used fish soluble pulp as a partial substitute for fish meal to feed *Macrobrachium rosenbergii* did not cause significant differences in the SOD and hepatopancreas ACP contents among all the groups [37]. In our experiment, there was no significant difference between the T-SOD and T-AOC contents among all the groups, indicating that using chicken meal and soybean products as substitutes for part of fish meal would not have a significant effect on the antioxidant capacity of *P. monodon*. The ACP content in the 80SC and 100SC groups was significantly increased compared to that in the FM group ($p < 0.05$), while that in the 40SC and 60SC groups had a slight increase, but there was no significant difference ($p > 0.05$). The AKP content in the 100SC group was significantly high, while there were no significant differences among the other four groups ($p > 0.05$), indicating that a long-term high substitution level would cause the organism to be continuously subjected to stress. The 40SC, 60SC and FM groups did not have significant differences among their ACP and AKP contents, which proved that the substitutions had no effect on the organisms.

Intestinal microbiota refers to the large number of microorganisms present in the intestinal tract of animals, which they rely on to live and help animals carry out a variety of physiological and biochemical functions. Changes in aquatic animal feed could have some impact on the intestinal microbiota [38]. 16SrRNA is located on the small subunit of the ribosome of prokaryotic cells, including ten conserved regions and nine hypervariable regions, among which the conserved regions have little difference among bacteria and the hypervariable regions are specific to the genus or species and vary between them. Therefore, 16SrDNA can be used as a characteristic nucleic acid sequence to reveal biological species and is the most suitable index for bacterial phylogeny and classification identification. Although the OTU clustering method can effectively overcome sequencing errors, it reduces the accuracy of classification, and some sequences below a set threshold cannot be accurately distinguished. The method of de-noising is recommended. OTUs have been given a new name, ASVs (Amplicon Sequence Variants) [39,40]. Intestinal probiotics can promote the body's digestion and absorption of food and competitively inhibit the growth of intestinal harmful microorganisms, thus maintaining the ecological balance in

the body and promoting the healthy growth of the body [41]. The functional prediction of FAPROTAX in this paper does not detect the functional components that are harmful to the body. Many studies have shown that Proteobacteria are the dominant bacteria in the gut of shrimp [42,43], and some members of this phyla are involved in the nitrogen cycle and the mineralization of organic compounds [44,45]. In this study, Proteobacteria were the most abundant bacteria in the five groups, accounting for 37.67%, 66%, 40%, 40% and 43.33%, respectively. Compared to the FM group, the abundance of Fusobacteriota in the other four groups decreased significantly. Fusobacteriota are conducive to food digestion and absorption [46]. In the present study, the abundance of Fusobacteriota in the substitution groups decreased significantly, so we speculated that it may be due to the anti-nutritional factors in soybean products, which can hinder the absorption and utilization of proteins. This also explains why the WG in the substitution groups decreased. The results in this study were consistent with the results in a previous study on *L. vannamei* [47].

5. Conclusions

Using soybean products and chicken meal as substitutes for 40% and 60% of fish meal did not affect the growth performance and antioxidant capacity of *P. monodon*, and the intestinal microbiota of *P. monodon* remained stable and did not cause damage to the organism. However, a high substitution ratio can affect the growth and immunoenzyme activity of *P. monodon*. Considering the effects on growth performance, antioxidant capacity and intestinal microbiota, it is feasible to use soybean products and chicken meal to replace 60% of fish meal.

Author Contributions: S.J. and F.Z. conceived and designed the experiments. W.Y. and J.S. performed the bioinformatics analysis and prepared the manuscript, the table and the figures. Q.Y. and J.H. conducted the experiment. Y.L., S.J. and Y.Y. collected the samples. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The present study was approved by the Animal Care and Use Committee of the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (Approval number SCSFRI2021-0731). All procedures were strictly carried out according to the regulations and guidelines approved by the committee.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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