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Research Article

Effects of Dietary American Cockroach *Periplaneta americana* Meal Inclusion on the Growth Performance, Antioxidant Capacity, and Immunity of Juvenile Rainbow Trout *Oncorhynchus mykiss*

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A feeding trial was conducted to assess the effects of dietary American cockroach *Periplaneta americana* meal (PAM) inclusion on the growth performance, antioxidant capacity, and immunity of juvenile rainbow trout *Oncorhynchus mykiss*. Five isonitrogenous and isolipidic diets were formulated to contain 100, 150, 200, 250, and 300 g/kg PAM (defined as PAM100-PAM300), respectively. The final body weight and weight gain rate of PAM250 were significantly higher than those of PAM100 and PAM150, and a higher specific growth rate was recorded in PAM200-PAM300. The highest levels of liver alanine aminotransferase and serum aspartate aminotransferase (AST) as well as serum total amino acids were detected in PAM150, while higher levels of liver AST and serum total protein were detected in PAM200-PAM300. The liver lipase activity of PAM150, PAM200, and PAM300 was significantly higher than that of PAM100. The liver glucose content in PAM150 was significantly higher than that in PAM250-PAM300. The highest liver catalase, alkaline phosphatase (AKP), and lysozyme activities were detected in PAM300; the lowest liver malondialdehyde content was detected in PAM250, while the highest serum total antioxidant capacity, catalase, and total superoxide dismutase activities were detected in PAM250. The highest serum AKP and acid phosphatase activities were detected in PAM150, while higher serum IgM and complement C3 contents were detected in PAM200-PAM300. In conclusion, dietary PAM inclusion at 200-300 g/kg could improve the growth performance, antioxidant capacity, and immunity of juvenile *O. mykiss*.

1. Introduction

Fishmeal is an important feed ingredient, and it is widely used in aquafeeds because of its high protein content, balanced amino acid composition, good palatability, digestibility and absorptivity, and low content of antinutritional factors (ANFs) [1]. However, due to overfishing, pollution, climate change, and other factors, a series of problems such as the sharp decline of wild fish resources, unstable supply of fishmeal, and increase in prices have raised [2, 3]. Therefore, looking for protein alternative sources has become one of the current hot issues in aquatic nutrition research [4, 5].

At present, the alternative sources to fishmeal mainly include animal by-products, plant proteins, insects, and unicellular algae ingredients. Among them, animal ingredients, such as hydrolyzed feather meal [6], poultry by-product meal [7], maggot meal [8], and earthworm meal [9], have high crude protein content, relatively balanced amino acid compositions, low antinutritional factors, some functional ingredients (cholesterol, taurine, etc.), and low price. Therefore, the animal protein sources were considered the potential alternatives to fishmeal in aquafeeds. Plant ingredients include soybean meal [10], cottonseed meal [11], and corn gluten [12]. Using plant ingredients to replace

Table 1: Formulations of five experimental diets (g/kg).

Ingredients	Diets				
	PAM100	PAM150	PAM200	PAM250	PAM300
Fishmeal	300.0	250.0	200.0	150.0	100.0
P. americana meal	100.0	150.0	200.0	250.0	300.0
Wheat flour	160.0	160.0	160.0	160.0	160.0
Soybean meal	150.0	150.0	150.0	150.0	150.0
Rapeseed meal	50.0	50.0	50.0	50.0	50.0
Cottonseed meal	50.0	50.0	50.0	55.0	55.0
Corn gluten meal	45.0	50.0	55.0	55.0	60.0
Soy lecithin	10.0	10.0	10.0	10.0	10.0
Fish oil	45.0	45.0	40.0	40.0	40.0
Soybean oil	55.0	50.0	50.0	45.0	40.0
Vitamin premix [1]	10.0	10.0	10.0	10.0	10.0
Mineral premix [2]	10.0	10.0	10.0	10.0	10.0
$Ca(H_2PO_4)_2$	11.5	11.5	11.5	11.5	11.5
Vitamin C phosphate (30%)	0.2	0.2	0.2	0.2	0.2
Antioxidant	0.3	0.3	0.3	0.3	0.3
Choline chloride (50%)	3.0	3.0	3.0	3.0	3.0

[1]Vitamin premix (per kg diet): vitamin A, 62500 IU; vitamin D_3, 15000 IU; vitamin E, 1.75 g; vitamin K_3, 35.4 mg; vitamin B_1, 100 mg; vitamin B_2, 150 mg; vitamin B_6, 150 mg; vitamin B_{12}, 0.2 mg; biotin, 4 mg; D-calcium pantothenate, 250 mg; folic acid, 25 mg; nicotinamide, 300 mg; vitamin C, 700 mg. [2]Mineral premix (per kg diet): $FeSO_4.H_2O$, 200 mg; $CuSO_4.5H_2O$, 96 mg; $ZnSO_4.H_2O$, 360 mg; $MnSO_4.H_2O$, 120 mg; $MgSO_4.H_2O$, 240 mg; KH_2PO_4, 4.2 g; NaH_2PO_4, 0.5 g; KI, 5.4 mg; $CoCl_2.6H_2O$, 2.1 mg; Na_2SeO_3, 3 mg.

fishmeal is an important strategy to alleviate the shortage of fishmeal. However, plant protein source has imbalanced amino acid compositions, lack of essential amino acids such as lysine and methionine, and high levels of antinutritional factors (protease inhibitors, glucoside, etc.) [13], which limited the use of plant protein source in aquafeeds.

Insect protein sources, such as the larva of house fly *Musca domestica*, soldier fly *Hermetia illucens*, and grain beetle *Tenebrio molitor*, have high protein contents (up to 50%-82% dry weight) [14, 15], and the essential amino acid content is 10%-30%, which accounts for 35%-50% of the total amino acids [16]. In addition, the insect protein source is also rich in palmitic acid, unsaturated fatty acids, vitamins, minerals, etc., and has good digestibility. Therefore, it is a high-quality feed protein resource [17, 18]. American cockroach *Periplaneta americana* is an insect widely distributed in the world with strong vitality. *P. americana* is mainly used for the treatment of food waste [19, 20]. In addition, *P. americana* is an important medicinal insect, which can be used to extract active ingredients such as polysaccharides, peptides, amino acids, and nucleosides [21]. A previous study has shown that the *P. americana* dry meal contains approximately 60% crude protein and 20% crude fat, and its amino acid composition is close to the fishmeal [20]. Several studies have shown that *P. americana* and its extract could significantly improve the antioxidant capacity and immunity of rats [22], broilers [23, 24], and rabbits [25]. Therefore, *P. americana* may be a potential alternative to fishmeal in aquafeeds. However, there have been limited studies about the dietary fishmeal replacement with *P. americana* in aquafeeds.

Rainbow trout *Oncorhynchus mykiss* is a commercially important fish species in the world. This fish species is a high-value and popular food source because of its high contents of protein and long-chain polyunsaturated fatty acids (LC-PUFA) [26, 27]. *O. mykiss* is a carnivorous fish, and the commercial feeds for this species have a characteristic of high protein and high fat (especially high LC-PUFA). Usually, a high proportion of fishmeal and fish oil must be added to the feed [28], which leads to an increase in feed costs, and is not conducive to the sustainable development of *O. mykiss* farming industry. Therefore, it is imperative to find a suitable alternative source to fishmeal. This study was conducted to investigate the effects of different dietary *P. americana* meal inclusion on the growth, feed utilization, antioxidant capacity, and immunity of juvenile *O. mykiss*. The results will provide a certain theoretical basis and practical reference for the application of *P. americana* in aquafeed.

2. Materials and Methods

2.1. Experimental Diets. Our pre-experiment showed that dietary PAM inclusion at 100 g/kg could improve the growth performance of juvenile *O. mykiss*; however, it is unclear whether higher fishmeal replacement levels affect the growth, metabolism, and biochemical composition of juvenile *O. mykiss*. In view of this, five isonitrogenous and isolipidic experimental diets were formulated to contain 100, 150, 200, 250, and 300 g/kg *P. americana* meal, respectively, defined as PAM100 (control), PAM150, PAM200, PAM250, and PAM300, respectively (Table 1). The *P.*

americana meal was purchased from a American cockroach farm in Dali, Yunnan Province, China. *P. americana* larvae develop into adults after 4 months of rearing. During the breeding period of *P. americana*, they were mainly fed corn meal, pumpkin, and potatoes. The adult *P. americana* were collected, dried at 80-90°C, and ground for the later experimental diets preparation. All the dry ingredients of experimental diets were ground to pass through a 250 μm sieve before mixing then combined and thoroughly mixed to homogeneity. Lipid ingredients were added to the dry components and thoroughly mixed for 10 min; then, distilled water (300 g/kg dry ingredients mixture) was added and mixed for 10 min. The diets were extruded through a 1.5 mm orifice, air-dried at room temperature, and then stored in plastic bags at -20°C until use.

2.2. Experimental Setup and Culture Management. In early November 2020, juvenile *O. mykiss* were transported from a commercial farm (Tanghao Aquaculture Co., Ltd., Kunming, China) to the Agronomy Experimental Center, College of Agriculture and Biological Sciences, Dali University. Three hundred healthy and active juvenile fish (mean weight 3.13 ± 0.02 g) were selected and randomly stocked into 15 aquariums (60 cm × 36 cm × 72 cm) with a water recirculation system. There were five treatments (PAM100-PAM300), with each treatment having three replicate aquariums (20 fish per tank). Prior to the feeding trial, all fish were acclimated to the aquarium conditions for 2 weeks and fed diet PAM100. This experiment started on November 25, 2020, and lasted 42 days.

During the feeding trial, experimental fish were hand-fed to apparent satiation twice (08 : 00 and 17 : 00) daily. All rearing tanks were provided with continuous aeration and maintained under a photoperiod cycle of 12 h light : 12 h dark. Fluorescent lamps (40 W) were used as the light source. Water in each tank was recirculated through a biological and mechanical filtration system containing quartz sand, sponge, and activated carbon. The water temperature was maintained at 15-20°C. Any uneaten feed was collected 1 h after each feeding with the siphoning method, and the dry matter content was recorded for feed consumption calculation. The ammonia-N, nitrite, dissolved oxygen (DO), and pH of water were monitored every three days. The water was exchanged according to the water quality. During the feeding trial, water quality parameters were maintained as follows: the ammonia-N and nitrite levels were not more than 0.5 mg/L and 0.15 mg/L, respectively; DO level was >5 mg/L; and the pH was 7.0 - 8.5.

2.3. Sample Collection. At the end of the feeding trial, fish fasted for 24 h before sampling. All experimental fish were anesthetized with eugenol (1: 12,000) and then were weighed and counted to calculate the growth performance and feed utilization efficiency. Five fish per tank were randomly collected and stored at -20°C for proximate composition analysis. Five more fish per tank were sampled for biochemical parameter analyses. Blood was collected from the caudal vein with a 1.0 mL syringe and transferred into a 1.5 mL Eppendorf tube. Serum was obtained after centrifugation (10,000 × g for 10 min at 4°C) and immediately stored at -80°C until analysis. The viscera and liver were removed from the abdominal cavity and then weighted.

The liver samples were frozen in liquid nitrogen and then stored at -80°C for later analysis. The survival rate (SR), daily feed intake (DFI), weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), viscerosomatic index (VSI), and hepatosomatic index (HSI) were calculated:

$$\begin{aligned}
\mathrm{SR}\,(\%) &= \frac{N_i - N_f}{N_i} \times 100,\\
\mathrm{DFI}\,(\%) &= \frac{2F}{T \times \left(W_i + W_f + W_d\right)} \times 100,\\
\mathrm{WGR}\,(\%) &= \frac{W_f - W_i}{W_i} \times 100,\\
\mathrm{SGR}\,(\%/\mathrm{d}) &= \frac{\ln W_f - \ln W_i}{T} \times 100,\\
F\mathrm{CR} &= \frac{F}{W_f - W_i},\\
F\mathrm{ER} &= \frac{W_f - W_i}{F},\\
\mathrm{PER} &= \frac{W_f - W_i}{P_f},\\
\mathrm{VSI}\,(\%) &= \frac{W_v}{W_b} \times 100,\\
\mathrm{HSI}\,(\%) &= \frac{W_l}{W_b} \times 100,
\end{aligned} \tag{1}$$

where N_f is the final number of fish, N_i is the initial number of fish; W_f is the final body weight of fish; W_i is the initial body weight of fish; W_d is the weight of dead fish; T is the culture time; F is the feed fed; P_f is the protein fed; W_v is the weight of viscera; W_b is the body weight of fish; W_l is the weight of the liver.

2.4. Proximate Composition and Amino Acid and Fatty Acid Composition Analyses. Prior to biochemical analysis, fish samples from a same tank were pooled and homogenized. The analysis of moisture content, crude protein (Kjeldahl method, using a N to protein conversion factor of 6.25), and ash content of fishmeal, *P. americana* meal, experimental diets, and fish samples was conducted according to AOAC procedures [29]. Total lipids were extracted with chloroform-methanol (2 : 1, *v*/*v*) according to the method described by Folch et al. [30]. The amino acid composition of the fishmeal, *P. americana* meal, experimental diets, and fish samples was analyzed following the methods described by Blackburn [31]. Approximately 0.1 g from each sample was placed separately in 40 mL ampules with 8 mL of 6 mol/L HCl. The ampules were vacuumed-sealed, and

samples were hydrolyzed at 110°C for 24 h. Hydrolysates were dissolved to a volume of 50 mL using distilled water and centrifuged at 2659 × *g*, and the supernatants were filtered through a 0.22 *μ*m filter (Millipore, Bedford, MA). 1 mL of the filtered supernatant was then retrieved and vacuum dried at 50°C to remove the initial HCl, followed by the addition of 2–5 mL of 0.02 mol/L HCl. Finally, 1 mL of the final sample was analyzed with a Hitachi L-8900 amino acid analyzer (Hitachi, Ltd., Tokyo, Japan). The concentration of tryptophan analysis was determined by hydrolyzation using a solution containing 5 M NaOH and 5% $SnCl_2$ (*w*/*v*) for 20 h at 110°C. After hydrolysis, the hydrolysate was neutralized with 6 M HCl, centrifuged at 2659 × *g*, and filtered using filter paper. The subsequent analysis was done similarly to the methods described above. The concentrations of methionine and cystine in the samples were determined according to the method of formic acid oxidation and hydrolyzation [32].

For the fatty acid analysis, total lipids of the experimental diets and fish samples were esterified with boiling 14% boron trifluoride/methanol (*w*/*w*), and the fatty acid methyl esters (FAMEs) were extracted with hexane [33]. FAMEs were analyzed using an Agilent 7890B GC/5977A gas chromatograph-mass spectrometer (GC-MS) with an Omegawax 320 fused silica capillary column (30 m × 0.32 mm × 0.25 um; Supelco, Bellefonte, PA, USA). The injector and detector temperature were maintained at 260°C. The column temperature was initially set at 40°C, and then increased at a rate of 10°C/min to 170°C and held for 1 min, followed by an increase at a rate of 2°C/min to 220°C and held for 1 min. The temperature was then further increased at a rate of 2°C/min to the final temperature of 230°C and held for 30 min until all FAMEs had been eluted. The peaks were identified by comparing retention times with a 37-component FAMEs standard mixture (18919-1AMP, Sigma-Aldrich Co., St. Louis, MO, USA). The fatty acid composition was expressed as the percentage of each fatty acid to the total fatty acids (g/kg total fatty acids).

2.5. Measurement of Antioxidant and Immune Indices. The wet liver tissue plus a 5-fold volume (*v*/*w*) of ice-cold physiological saline solution was added to a 5 ml test tube and homogenized using an IKA homogenizer (T10B, IKA Co., Germany), and the homogenate was centrifuged at 10,000 × *g* for 10 min at 4°C. Then, the supernatant was collected for later analysis.

The total dissoluble protein (TP), total amino acid (T-AA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, lipase (LPS), triglyceride (TG), total cholesterol (T-CHO), glucose (GLU), lactate dehydrogenase (LDH), total antioxidant capacity (T-AOC), superoxide dismutase (SOD), malondialdehyde (MDA), acid phosphatase (ACP), alkaline phosphatase (AKP), and lysozyme (LZM) in the serum and liver were analyzed with a spectrophotometer (T6 New Century, Beijing Purkinje General Instrument Co., Ltd, Beijing, China) and commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The contents of immunoglobulin M (IgM) and complement (C3) in the serum were measured using commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocols.

2.6. Statistical Analysis. Data are presented as mean ± standard error (SE). Homogeneity of variance was tested with Levene's test. When necessary, arcsine-square root or logarithmic transformation was performed prior to analysis. Statistical analyses were conducted using one-way analysis of variance (ANOVA) and compared with Duncan's multiple range tests. When a normal distribution and/or homogeneity of the variances was not achieved, data were subjected to the Kruskal-Wallis *H* nonparametric test, followed by the Games-Howell nonparametric multiple comparison test. $P < 0.05$ was regarded as the statistically significant level. The linear or quadratic regression analysis was performed based on the dietary PAM inclusion and the growth performance, body composition, metabolism indices, antioxidant capacity, and immunity parameters of the experimental fish. All statistical analyses were performed using the SPSS statistics package software (version 16.0).

3. Results

3.1. Survival Rate, Growth Performance, and Feed Utilization. The SR, final body weight (FBW), WGR, SGR, FER, and PER of *O. mykiss* firstly increased with increasing dietary PAM inclusion levels, reaching a peak at the PAM250 group but then decreased with increasing dietary PAM inclusion levels. There were significant correlations between the FBW, WGR, SGR, FER, or PER of *O. mykiss* and dietary PAM content ($P < 0.05$). The SR, FBW, and WGR of *O. mykiss* of the PAM250 group were significantly higher than those of the PAM100 and PAM150 groups, while a higher SGR was also recorded at the PAM200, PAM250, and PAM300 groups (Table 2). The FCR decreased firstly and then increased with increasing dietary PAM levels, and showing a significant negative correlation with dietary PAM contents ($P < 0.05$), and the FCR of the PAM250 group was the lowest. The lowest and highest VSI were found at the PAM200 and PAM300 groups, respectively.

3.2. Proximate Composition and Amino Acid and Fatty Acid Contents of Whole Fish Body. The contents of moisture and ash of whole fish body were significantly negatively correlated with dietary PAM content, while the contents of total lipid and crude protein were significantly negatively correlated with dietary PAM content ($P < 0.05$).

The PAM100 group has the highest moisture content of whole fish body, while a lower value was observed at the PAM200 and PAM300 groups. The content of total lipid firstly increased with increasing dietary PAM inclusion levels to the peak at the PAM200 group and then decreased at the PAM250 and PAM300 groups. The crude protein content of whole fish body in the PAM300 group was significantly higher than that of the PAM100 and PAM150 groups, while no significant difference was found in the whole-body ash content among the dietary treatment groups (Table 3).

Table 2: Growth performance of juvenile *Oncorhynchus mykiss*.

Items	Diets					Regression analysis		
	PAM100	PAM150	PAM200	PAM250	PAM300	Regression equation	R^2	P value
SR (%)	69.10 ± 1.64^{c}	79.82 ± 1.36^{b}	84.21 ± 1.12^{ab}	89.28 ± 1.19^{a}	83.53 ± 1.61^{ab}	$y = 0.0766x + 65.867$	0.174	0.122
DFI (%)	4.08 ± 0.12	4.39 ± 0.23	4.31 ± 0.47	4.09 ± 0.13	4.86 ± 0.66	$y = 0.0025x + 3.8346$	0.086	0.289
FBW (g)	20.40 ± 1.59^{c}	22.34 ± 0.75^{bc}	23.85 ± 0.33^{ab}	25.53 ± 0.65^{a}	24.76 ± 0.66^{ab}	$y = -0.00015x^2 + 0.08378x + 13.365$	0.650	0.002
WGR (%)	547.08 ± 46.10^{c}	621.72 ± 25.55^{bc}	665.41 ± 11.19^{ab}	716.48 ± 22.48^{a}	688.66 ± 20.45^{ab}	$y = -0.005643x^2 + 3.0132x + 299.19$	0.678	0.001
SGR (%/d)	4.33 ± 0.16^{b}	4.59 ± 0.08^{ab}	4.73 ± 0.03^{a}	4.88 ± 0.06^{a}	4.80 ± 0.06^{a}	$y = -0.000019x^2 + 0.01018x + 3.5013$	0.681	0.001
FCR	1.19 ± 0.04	1.13 ± 0.02	1.12 ± 0.03	1.08 ± 0.03	1.10 ± 0.03	$y = -0.00045x + 1.2136$	0.294	0.037
FER	0.84 ± 0.03	0.89 ± 0.01	0.89 ± 0.03	0.92 ± 0.02	0.91 ± 0.03	$y = 0.0003x + 0.8226$	0.293	0.037
PER	2.02 ± 0.07	2.10 ± 0.02	2.12 ± 0.07	2.21 ± 0.06	2.20 ± 0.07	$y = 0.0009x + 1.9468$	0.352	0.020
VSI (%)	11.81 ± 0.29^{ab}	12.38 ± 0.20^{ab}	11.49 ± 0.66^{b}	12.36 ± 0.50^{ab}	13.06 ± 0.28^{a}	$y = 0.000058x^2 - 0.0183x + 13.263$	0.113	0.013
HSI (%)	1.67 ± 0.16	1.72 ± 0.02	1.76 ± 0.18	1.73 ± 0.06	1.86 ± 0.09	$y = 0.000003x^2 - 0.00025x + 1.6834$	0.032	0.307

Data are presented as mean ± SE ($n = 3$). Values within the same row with different letters mean significant difference ($P < 0.05$). SR: survival rate; DFI: daily feed intake; FBW: final body weight; WGR: weight gain rate; SGR: specific growth rate; FCR: feed conversion ratio; FER: feed efficiency ratio; PER: protein efficiency ratio; VSI: viscerosomatic index; HSI: hepatosomatic index.

Table 3: Proximate composition of whole body (g/kg wet body) of juvenile *Oncorhynchus mykiss*.

Items	Diets					Regression analysis		
	PAM100	PAM150	PAM200	PAM250	PAM300	Regression equation	R^2	P value
Moisture	706.1 ± 3.6^{a}	701.5 ± 4.5^{ab}	686.6 ± 1.8^{b}	694.5 ± 4.6^{ab}	685.8 ± 8.3^{b}	$y = -0.00985x + 71.494$	0.383	0.014
Total lipid	134.1 ± 8.0^{b}	142.5 ± 5.5^{ab}	156.0 ± 4.1^{a}	154.8 ± 3.9^{a}	147.0 ± 3.4^{ab}	$y = -0.000139x^2 + 0.06291x + 8.3981$	0.536	0.010
Crude protein	139.1 ± 2.9^{bc}	137.6 ± 2.6^{c}	145.2 ± 3.2^{abc}	147.4 ± 2.3^{ab}	149.9 ± 2.6^{a}	$y = 0.00546x + 12.712$	0.472	0.005
Ash	29.2 ± 0.5	26.7 ± 1.9	27.4 ± 0.3	25.4 ± 0.6	25.6 ± 1.6	$y = -0.00165x + 3.0181$	0.300	0.034

Data are presented as mean ± SE (n =3). Values within the same row with different letters mean significant difference ($P < 0.05$).

Table 4: The amino acid compositions (g/kg dry matter) of whole body of juvenile *O. mykiss*.

Amino acids	Diets					Regression analysis		
	PAM100	PAM150	PAM200	PAM250	PAM300	Regression equation	R^2	P value
Arginine	42.7 ± 3.2	39.6 ± 4.8	41.1 ± 0.1	40.8 ± 1.6	40.5 ± 0.1	$y = -0.00063x + 4.2194$	0.025	0.661
Histidine	11.4 ± 0.1	16.1 ± 4.9	10.0 ± 2.6	10.4 ± 0.3	11.0 ± 0.3	$y = -0.00132x + 1.4424$	0.091	0.397
Isoleucine	27.1 ± 1.8	25.5 ± 2.5	26.1 ± 0.1	26.8 ± 0.8	27.1 ± 0.1	$y = 0.00027x + 2.5977$	0.016	0.729
Leucine	29.1 ± 2.8	26.7 ± 4.0	28.1 ± 0.1	28.5 ± 1.2	27.2 ± 0.1	$y = -0.00042x + 2.8764$	0.014	0.742
Lysine	19.2 ± 1.3	18.3 ± 1.7	18.8 ± 0.2	19.2 ± 0.6	19.7 ± 0.2	$y = 0.00037x + 1.8293$	0.057	0.505
Methionine	8.5 ± 0.1	9.4 ± 0.9	8.9 ± 0.7	10.1 ± 0.2	10.2 ± 0.3	$y = 0.00084x + 0.7755$	0.496	0.023
Phenylamine	14.5 ± 0.5	14.2 ± 0.7	13.9 ± 0.1	14.4 ± 0.3	15.2 ± 0.3	$y = 0.00031x + 1.3829$	0.377	0.283
Threonine	18.3 ± 1.1	13.4 ± 5.1	18.1 ± 0.2	16.9 ± 0.1	16.8 ± 0.1	$y = 0.000011x^2 - 0.00427x + 2.0344$	0.023	0.920
Tryptophan	0.42 ± 0.3	4.0 ± 0.4	4.1 ± 0.0	4.1 ± 0.2	4.1 ± 0.0	$y = -0.00005x + 0.4199$	0.025	0.661
Valine	19.3 ± 1.4	18.3 ± 1.8	1.86 ± 0.1	19.0 ± 0.6	19.5 ± 0.1	$y = 0.00024x + 1.8461$	0.021	0.689
∑EAA	194.3 ± 12.1	185.6 ± 16.7	187.7 ± 1.4	190.2 ± 7.1	191.3 ± 0.7	$y = 0.000057x^2 - 0.02304x + 21.032$	0.232	0.824
Alanine	12.2 ± 0.5	11.5 ± 0.8	11.7 ± 1.0	11.6 ± 0.3	12.0 ± 0.1	$y = 0.000005x^2 - 0.00216x + 1.372$	0.199	0.460
Aspartic acid	36.9 ± 4.1	33.7 ± 5.6	35.6 ± 0.1	35.4 ± 1.8	33.5 ± 0.6	$y = -0.001x + 3.7018$	0.045	0.557
Cystine	0.5 ± 0.0^{b}	0.8 ± 0.4^{ab}	1.6 ± 0.6^{a}	0.9 ± 0.1^{ab}	0.8 ± 0.1^{ab}	$y = -0.000006x^2 + 0.00256x - 0.1474$	0.033	0.615
Glutamic acid	19.4 ± 0.5	18.3 ± 1.4	19.3 ± 0.0	17.0 ± 0.3	15.9 ± 0.3	$y = -0.00166x + 2.1324$	0.409	0.047
Glycine	26.8 ± 0.3^{a}	25.4 ± 1.0^{ab}	24.9 ± 0.6^{ab}	24.3 ± 0.6^{b}	24.9 ± 0.2^{ab}	$y = -0.00096x + 2.7133$	0.459	0.031
Proline	15.6 ± 0.1^{ab}	15.4 ± 0.0^{bc}	14.8 ± 0.3^{c}	15.2 ± 0.2^{bc}	16.3 ± 0.3^{a}	$y = 0.00001x^2 - 0.00375x + 1.846$	0.736	0.009
Serine	37.2 ± 3.4	34.3 ± 5.0	36.2 ± 0.0	36.0 ± 1.8	34.6 ± 0.1	$y = -0.00071x + 3.7068$	0.028	0.645
Tyrosine	12.8 ± 0.5	12.5 ± 0.5	12.3 ± 1.0	12.7 ± 2.5	13.5 ± 0.2	$y = 0.000008x^2 - 0.00271x + 1.4777$	0.564	0.055
∑NEAA	161.3 ± 9.2	151.8 ± 13.5	156.3 ± 1.4	153.0 ± 7.3	151.5 ± 0.3	$y = -0.00369x + 16.217$	0.088	0.405
∑aa	355.6 ± 21.2	337.4 ± 30.2	344.0 ± 2.7	343.3 ± 14.3	342.8 ± 0.9	$y = -0.00398x + 35.259$	0.023	0.679
∑EAA/∑AA	0.54 ± 0.1^{b}	0.55 ± 0.00^{ab}	0.55 ± 0.00^{ab}	0.55 ± 0.01^{ab}	0.56 ± 0.00^{a}	$y = 0.00006x + 0.5398$	0.681	0.003

Data are presented as mean ± SE (n =3). ∑EAA: total essential amino acids; ∑NEAA: total nonessential amino acids; ∑AA: total amino acids.

There were no significant differences in the contents of total essential amino acids (∑EAA), total nonessential amino acids (∑NEAA), and total amino acids (∑AA) of whole fish body among the dietary treatment groups (Table 4). There were significant positive correlations between the contents of methionine, proline, or ∑EAA/∑AA in the whole fish body and dietary PAM content, while significant negative correlations were observed between the contents of glutamic acid or glycine and dietary PAM content ($P < 0.05$). The whole-body cystine content of the PAM200 group was significantly higher than that of the PAM100 group, while the highest glycine content was observed at the PAM100 group ($P < 0.05$). The whole-body proline content of the PAM300 group was significantly higher than that of the PAM150, PAM200, and PAM250 groups, while the ∑EAA/∑AA ratio increased significantly with increasing dietary PAM inclusion levels ($P < 0.05$).

The fatty acid composition of the whole fish body is shown in Table 5. The contents of C20:1n9, C20:3n6, C20:2n6, and ∑n-6PUFA in the whole fish body showed significantly positive correlations with dietary PAM content, while the contents of C14:0, C15:0, C17:0, C16:2n6, C18:4n3, C20:4n6, C20:5n3, C20:4n3, C22:6n3, C22:5n3, ∑PUFA, ∑n-3PUFA, n-3/n-6 PUFA, and ∑LC-PUFA showed significantly negative correlations with dietary PAM content ($P < 0.05$). For the saturated fatty acid, the contents of C14:0, C15:0, and C17:0 decreased significantly with increasing dietary PAM inclusion levels, while the highest contents of C18:0, C20:0, and C22:0 were observed in the PAM250 group ($P < 0.05$). For the monounsaturated fatty acids, the highest content of C20:1n9 was recorded in the PAM250 group, while no significant differences were found in the contents of other monounsaturated fatty acids and total monounsaturated fatty acids (∑MUFA). For polyunsaturated fatty acids, the highest contents of C16:2n6, C18:3n6, C18:4n3, C20:5n3, C22:5n3, C22:2n6, total polyunsaturated fatty acids (∑PUFA), ∑n-3PUFA, and total long-chain polyunsaturated fatty acids (∑LC-PUFA), as well as n-3/n-6PUFA ratio, were detected at the PAM100 group, while the highest ∑n-6PUFA was observed in the PAM300 group ($P < 0.05$).

3.3. Metabolism-Related Parameter in the Liver and Serum. There were significant positive correlations between the levels of liver AST, liver LPS, serum TP, or serum AST and dietary PAM content ($P < 0.05$), while no significant correlation was found for the other metabolism-related parameters (Table 6). For amino acid metabolism, the highest liver ALT

Table 5: The fatty acid compositions (g/kg total fatty acids) of whole body of juvenile *O. mykiss*.

Fatty acids	Diets					Regression analysis		
	PAM100	PAM150	PAM200	PAM250	PAM300	Regression equation	R^2	P value
C14:0	32.3 ± 0.5^{a}	30.8 ± 0.7^{ab}	30.7 ± 0.3^{b}	30.2 ± 0.0^{b}	29.0 ± 0.5^{b}	$y = -0.00145x + 3.3532$	0.667	0.000
C15:0	5.9 ± 0.1^{a}	5.5 ± 0.2^{ab}	5.1 ± 0.1^{b}	5.0 ± 0.1^{bc}	4.5 ± 0.2^{c}	$y = -0.0007x + 0.6582$	0.808	0.000
C16:0	128.6 ± 2.8	132.3 ± 5.1	128.0 ± 2.6	128.6 ± 1.0	136.4 ± 5.1	$y = 0.00382x + 12.387$	0.157	0.128
C17:0	7.1 ± 0.1^{a}	6.5 ± 0.2^{b}	6.4 ± 0.1^{b}	6.2 ± 0.2^{b}	5.5 ± 0.03^{c}	$y = -0.00069x + 0.77$	0.736	0.000
C18:0	76.5 ± 0.2^{ab}	73.8 ± 0.9^{b}	79.0 ± 1.2^{a}	79.4 ± 1.4^{a}	74.3 ± 1.6^{b}	$y = -0.000029x^2 + 0.01171x + 6.6181$	0.181	0.274
C20:0	4.9 ± 0.1^{bc}	4.5 ± 0.1^{c}	4.9 ± 0.1^{bc}	5.4 ± 0.2^{a}	5.0 ± 0.2^{ab}	$y = 0.00017x + 0.4578$	0.121	0.188
C22:0	2.7 ± 0.1^{ab}	2.4 ± 0.1^{b}	2.6 ± 0.0^{b}	2.9 ± 0.1^{a}	2.7 ± 0.1^{ab}	$y = 0.00008x + 0.2476$	0.096	0.242
∑SFA	258.0 ± 2.0	255.9 ± 2.8	256.7 ± 0.9	257.7 ± 1.2	258.5 ± 2.4	$y = 0.000023x^2 - 0.00833x + 26.371$	0.151	0.345
C16:1n7	75.5 ± 3.1	71.3 ± 2.9	73.8 ± 1.7	72.1 ± 1.9	69.2 ± 2.6	$y = -0.00303x + 7.8099$	0.240	0.054
C17:1n7	4.6 ± 0.3	4.4 ± 0.3	4.9 ± 0.1	4.4 ± 0.20	4.3 ± 0.3	$y = -0.0002x + 0.4902$	0.116	0.197
C18:1n9	169.3 ± 3.3	172.6 ± 4.4	160.5 ± 2.4	161.7 ± 1.7	167.2 ± 5.2	$y = 0.000068x^2 - 0.02891x + 19.418$	0.178	0.280
C20:1n9	40.6 ± 1.1^{b}	40.5 ± 1.7^{b}	43.8 ± 1.2^{ab}	46.3 ± 0.5^{a}	44.7 ± 1.4^{ab}	$y = 0.00245x + 3.8096$	0.400	0.009
C22:1n9	7.9 ± 0.6	8.0 ± 0.2	7.4 ± 0.3	7.6 ± 0.6	7.4 ± 0.4	$y = -0.00037x + 0.8366$	0.139	0.156
∑MUFA	298.0 ± 1.6	296.8 ± 2.8	290.3 ± 1.7	292.1 ± 3.2	292.8 ± 1.3	$y = -0.00257x + 29.936$	0.190	0.092
C16:2n6	2.3 ± 0.0^{a}	2.2 ± 0.0^{ab}	2.2 ± 0.0^{ab}	2.0 ± 0.1^{b}	1.9 ± 0.1^{b}	$y = -0.0002x + 0.2536$	0.536	0.001
C18:3n6	6.4 ± 0.4^{a}	5.3 ± 0.2^{b}	5.7 ± 0.1^{ab}	6.1 ± 0.0^{ab}	5.5 ± 0.3^{b}	$y = -0.00028x + 0.6323$	0.137	0.159
C18:4n3	14.9 ± 0.7^{a}	12.9 ± 0.4^{b}	8.4 ± 0.1^{c}	8.2 ± 0.1^{c}	8.5 ± 0.4^{c}	$y = -0.00323x + 1.7158$	0.730	0.000
C18:2n6	128.6 ± 2.8	132.3 ± 5.1	128.0 ± 2.6	128.6 ± 1.0	136.4 ± 5.1	$y = 0.00382x + 12.387$	0.157	0.128
C20:4n6	14.4 ± 0.5	13.6 ± 0.1	14.2 ± 0.7	13.7 ± 0.1	13.0 ± 0.6	$y = -0.00064x + 1.4998$	0.278	0.036
C20:5n3	26.8 ± 1.2^{a}	23.4 ± 0.7^{b}	20.6 ± 0.3^{c}	19.5 ± 0.3^{cd}	17.7 ± 0.3^{d}	$y = -0.0044x + 3.0409$	0.893	0.000
C20:3n6	14.2 ± 0.7^{bc}	13.6 ± 0.5^{c}	16.1 ± 0.7^{ab}	15.7 ± 0.3^{ab}	16.3 ± 0.7^{a}	$y = 0.00111x + 1.2877$	0.368	0.013
C20:4n3	12.4 ± 0.9	12.1 ± 0.9	11.3 ± 0.4	10.9 ± 0.4	11.2 ± 0.5	$y = -0.00079x + 1.3124$	0.254	0.046
C20:2n6	34.7 ± 1.1^{b}	35.7 ± 1.4^{b}	40.5 ± 1.7^{a}	41.3 ± 0.2^{a}	40.3 ± 1.3^{a}	$y = 0.003x + 3.231$	0.478	0.003
C22:5n6	6.1 ± 0.5	5.8 ± 0.2	6.3 ± 0.2	6.3 ± 0.1	5.7 ± 0.2	$y = -0.000003x^2 + 0.00128x + 0.5014$	0.125	0.419
C22:6n3	126.3 ± 2.1^{a}	126.4 ± 2.4^{a}	126.1 ± 1.1^{a}	126.3 ± 1.0^{a}	119.4 ± 1.0^{b}	$y = -0.00333x + 13.129$	0.373	0.012
C22:5n3	12.3 ± 0.7^{a}	11.2 ± 0.2^{b}	10.6 ± 0.1^{bc}	10.2 ± 0.1^{bc}	9.6 ± 0.3^{c}	$y = -0.00135x + 1.3465$	0.773	0.000
C22:2n6	26.5 ± 0.8^{a}	24.8 ± 0.7^{ab}	23.4 ± 0.1^{b}	25.5 ± 0.7^{ab}	24.4 ± 0.8^{ab}	$y = -0.00078x + 2.6442$	0.157	0.129
∑PUFA	426.1 ± 2.4^{a}	418.4 ± 2.5^{b}	413.5 ± 0.7^{bc}	414.2 ± 0.4^{bc}	410.2 ± 0.5^{c}	$y = -0.00703x + 43.06$	0.704	0.000
∑n-3PUFA	192.8 ± 2.8^{a}	185.9 ± 0.8^{b}	177.0 ± 0.8^{c}	175.1 ± 0.5^{c}	166.4 ± 1.7^{d}	$y = -0.0131x + 20.545$	0.922	0.000
∑n-6PUFA	233.3 ± 0.6^{c}	232.5 ± 1.9^{c}	236.5 ± 1.3^{bc}	239.1 ± 0.9^{b}	243.8 ± 1.9^{a}	$y = 0.00607x + 22.515$	0.726	0.000
n-3/n-6PUFA	0.83 ± 0.01^{a}	0.80 ± 0.01^{a}	0.75 ± 0.01^{b}	0.73 ± 0.00^{b}	0.68 ± 0.01^{c}	$y = -0.00074x + 0.9045$	0.921	0.000
∑LC-PUFA	239.2 ± 2.9^{a}	230.7 ± 1.9^{b}	228.6 ± 0.4^{b}	228.0 ± 0.4^{b}	217.5 ± 3.9^{c}	$y = -0.01028x + 24.884$	0.729	0.000

Data are presented as mean ± SE (n =3). ∑SFA: total saturated fatty acids; ∑MUFA: total monounsaturated fatty acids; ∑PUFA: total polyunsaturated fatty acids; ∑LC-PUFA: total long-chain polyunsaturated fatty acids.

activity was detected in the PAM150 group, while a significantly higher liver AST activity was detected in the PAM200, PAM250, and PAM300 groups compared with the PAM100 group (Table 6). Significantly higher serum TP content was detected in the PAM200, PAM250, and PAM300 groups as compared to the PAM100 and PAM150 groups, while the highest serum T-AA content was detected in the PAM150 group. The serum ALT activity of the PAM200 group was significantly higher than that of the PAM150 and PAM300 groups but was not significantly different from the PAM100 and PAM200 groups. The serum AST activity of the PAM100 group was significantly lower than that of other dietary groups. For the lipid metabolism, the highest and the lowest liver LPS activity was detected in the PAM300 and PAM100 groups, respectively, while no significant differences were found for the TG and T-CHO contents in the liver and serum among the dietary treatment groups. For the carbohydrate metabolism, there were no significant differences in the liver and serum LDH activity as well as serum GLU content among the dietary groups, while the liver GLU content of the PAM150 group was significantly higher than that of other dietary groups.

Table 6: Metabolism indices of liver and serum of juvenile *O. mykiss*.

Items	Diets					Regression analysis		
	PAM100	PAM150	PAM200	PAM250	PAM300	Regression equation	R^2	*P* value
Amino acid metabolism								
Liver								
Trypsin (U/mg protein)	2334.26 ± 259.57	2180.06 ± 186.51	1814.83 ± 336.62	1756.27 ± 191.84	1874.90 ± 234.61	$y = -2.7428x + 2534.9$	0.103	0.089
ALT (U/g protein)	19.60 ± 3.43^{b}	38.33 ± 3.38^{a}	22.21 ± 1.68^{b}	26.91 ± 3.50^{b}	25.99 ± 4.78^{b}	$y = -0.000548x^2 + 0.22044x + 7.0959$	0.053	0.493
AST (U/g protein)	38.99 ± 3.16^{b}	45.51 ± 2.34^{ab}	47.85 ± 1.02^{a}	49.03 ± 2.71^{a}	50.68 ± 3.74^{a}	$y = 0.06198x + 33.605$	0.335	0.001
Serum								
TP (mg/mL)	15.47 ± 0.68^{b}	15.31 ± 0.45^{b}	18.37 ± 0.57^{a}	17.75 ± 0.67^{a}	18.50 ± 0.80^{a}	$y = 0.01697x + 13.686$	0.356	0.001
T-AA (umol/mL)	34.38 ± 1.51^{b}	55.00 ± 7.29^{a}	46.88 ± 5.46^{ab}	45.63 ± 5.60^{ab}	45.63 ± 4.69^{ab}	$y = -0.000982x^2 + 0.41911x + 5.875$	0.112	0.201
ALT (U/mL)	3.35 ± 0.48^{ab}	2.55 ± 0.38^{b}	3.96 ± 0.89^{a}	3.34 ± 0.43^{ab}	2.58 ± 0.56^{b}	$y = -0.000056x^2 + 0.02098x + 1.4863$	0.034	0.627
AST (U/mL)	13.21 ± 0.80^{b}	23.72 ± 2.27^{a}	31.65 ± 2.92^{a}	28.25 ± 4.22^{a}	33.52 ± 4.70^{a}	$y = 0.09032x + 8.0053$	0.3824	0.000
Urea (mmol/mL)	6.13 ± 0.13	5.96 ± 0.33	6.62 ± 0.41	6.35 ± 0.14	5.70 ± 0.33	$y = -0.000055x^2 + 0.02091x + 4.4305$	0.109	0.211
Lipid metabolism								
Liver								
LPS (U/mg protein)	9.96 ± 1.00^{c}	13.26 ± 1.69^{ab}	15.88 ± 2.12^{ab}	11.73 ± 0.75^{bc}	17.73 ± 1.98^{a}	$y = 0.02718x + 8.106$	0.210	0.021
TG (mmol/g protein)	0.30 ± 0.05	0.25 ± 0.04	0.46 ± 0.11	0.26 ± 0.02	0.30 ± 0.06	$y = -0.000007x^2 + 0.00265x + 0.0795$	0.030	0.665
T-CHO (mmol/g protein)	0.26 ± 0.07	0.24 ± 0.10	0.26 ± 0.07	0.22 ± 0.04	0.27 ± 0.07	$y = 0.000003x^2 - 0.00104x + 0.3378$	0.010	0.930
Serum								
TG (mmol/L)	4.34 ± 0.82	5.60 ± .069	4.66 ± 0.50	4.65 ± 0.70	3.62 ± 0.92	$y = -0.00476x + 5.5289$	0.0362	0.314
T-CHO (mmol/L)	13.19 ± 1.30	14.34 ± 1.55	15.56 ± 1.14	14.76 ± 1.49	12.12 ± 1.10	$y = -0.000274x^2 + 0.1062x + 5.0912$	0.134	0.143
Carbohydrate metabolism								
Liver								
LDH (U/g protein)	141.78 ± 36.93	132.54 ± 13.46	171.25 ± 29.99	105.36 ± 10.46	169.30 ± 33.72	$y = 0.08093x + 134.85$	0.005	0.699
GLU (mmol/g protein)	1.10 ± 0.25^{ab}	1.48 ± 0.23^{a}	1.22 ± 0.16^{ab}	0.92 ± 0.07^{b}	0.84 ± 0.08^{b}	$y = -0.00214x + 1.5414$	0.112	0.071
Serum								
LDH (U/mL)	24.91 ± 0.54	24.23 ± 0.64	25.04 ± 0.30	24.12 ± 0.21	25.61 ± 0.55	$y = 0.000075x^2 - 0.02723x + 26.871$	0.302	0.288
GLU (mmol/mL)	5.12 ± 0.27	5.56 ± 0.34	5.77 ± 0.34	4.80 ± 0.45	5.26 ± 0.46	$y = -0.000033x^2 + 0.01223x + 4.3359$	0.028	0.682

Data are presented as mean ± SE (n =3). Values within the same row with different letters mean significant difference ($P < 0.05$). TP: total dissoluble protein; T-AA: total amino acid; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LPS: lipase; TG: triglyceride; T-CHO: total cholesterol; LDH: lactic dehydrogenase; GLU: glucose.

3.4. Antioxidant and Immune-Related Parameters in the Liver and Serum. There were significant positive correlations between the levels of liver CAT, AKP, and LZM, serum T-AOC, CAT, LZM, IgM, complement C3, and dietary PAM content, while a significant negative correlation was observed between the serum AKP activity and dietary PAM content ($P < 0.05$, Table 7). For the liver, there were no significant differences in the activities of T-AOC and SOD among the dietary treatment groups (Table 7). The highest CAT and AKP activities were detected in the PAM300 group, and the lowest MDA and ACP levels were detected in the PAM250 group, while the LZM activity showed an increasing trend with elevated dietary PAM inclusion levels. For the serum, the highest activities of T-AOC, CAT, and SOD were detected in the PAM250 group, while there were no significant differences in the MDA and LZM levels among the dietary groups. The ACP and AKP activities of the PAM150 group were significantly higher than those of the PAM200, PAM250, and PAM300 groups, while higher contents of IgM and complement C3 were detected in the PAM200, PAM250, and PAM300 groups.

4. Discussion

A series of studies have reported the effects of dietary fishmeal replacement with insect proteins, such as *H. illucens* [34] and *T. molitor* [35], on fish growth performance, but limited studies are available about the substitution of *P. americana* meal for dietary fishmeal. Jeong et al. [35] found that dietary supplementation with 14% of *T. molitor* meal could significantly improve the weight gain rate, specific growth rate, and feed utilization of *O. mykiss*. Partial dietary fishmeal replacement with *H. illucens* meal could increase the growth performance and feed utilization of juvenile *O. mykiss* [36] and Siberian sturgeon *Acipenser baerii* [37]. Similarly, 50% dietary fishmeal replacement with silkworm pupae *Bombyx mori* could improve the growth performance and body protein deposition of genetically improved farmed tilapia (GIFT) *Oreochromis niloticus* [38]. In this study, diets containing 200, 250, and 300 g/kg *P. americana* meal could improve the growth performance of juvenile *O. mykiss*. Possible explanations for such results include the following: (1) the *P. americana* meal is rich in protein (similar to the fishmeal) and essential amino acids (Table 8), and the addition of *P. americana* meal to feed may improve the dietary protein diversity and amino acid balance, which improved the dietary protein utilization in juvenile *O. mykiss*; (2) *P. americana* is rich in antibacterial peptides, polysaccharides, nucleosides, polyols, and other active ingredients [39], which may improve the health of *O. mykiss*, thereby facilitating their growth [40].

Previous studies have shown that dietary fishmeal replacement with insect proteins has different effects on the proximate compositions of different aquatic animals. The content of whole-body lipid of juvenile turbot *Psetta maxima* showed a decreasing trend with increasing dietary fishmeal replacement levels with *H. illucens* [41], while dietary fishmeal replacement with larvae *H. illucens* has no significant effects on the contents of crude protein and crude lipid in juvenile obscure puffer *Takifugu obscures* [42]. Similar results were found in hybrid yellow catfish *Pelteobagrus fulvidraco* [43]. In this study, the crude protein content of whole fish body showed an increasing trend with the elevated dietary *P. americana* meal inclusion levels. This may be because that appropriate dietary *P. americana* meal ratio could improve the protein diversity and the balance of amino acids, which is conducive to the digestion, absorption, and utilization of feed protein, and thereby is conducive to the body protein deposition. The reasons for the increasing body total lipid content may be because that the *P. americana* meal is rich in n-6 polyunsaturated fatty acids (n-6 PUFA), and dietary PAM inclusion increases the n-6PUFA content in the feed, which may promote the synthesis of triglycerides and lipid deposition in the fish body. Similar results have been found in black tiger shrimp *Penaeus monodon* [44] and Chinese mitten crab *Eriocheir sinensis* [45], but the underlying mechanism remains to be further studied.

For the fatty acid composition of the whole fish body, the contents of EPA, DHA, ∑PUFA, and ∑n-3PUTA decreased with increasing dietary PAM inclusion levels, which was opposite to the corresponding fatty acid content in the feed. The ∑n-6PUFA content increased with increasing dietary PAM inclusion levels. The possible reasons for such results may be that the *P. americana* meal contains a certain amount of chitin, and dietary PAM inclusion increased the chitin content in the feed, thereby inhibiting the digestion and absorption of fatty acids and other lipids [46, 47]. Dietary PAM inclusion reduced the content of long-chain polyunsaturated fatty acids (∑LC-PUFA) in the whole fish body. The possible reasons might be higher dietary contents of EPA and DHA, which inhibit the synthesis of LC-PUFA [48–50].

The amino acid composition in the fish body generally reflects the dietary protein level and amino acid composition. Dietary fishmeal replacement with *B. mori* meal could meet the amino acid requirements of mirror carp *Cyprinus carpio* var. *specularis* fingerlings, while 50% dietary fishmeal replacement has a significant effect on several amino acids in the fish body [51]. Similarly, 0%-24% dietary fishmeal replacement with larvae *H. illucens* meal has no significant effect on the amino acid composition of juvenile *T. obscures* [42]. Our study showed that the ratio of total essential amino acids to total amino acids in the whole body of juvenile *O. mykiss* increased with the increase of dietary PAM inclusion levels. This may be because that the dietary supplementation with *P. americana* meal improved the balance of amino acids in the feed, which was conducive to the deposition of essential amino acids.

The metabolism of aquatic animals can be understood by detecting the metabolic indices. For amino acid metabolism indices, the AST and ALT are two important aminotransferases, which play important roles in the decomposition and synthesis of amino acids [52, 53]. When the dietary amino acid is insufficient, the activities of AST and ALT of animals are lower, and the transamination is weak; conversely, when the dietary amino acid is appropriate, the activities of AST and ALT increase, and the transamination is enhanced correspondingly [54]. In this study, dietary

Table 7: Antioxidant and immune-related indices in serum and liver of juvenile *O. mykiss*.

Items	Diets					Regression analysis		
	PAM100	PAM150	PAM200	PAM250	PAM300	Regression equation	R^2	*P* value
Liver								
T-AOC (U/mg protein)	0.89 ± 0.04	0.92 ± 0.22	0.76 ± 0.05	1.03 ± 0.14	0.81 ± 0.12	$y = -0.00014x + 0.9109$	0.001	0.863
CAT (U/mg protein)	13.14 ± 0.69^{b}	16.28 ± 0.63^{ab}	12.93 ± 0.36^{b}	16.65 ± 2.19^{ab}	17.87 ± 1.99^{a}	$y = 0.01968x + 11.439$	0.141	0.041
SOD (U/mg protein)	1749.28 ± 152.76	1701.35 ± 97.11	1836.84 ± 108.09	2075.24 ± 154.80	1932.04 ± 176.54	$y = 1.4788x + 1563.2$	0.093	0.101
MDA (nmol/mg protein)	2.58 ± 0.54^{b}	3.46 ± 0.23^{ab}	3.54 ± 0.20^{ab}	2.43 ± 0.33^{b}	3.86 ± 0.43^{a}	$y = 0.00307x + 2.5596$	0.047	0.250
ACP (U/100 g protein)	23.73 ± 2.18^{a}	23.32 ± 1.93^{a}	20.11 ± 0.57^{ab}	17.83 ± 0.89^{b}	21.47 ± 2.00^{ab}	$y = -0.02003x + 25.296$	0.110	0.074
AKP (100 g protein)	3.94 ± 0.39^{ab}	3.67 ± 0.59^{ab}	2.57 ± 0.15^{b}	3.06 ± 0.34^{ab}	4.40 ± 0.65^{a}	$y = 0.000137x^2 - 0.05422x + 8.2048$	0.223	0.033
LZM (U/mg protein)	119.73 ± 9.38^{c}	113.39 ± 8.91^{c}	233.98 ± 4.61^{b}	219.68 ± 7.95^{b}	412.02 ± 6.96^{a}	$y = 1.3817x - 56.582$	0.796	0.000
Serum								
T-AOC (U/mL)	7.96 ± 0.58^{b}	7.30 ± 0.23^{b}	7.73 ± 0.62^{b}	9.81 ± 0.51^{a}	8.83 ± 0.58^{ab}	$y = 0.00851x + 6.6206$	0.167	0.025
CAT (U/mL)	14.52 ± 0.69^{bc}	15.12 ± 0.65^{bc}	13.14 ± 0.21^{c}	23.68 ± 0.73^{a}	16.06 ± 1.64^{b}	$y = 0.02332x + 11.84$	0.152	0.033
SOD (U/mL)	136.77 ± 5.62^{a}	148.07 ± 2.77^{a}	114.39 ± 8.72^{b}	141.91 ± 5.68^{a}	141.50 ± 6.23^{a}	$y = 0.00108x^2 - 0.42528x + 173$	0.068	0.384
MDA (nmol/mL)	12.79 ± 0.63	14.68 ± 1.04	14.18 ± 1.05	15.51 ± 1.96	15.27 ± 1.14	$y = 0.0116x + 12.165$	0.078	0.136
ACP (U/100 mL)	5.62 ± 0.43^{ab}	6.97 ± 0.40^{a}	5.08 ± 0.57^{b}	4.96 ± 0.56^{b}	5.41 ± 0.48^{b}	$y = -0.00487x + 6.58$	0.068	0.162
AKP (100 mL)	22.16 ± 1.52^{ab}	26.68 ± 3.36^{a}	12.77 ± 0.82^{c}	16.96 ± 1.03^{bc}	17.86 ± 1.48^{bc}	$y = -0.03666x + 26.62$	0.210	0.042
LZM ($\times 10^3$ U/mL)	5.72 ± 0.68	4.75 ± 0.52	6.71 ± 1.47	8.87 ± 1.81	8.31 ± 1.82	$y = 18.627x + 3146.7$	0.146	0.037
IgM (μg/mL)	577.36 ± 94.84^{b}	778.02 ± 171.18^{ab}	1248.07 ± 287.21^{a}	1164.51 ± 103.46^{a}	1037.03 ± 136.52^{ab}	$y = 2.6116x + 438.67$	0.279	0.043
Complement C3 (μg/mL)	599.38 ± 132.60^{b}	605.05 ± 161.28^{b}	1496.72 ± 249.84^{a}	1229.38 ± 163.04^{a}	1419.21 ± 93.90^{a}	$y = 4.5279x + 164.36$	0.488	0.004

Data are presented as mean ± SE ($n = 3$). Values within the same row with different letters mean significant difference ($P < 0.05$). T-AOC: total antioxidant capacity; SOD: superoxide dismutase; MDA: malondialdehyde; ACP: acid phosphatase; AKP: alkaline phosphatase; LZM: lysozyme; IgM: immunoglobulin M.

Table 8: Nutritional components of fishmeal, *P. americana* meal, and five experimental diets.

Items	Fishmeal	PAM	Diets				
			PAM100	PAM150	PAM200	PAM250	PAM300
Proximate composition (g/kg dry diet)							
Moisture	63.7	57.9	78.4	63.9	73.3	72.6	97.9
Crude protein	668.7	691.1	416.8	423.4	419.6	419.4	414.7
Total lipid	83.0	165.6	224.7	227.0	234.1	227.9	233.5
Ash	128.0	45.0	79.8	77.7	72.7	69.0	62.4
Gross energy (KJ/g)			22.22	22.18	22.19	22.23	22.20
Fatty acid composition (g/kg total fatty acids)							
C14:0	32.4	15.3	28.7	28.7	29.8	31.2	30.7
C15:0	7.5	2.1	5.0	5.0	5.4	5.7	5.4
C16:0	185.3	231.2	179.7	179.7	165.4	166.3	170.5
C17:0	14.4	6.8	7.4	7.4	8.4	8.6	8.2
C18:0	99.2	127	75.4	75.4	86.6	91.2	90.4
C20:0	12.4	12.9	8.0	8.9	9.2	10.0	9.6
C22:0	6.1	4.6	5.2	5.2	5.9	6.3	5.8
∑SFA	357.2	399.9	309.5	309.5	310.7	319.4	320.6
C16:1n7	59.2	34.8	39.9	39.9	43.5	46.0	44.4
C17:1n7	3.0	3.0	3.0	3.0	3.5	3.3	3.2
C18:1n9	250.2	231.2	207.6	207.6	191.9	166.3	170.5
C20:1n7	9.1	5.5	26.3	26.3	38.1	31.4	31.0
C22:1n9	3.3	0.0	5.4	5.4	5.4	6.0	6.8
∑MUFA	324.8	274.5	282.2	282.2	282.5	253	256
C16:2n6	2.1	1.7	2.2	2.2	2.5	2.8	2.7
C18:4n3	5.7	0.0	14.1	14.1	14.2	15.2	15.0
C18:2n6	88.4	231.2	179.7	179.7	165.4	166.3	170.5
C20:4n6	14.8	13.2	6.6	6.6	7.6	8.3	8.0
C20:5n3	38.0	5.8	40.6	40.6	43.2	45.4	45.0
C22:6n3	85.4	3.1	72.8	72.8	77.3	80.3	76.8
C22:2n6	2.6	9.5	37.3	37.3	40.9	44.8	47.1
∑PUFA	236.9	264.4	353.3	353.3	351.1	363.1	365.2
∑n-3PUFA	129.1	8.9	127.5	127.5	134.7	141.0	136.8
∑n-6PUFA	107.8	255.6	225.8	225.8	216.4	222.2	228.4
n-3/n-6PUFA	1.20	0.03	0.56	0.56	0.62	0.63	0.60
∑LC-PUFA	140.7	31.6	157.3	157.3	169.0	178.8	176.9
Amino acid composition (g/kg dry diet)							
Essential amino acids							
Arginine	27.7	20.5	44.1	39.9	42.3	45.1	41.6
Histidine	2.9	6.6	4.4	4.5	11.6	12.2	11.5
Isoleucine	54.4	36.4	31.8	29.6	30.6	31.0	28.8
Leucine	30.7	21.4	18.2	16.7	17.5	19.8	17.7
Lysine	6.7	20.4	20.4	19.1	19.3	19.7	18.2
Methionine	4.6	3.3	6.0	6.2	6.3	6.8	5.6
Phenylalanine	31.0	19.0	17.4	17.3	17.2	16.9	15.9
Threonine	23.9	14.6	20.1	19.5	15.1	15.1	14.2
Tryptophan	6.7	4.8	4.5	4.2	4.3	4.5	4.1
Valine	48.3	30.6	24.0	22.0	22.4	22.6	20.8
∑EAA	236.8	177.6	190.9	179.0	186.6	193.5	178.2

Table 8: Continued.

Items	Fishmeal	PAM	Diets				
			PAM100	PAM150	PAM200	PAM250	PAM300
Nonessential amino acids							
Alanine	34.9	32.3	13.2	12.6	12.7	12.9	11.7
Aspartic acid	45.1	38.5	34.3	28.7	30.1	33.9	29.5
Cystine	7.9	2.1	1.2	1.1	1.1	1.6	1.0
Glutamic acid	68.8	54.9	29.7	26.6	26.6	26.7	22.5
Glycine	54.9	40.5	26.0	24.5	24.1	23.5	21.6
Proline	59.0	26.4	25.7	24.2	23.4	23.0	21.1
Serine	54.2	18.4	45.4	39.2	41.7	46.4	40.8
Tyrosine	19.7	30.2	14.8	15.7	16.4	16.9	16.5
∑NEAA	344.4	243.2	190.3	172.6	176	184.9	164.6
∑AA	581.3	420.8	381.2	351.6	362.6	378.4	342.9
∑EAA/∑AA	0.41	0.42	0.50	0.51	0.51	0.51	0.52

∑SFA: total saturated fatty acids; ∑MUFA: total monounsaturated fatty acids; ∑PUFA: total polyunsaturated fatty acids; ∑LC-PUFA: total long-chain polyunsaturated fatty acids; ∑EAA: total essential amino acids; ∑NEAA: total nonessential amino acids; ∑AA: total amino acids.

PAM inclusion increased the activities of AST and ALT in the liver of juvenile *O. mykiss*, which indicates that dietary PAM inclusion could promote the amino acid metabolism of juvenile *O. mykiss*. Urea is the main metabolite of protein metabolism, which is believed to directly reflect protein metabolism [55]. In this study, dietary PAM inclusion increased the urea content in the liver of juvenile *O. mykiss*, which may reflect decreasing ammonia excretion rate of *O. mykiss* fed diets with PAM inclusion. A previous study has reported that elevated serum total protein (TP) may imply an improved innate immune system in the aquatic animal [56]. In this study, the serum TP content of juvenile *O. mykiss* from PAM200, PAM250, and PAM300 groups was significantly higher compared to the PAM100 and PAM150 groups. Such a result suggests that the metabolism of *O. mykiss* was enhanced and fish are in good health condition.

For lipid metabolism indices, the lipase (LPS) is an important enzyme for lipid digestion and absorption in aquatic animals [57, 58]. Previous studies showed that the 40%-80% of dietary fishmeal replacement with maggot meal could increase the liver LPS activity of *P. fulvidraco*, and the authors suggested that such result might be related to the lower content of polyunsaturated fatty acids in the feed lipid source and fly maggot meal [59]. Dietary fishmeal replacement by *T. molitor* meal significantly increased the intestine LPS activity of bullfrog, and the authors concluded that dietary fishmeal replacement increased the crude lipid content of the feed and therefore required more lipase for digestion [60]. Our study showed that higher liver LPS activity was detected in the PAM150, PAM200, PAM250, and PAM300 groups. Such results indicate that partial dietary PAM inclusion may be beneficial for lipid digestion and absorption of juvenile *O. mykiss*.

The carbohydrates metabolism is an important physiological metabolic process, and the carbohydrates can provide energy and carbon sources for the growth and metabolism of aquatic animals [61]. Previous studies showed that partially dietary fishmeal replacement with defatted *H. illucens* meal had no significant effects on the serum glucose level of juvenile *O. mykiss* [62], while replacing 66% of dietary fishmeal with black soldier fly larvae meal significantly increased the serum glucose levels in Atlantic salmon *Salmo salar* [18]. In addition, replacing 30% of dietary fishmeal with *M. domestica* meal significantly increased serum glucose levels in European sea bass *Dicentrarchus labrax*, while using *T. molitor* meal to replace 30% of dietary fishmeal significantly decreased the serum glucose level [63]. In this study, the liver glucose content of the PAM150 group was significantly higher than that of other dietary groups, which indicates that appropriate dietary PAM inclusion could increase the liver glucose of juvenile *O. mykiss*. This may be because that the *P. americana* meal contains a certain amount of chitin [64], which is digested and decomposed into glucose by juvenile *O. mykiss*, and causes the increasing liver glucose levels.

Antioxidative enzymes, such as CAT and SOD, are important components in the antioxidant defense system of fish [65]. T-AOC is a comprehensive reflection of the antioxidant capacity of aquatic animals [66]. MDA content can be used to assess the degree of lipids peroxidation in fish [67]. In this study, the highest levels of antioxidant enzyme activities and the lowest serum MDA content were detected in PAM250 group. Such results suggested that the dietary 250 g/kg PAM inclusion could improve the antioxidant capacity of juvenile *O. mykiss*. This may be closely related to the fact that *P. americana* L contains organic acids, polysaccharides, and mineral elements [68–70].

Fish have nonspecific and specific immunity, and the protection of fish against diseases is mainly carried out by the nonspecific immune system [71]. AKP is related to the metabolism of RNA, DNA, protein, and lipid in the body and plays an important role in the immune system of fish [72]. Lower AKP activity in the serum and liver was detected at the PAM200 and PAM250 groups. This may be related to the high content of chitosan in *P. americana*. Previous studies have shown that high levels of chitosan in feed can

significantly reduce AKP activity in juvenile *O. mykiss* [73]. However, the specific reasons remain to be further studied. Lysozyme (LZM) is one of the important indicators of fish immunity [74]. In this study, dietary PAM inclusion levels at 200-300 g/kg significantly increased the LZM activity in the liver and serum of juvenile *O. mykiss*, which indicates that appropriate dietary PAM inclusion can increase immune function. Immunoglobulin is a glycoprotein produced by the proliferation and differentiation of B lymphocytes into plasma cells after receiving antigen stimulation, and five types of immunoglobulin molecules are found in teleost fish, namely, IgM, IgD, IgZ, IgT, and IgM-Z, of which IgM is the main immunoglobulin in fish [75]. Complement is a biologically active protein present in vertebrate serum and other body fluids, and fish mainly use alternative pathway complement to defend against virus invasion [76]. In this study, dietary PAM inclusion at 200, 250, and 300 g/kg significantly increased the contents of IgM and complement 3 (C3) in the serum of juvenile *O. mykiss*. Such results suggested that dietary PAM inclusion could improve the immune function of juvenile *O. mykiss*, which may be related to the active ingredients such as polyols, polysaccharides, and antimicrobial peptides contained in *P. americana* meal [21].

In summary, dietary PAM inclusion at 200-300 g/kg can improve the growth performance, and feed utilization of juvenile *O. mykiss*, facilitate the accumulation of protein, amino acids, and glucose in the body, and significantly improve the antioxidant capacity and immunity of juvenile *O. mykiss*. The optimal dietary PAM inclusion level was approximately 250 g/kg for juvenile *O. mykiss*.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

Xiaowen Long and Jungming Deng designed the experiment. Xiaowen Long, Yuting Liu, Yiqing Li, Junjie Li, and Chengfa Zhao performed the experiment. Xiaowen Long and Chengfa Zhao analyzed the data. Xiaowen Long and Junming Deng drafted the paper. Junming Deng critically reviewed the final manuscript.

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