Evaluation of Six Novel Protein Sources on Apparent Digestibility in Pacific White Shrimp, *Litopenaeus vannamei*

Xiaoyue Li, Yongkang Chen, Chaozhong Zheng, Shuyan Chi, Shuang Zhang, Beiping Tan, and Shiwei Xie

1Laboratory of Aquatic Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524088, China
2Guangdong Provincial Key Laboratory of Improved Variety Reproduction in Aquatic Economic Animals, Institute of Aquatic Economic Animals, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China
3Aquatic Animals Precision Nutrition and High-Efficiency Feed Engineering Research Centre of Guangdong Province, Zhanjiang 524088, China
4Key Laboratory of Aquatic, Livestock and Poultry Feed Science and Technology in South China, Ministry of Agriculture, Zhanjiang 524088, China

Correspondence should be addressed to Beiping Tan; bptan@126.com and Shiwei Xie; xswzsdx@163.com

Received 7 July 2022; Accepted 18 October 2022; Published 2 November 2022

Academic Editor: Mahmoud Dawood

Copyright © 2022 Xiaoyue Li et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

This study is aimed at evaluating the apparent digestibility coefficients (ADC) of six novel protein sources in Pacific white shrimp (*Litopenaeus vannamei*), including black soldier fly larvae meal (BSFLM), *Chlorella vulgaris* meal (CM), cottonseed protein concentrate (CPC), *Tenebrio molitor* meal (TM), *Clostridium autoethanogenum* protein (CAP), and methanotroph (*Methylococcus capsulatus*, Bath) bacteria meal (BPM). The control diet (CD) was formulated to contain 448.8 g/kg crude protein and 71.8 g/kg crude lipid. Then, six experimental diets were formulated to contain 70% CD and 30% test ingredients. The yttrium oxide was used as an exogenous indicator for apparent digestibility detection. Six hundred and thirty healthy and uniform-sized shrimp (approximately 3.04 ± 0.01 g) were randomly distributed into triplicate groups of 30 shrimp and they were fed three times daily. After the shrimp was acclimating for one week, their feces were collected 2 hours after the morning feeding until sufficient samples were available for compositional analysis to calculate apparent digestibility. The apparent digestibility coefficients for a dry matter of diets (ADC_D) and ingredients (ADC_I) as well as the apparent digestibility coefficients for crude protein (ADC_Pro), crude lipid (ADC_L), and phosphorus (ADC_P) of test ingredients were calculated. Results showed that the growth performance of shrimp fed BSFLM, TM, and BPM diets significantly decreased compared to that fed the CD (P < 0.05), and no significant differences were found among those fed CD, CM, CAP, and CPC diets (P > 0.05). There were no significant differences in survival among each group (P > 0.05). As for the diets, results showed that the ADC_D of BSFLM, CM, CPC, and TM diets was significantly lower than that of CD, while that of the CAP diet was significantly higher than that of CD (P < 0.05) and there were no significant differences between BPM and CD diets (P > 0.05). As for the test ingredients, the ADC_Pro and ADC_L of BSFLM, CM, CPC, and TM were significantly lower than those of CD in *Litopenaeus vannamei* (P < 0.05). The ADC_Pro of CAP was significantly higher than that of CD (P < 0.05), but no significant differences were found in ADC_L between CAP and CD (P > 0.05). The ADC_Pro of BPM was significantly lower than that of CD (P < 0.05), but there were no significant differences in ADC_L between BPM and CD (P > 0.05). The ADC_P of CM, CAP, and BPM were significantly higher than that of CD, while that of BSFLM was significantly lower than that of CD (P < 0.05), and no significant differences were found in ADC_P between TM and CD (P > 0.05). To conclude, newly developed protein sources such as single-cell protein (CAP, BPM, and CM) showed great potential as a fishmeal alternative, and insect protein meals (TM and BSFLM) were less effective for shrimp compared to the CD. Although the utilization of CPC by shrimp was lower than other protein sources, it had been much improved compared to the untreated cottonseed meal. The present study will contribute to the application of novel protein sources in shrimp feeds.
1. Introduction

The production of the Pacific white shrimp, Litopenaeus vannamei, reached 4.9 million tonnes in 2018, accounting for 52.9% of the crustacean production and 4.3% of the total aquaculture production in the world, making it one of the most important traded aquatic species globally (data from https://www.fao.org/fishery/en/collection/aquaculture?lang=en). The increasing shrimp production has stimulated the demand for shrimp feed. In terms of nutrients, the crude protein content accounts for 25%-50% of shrimp feed and it is also one of the most expensive constitutions [1]. And fishmeal (FM) has been considered the key ingredient in the feed due to its high protein content, balanced amino acid profile, high digestibility, and good palatability [2] and usually makes up 15%-35% of shrimp feed [3]. However, FM production has been stagnant during the last decades, and the catches used for FM production have decreased due to the El Niño phenomenon [4]. Therefore, the development of alternative protein sources has become an urgent issue to address and studies involving fishmeal alternatives are being carried out [5].

To assess a novel protein source, apparent digestibility is an important indicator for nutrient digestibility and absorption in animals [6]. High apparent digestibility can not only reduce the feed coefficients but also decrease the pollution of the water environment [7]. In this study, the apparent digestibility of six novel protein sources was evaluated, including black soldier fly (Hermetia illucens) larvae meal (BSFLM), Chlorella vulgaris meal (CM), cottonseed protein concentrate (CPC), Tenebrio molitor meal (TM), Clostridium autoethanogenum protein (CAP), and methanotroph (Methylococcus capsulatus, Bath) bacteria meal (BPM). The black soldier fly larvae are usually fed on waste organic matter such as kitchen waste and livestock manure. Based on high bioconversion capacity, black soldier fly larvae can convert vast amounts of organic waste into their biomass, which can be used for commercial solutions to environmental problems associated with manure and other organic waste [8]. On account of the abundant nutrient value, the black soldier fly larvae meal is currently studied as feed ingredients [9, 10]. In a previous study, dietary BSFLM reduced lipid digestibility but had no effects on the apparent digestibility of protein and dry matter in rainbow trout (Oncorhynchus mykiss) [11]. Also, the activities of lipase and amylase in the intestine of grass carp (Ctenopharyngodon idella) were significantly reduced after more than 50% of soybean meal was replaced with BSFLM in the feed [12]. These results indicated a decreasing trend in digestibility after feeding with dietary BSFLM. Another insect species, Tenebrio molitor, is initially considered a storage pest. However, due to its high nutrient value (crude protein 47%-60%), it is also studied as feed ingredients in pets, livestock, and aquatic animals [13]. Nevertheless, the effects of dietary TM on the digestibility of different animals were inconsistent. For example, the digestibility of protein, lipid, and dry matter was significantly reduced in gilthead sea bream (Sparus aurata) when fed with dietary TM, while these digestibility indicators were slightly improved in European sea bass (Dicentrarchus labrax L.) [14, 15].

Single-cell protein (SCP) is a mixture obtained from the cytoplasm of algae, yeast, or bacteria, with the advantages of high production efficiency, wide sources of production materials, land saving, and less influence by seasonal and climatic changes [16]. Generally, SCP contains high protein content (30%-50% in yeasts, 40%-70% in microalgae, and 50%-80% in bacteria), carbohydrates, nucleic acids, polyunsaturated fatty acids, minerals, and vitamins. Chlorella vulgaris is a unicellular microalga belonging to the Chlorellaceae family that can grow in autotrophic and heterotrophic conditions. Chlorella meal normally contains 50%-60% crude protein and 15%-22% crude lipid [17]. In addition, microalgae are rich in astaxanthin, which can be added to the feed to effectively improve the color of shrimp and increase their antioxidant capacity and resistance to stress during harvest and transport [18]. It is still unclear how dietary CM affects digestibility in shrimp, and inconsistent results have been reported [19, 20]. The production of bacterial protein meal is less dependent on land, water, and climate conditions than CM and has high production efficiency and pure nutritional value [21]. Research on BPM and CAP is gaining popularity, and both are showing good application prospects. Methane-oxidizing bacteria (Methylococcus capsulatus) are gram-negative bacteria capable of producing BPM by fermentation using methane as the carbon source, and the BPM products usually contain 71% crude protein and 8% crude lipid [22]. In previous studies, the digestibility significantly decreased when 40% of fishmeal was replaced with BPM in turbot juveniles (Scophthalmus maximus L.), while no significant differences were found in Japanese yellowtail (Seriola quinquergiata) [23, 24]. Clostridium autoethanogenum, an anaerobic gram-positive bacterium, can produce both ethanol and protein byproducts by consuming carbon monoxide from steelmaking converter gas as the carbon source through gas pretreatment, fermentation, distillation, filtration, and spray drying steps [25]. The CAP contains more than 80% crude protein, is rich in lysine, and can replace 30% of fishmeal in the feed without affecting the growth performance of Litopenaeus vannamei [26]. Several studies demonstrated that dietary CAP does not affect digestibility at low levels of inclusion, but high inclusion levels may reduce digestibility in animals [27, 28]. The CPC is a protein concentrate product obtained through low-temperature leaching and solvent extraction of traditional cottonseed meal, which has lower cotton phenol content and higher protein content (60%-70%) than cottonseed meal, and is a more ideal substitute for FM [29, 30]. Since few studies have evaluated the digestibility of these six novel protein sources in Pacific white shrimp, in this study, apparent digestibility was determined by the exogenous indicator method to provide valid data to support better utilization of novel protein sources in Pacific white shrimp feed.

2. Materials and Methods

2.1. Preparation of Experimental Diets. The control diet (CD) was formulated according to the nutritional requirements of the shrimp [31], containing 448.8 g/kg crude
protein and 71.8 g/kg crude lipid. As shown in Table 1, the experimental feed was formulated to contain 70% of CD and 30% of test ingredients, and yttrium trioxide was added as an exogenous indicator. All ingredients were sieved through an 80-mesh screen, weighed accurately, and configured into a homogeneous mixture in accordance with the step-by-step amplification. After being sieved through a 60-mesh screen, the mixture was extruded into 1.0 mm diameter pellets using a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangdong, China) and then ripened in an electric oven at 60°C for 30 min and stored at -20°C before use. The nutrient level and amino acid composition of the ingredients are shown in Table 2.

2.2. Shrimp Feeding and Management. The experimental shrimp were purchased from Guangdong Haida Group (Zhanjiang) and fed with commercial feed for two months. The formal experiment was conducted in an indoor seawater culture system; 630 healthy and uniform-sized shrimp (initial weight 3.04 ± 0.01 g) were randomly divided into 7 groups, with triplicate fiberglass tanks (300 L) per group and 30 shrimp in each tank. Shrimp were fed three times a day at 7:00, 12:00, and 20:00 with 6%-8% of body weight per day. During the experiment, 50% of seawater was changed every day. The water temperature was 25-28°C and the salinity was 25-30‰.

2.3. Feces Collection. Feces collection was conducted after a one-week feeding trial. To be specific, the residual feed was cleaned up 0.5 h after morning feeding. The fresh feces were collected from each tank by siphoning 2 h after feeding. Furthermore, the intact and coated feces were selected and stored in a sterile tube at -20°C prior to determination. After sufficient samples were collected, they were dried at 105°C and ground before analysis.

2.4. Sample Analysis. After a 4-week feeding trial, shrimp in each tank were counted and weighed. Moisture of diets was determined by oven drying at 105°C: weight reduction of feed after drying. Crude protein of feces and diets was detected by Primacs100 analyzer (Skalar, Dutch): after full combustion of the feed, the nitrogen oxides are reduced to nitrogen (crude protein = Total – N × 6.25). Crude lipid was detected by an XT15 extractor (Ankom, USA): weight reduction of feed after extraction by petroleum ether. Ash was detected by burning at 550°C: weight reduction of feed after fully burning [32, 33]. The amino acid compositions of ingredients were determined by an automatic amino acid analyzer 433D (Sykam, Germany) after hydrolysis in 6 M HCl for 24 h at 110°C. After being digested with nitric acid and hydrogen peroxide (6 mL 68% nitric acid and 1 mL 30% hydrogen peroxide) by microwave digestion (Anton Paar Multiwave PRO 41HVT56, Austria), samples were conducted in an inductively coupled plasma-mass spectrometer (ICP-MS, Agilent 7500cx, USA) to determine the phosphorus content. The nutrient levels and amino acid composition of diets are shown in Table 3.

2.5. Calculations and Statistical Analysis. The parameters of growth performance were calculated as follows [34]:

\[
\text{Weight gain rate (WGR, %)} = \left(\frac{\text{final body weight} - \text{initial body weight}}{\text{initial body weight}}\right) \times 100% \\
\text{Survival (\%)} = \left(\frac{\text{final shrimp number}}{\text{initial shrimp number}}\right) \times 100% \\
\text{Specific growth rate (SGR, %day}^{-1}\text{)} = \left(\frac{\ln (\text{final body weight}) - \ln (\text{initial body weight})}{\text{days}}\right) \times 100%, \\
\text{Feed efficiency (FE)} = \frac{\text{feed consumption}}{\text{body weight gain}}.
\]

The apparent digestibility coefficient (ADC) of dry matter (ADC_D), ingredients (ADC_I), crude protein (ADC_pro), crude lipid (ADC_L), phosphorus (ADC_P), and amino acids (ADC_AA) were calculated as follows: [35]:

\[
\text{ADC}_D(\%) = 100\% \times \left[1 - \frac{\text{Md}}{\text{Mf}}\right], \\
\text{ADC of nutrients in ingredients (\%)} = 100\% \times \left(1 - \frac{\text{Nd}}{\text{Nf}} \times \frac{\text{Md}}{\text{Mf}}\right), \\
\text{ADC of nutrients in diets (\%)} = 100\% \times \left(\frac{\text{Nd}}{\text{Nf}} \times \frac{\text{Md}}{\text{Mf}}\right),
\]

where Md and Mf are the percentage of yttrium oxide in diets and feces, respectively, and Nd and Nf are the percentage of nutrient in diets and feces, respectively.

\[
\text{ADC of nutrients in ingredients (\%)} = \text{ADC}_t + \left[(\text{ADC}_t - \text{ADC}_r) \times \frac{0.7 \times \text{Nr}}{0.3 \times \text{Ni}}\right], (3)
\]

where ADC_t is the ADC of nutrients in test diets and ADC_r is the ADC of nutrients in CD, while Nr and Ni are the nutrient contents of the CD and test diets, respectively.
Table 1: Formulation of experimental diets (g/kg dry matter).

| Ingredient                  | Control diet | Diets       | Test diet |
|-----------------------------|--------------|-------------|-----------|
| Brown fish meal             | 250.0        | 175.0       |
| Soybean meal                | 250.0        | 175.0       |
| Peanut meal                 | 100.0        | 70.0        |
| Wheat flour                 | 240.6        | 168.3       |
| Beer yeast                  | 30.0         | 21.0        |
| Shrimp shell meal           | 50.0         | 35.0        |
| Fish oil                    | 20.0         | 14.0        |
| Soybean oil                 | 20.0         | 14.0        |
| Choline chloride            | 10.0         | 7.0         |
| Vitamin and mineral premix a| 10.0         | 7.0         |
| Calcium monophosphate       | 15.0         | 10.5        |
| Vitamin C                   | 1.0          | 0.7         |
| Yttrium oxide               | 0.4          | 0.4         |
| Testing ingredients         |              | 300.0       |
| Total                       | 1000.0       | 1000.0      |

*aVitamin and mineral premix (kg\(^{-1}\) of diet): thiamine, 5 mg; riboflavin, 10 mg; vitamin A, 5000 IU; vitamin E, 40 mg; vitamin D₃, 1000 IU; menadione, 10 mg; pyridoxine, 10 mg; biotin, 0.1 mg; cyanocobalamin, 0.02 mg; calcium pantothenate, 20 mg; folic acid, 1 mg; niacin, 40 mg; vitamin C, 150 mg; iron, 100 mg; iodine, 0.8 mg; copper, 3 mg; zinc, 50 mg; manganese, 12 mg; selenium, 0.3 mg; cobalt, 0.2 mg.

Table 2: Nutrient level and amino acid composition of test ingredients (g/kg dry matter).

| Index                              | FM    | BSFLM | CM    | CPC   | TM    | CAP   | BPM   |
|------------------------------------|-------|-------|-------|-------|-------|-------|-------|
| Nutrient level of ingredients      |       |       |       |       |       |       |       |
| Dry matter                         | 932.0 | 911.0 | 937.9 | 947.5 | 915.7 | 928.6 | 954.0 |
| Crude protein                      | 682.1 | 351.7 | 515.3 | 615.1 | 658.8 | 842.1 | 741.0 |
| Crude lipid                        | 90.0  | 326.0 | 55.0  | 23.6  | 41.9  | 1.9   | 81.7  |
| Amino acid composition of ingredients |     |     |     |       |       |       |       |
| Methionine                         | 18.0  | 6.5   | 9.0   | 8.5   | 12.9  | 22.9  | 17.3  |
| Lysine                             | 50.6  | 17.5  | 32.0  | 24.7  | 48.5  | 87.0  | 37.8  |
| Leucine                            | 45.4  | 21.3  | 42.4  | 34.4  | 50.8  | 63.8  | 50.4  |
| Isoleucine                         | 26.2  | 13.0  | 18.6  | 18.9  | 28.0  | 52.8  | 29.4  |
| Histidine                          | 20.2  | 10.6  | 12.9  | 18.0  | 9.0   | 16.8  | 14.2  |
| Phenylalanine                      | 26.3  | 14.5  | 28.2  | 35.3  | 25.7  | 33.0  | 29.1  |
| Valine                             | 31.0  | 20.2  | 29.5  | 26.6  | 39.2  | 54.4  | 38.9  |
| Arginine                           | 37.1  | 15.8  | 31.0  | 78.9  | 37.3  | 34.0  | 42.1  |
| Threonine                          | 29.0  | 14.8  | 25.7  | 19.0  | 24.6  | 40.2  | 28.7  |
| Tyrosine                           | 22.3  | 18.3  | 20.8  | 13.5  | 20.5  | 31.4  | 18.1  |
| Aspartic acid                      | 59.1  | 27.8  | 50.5  | 56.6  | 48.5  | 95.4  | 58.2  |
| Serine                             | 23.5  | 13.6  | 20.4  | 26.5  | 57.4  | 32.1  | 22.0  |
| Glutamic acid                      | 86.4  | 44.9  | 67.8  | 123.7 | 77.4  | 97.8  | 72.8  |
| Glycine                            | 38.2  | 17.1  | 27.3  | 25.0  | 53.1  | 38.7  | 33.3  |
| Alanine                            | 38.4  | 22.4  | 39.3  | 23.6  | 12.9  | 46.3  | 47.0  |
| Proline                            | 26.2  | 18.9  | 19.9  | 21.7  | 44.3  | 24.0  | 25.2  |
| Cystine                            | 6.5   | 4.4   | 5.8   | 9.5   | 40.5  | 7.1   | 3.5   |
| Total                              | 584.4 | 301.6 | 481.1 | 550.9 | 630.6 | 777.7 | 568.0 |

FM: fishmeal; BSFLM: black soldier fly larvae meal; CM: Chlorella vulgaris meal; CPC: cottonseed protein concentrate; TM: Tenebrio molitor meal; CAP: Clostridium autoethanogenum protein; BPM: methanotroph bacterial meal.
Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan’s test to determine significant differences among treatments using SPSS 21.0 (SPSS, Chicago, IL, USA). Probability value of $P < 0.05$ was deemed to be statistically significant.

### 3. Results

#### 3.1. Growth Performance

As shown in Table 4, there were no significant differences in final body weight (FBW), weight gain rate (WGR), and specific growth rate (SGR) of shrimp fed the CD, CM, CPC, and CAP diets ($P > 0.05$). But the FBW, WGR, and SGR of shrimp fed the BSFLM, TM, and BPM diets significantly decreased compared to those fed the CD ($P < 0.05$). There was no significant difference in survival among shrimp fed different diets ($P > 0.05$). The feed efficiency (FE) of shrimp fed the BSFLM diet was significantly higher than that fed the CD ($P < 0.05$). There were no significant differences in FE among the shrimp fed the CM, CPC, TM, CAP, and BPM diets compared to those fed the CD ($P > 0.05$).

#### 3.2. Apparent Digestibility of Dry Matter, Ingredients, Crude Protein, Crude Lipid, and Phosphorus

As shown in Table 5, the apparent digestibility coefficients of dry matter (ADC$_{DM}$) in diets ranged from 67.52% to 83.46% and the apparent digestibility coefficients of ingredients (ADC$_{I}$) in test ingredients ranged from 39.67% to 97.41%. To be specific, the ADC$_{DM}$ of BSFLM, CM, CPC, and TM diets was significantly lower than that of CD, while the ADC$_{DM}$ of CAP diets was significantly higher than that of CD ($P < 0.05$). There was no significant difference in ADC$_{DM}$ between the CD and BPM diets ($P > 0.05$). The apparent digestibility coefficients of protein (ADC$_{Pro}$) in test ingredients ranged from 56.50% to 97.74%. Briefly, the ADC$_{Pro}$ of BSFLM, CM, CPC, TM, and BPM was significantly lower than that of CD, while ADC$_{Pro}$ of CAP was significantly higher than that of CD ($P < 0.05$). The apparent digestibility coefficients of lipid (ADC$_{L}$) in test ingredients ranged from 70.53% to 94.05%. Briefly, the ADC$_{L}$ of BSFLM, CM, CPC, and TM was significantly lower than that of CD ($P < 0.05$), while there were no significant differences in ADC$_{L}$ among CD, BPM, and CAP ($P > 0.05$). The apparent digestibility coefficients of phosphorus (ADC$_{P}$) in test ingredients ranged from 41.40% to 90.95%. Briefly, the ADC$_{P}$ of BSFLM was significantly lower than that of CD, while ADC$_{P}$ of CM, BPM, and CAP was significantly higher than that of CD ($P < 0.05$). There were no significant differences in ADC$_{P}$.

| Index                        | CD     | BSFLM | CM    | CPC   | TM    | CAP   | BPM   |
|------------------------------|--------|-------|-------|-------|-------|-------|-------|
| Nutrient level of diets      |        |       |       |       |       |       |       |
| Dry matter                   | 919.5  | 931.9 | 920.5 | 917.3 | 919.1 | 912.4 | 923.4 |
| Crude protein                | 448.8  | 431.5 | 500.9 | 517.0 | 548.1 | 593.6 | 551.2 |
| Crude lipid                  | 71.8   | 141.5 | 75.0  | 52.1  | 65.0  | 48.7  | 67.0  |
| Ash                          | 106.4  | 101.6 | 90.8  | 100.6 | 104.1 | 86.5  | 93.0  |
| Phosphorus                   | 14.5   | 14.7  | 15.5  | 16.4  | 11.4  | 15.3  | 17.0  |
| Amino acid composition of diets |      |       |       |       |       |       |       |
| Methionine                   | 6.8    | 6.4   | 7.9   | 8.0   | 9.3   | 12.2  | 11.3  |
| Lysine                       | 25.2   | 23.5  | 26.3  | 26.6  | 30.8  | 42.9  | 32.2  |
| Leucine                      | 31.4   | 29.7  | 35.8  | 34.3  | 37.1  | 44.8  | 39.9  |
| Isoleucine                   | 17.6   | 17.4  | 17.8  | 18.5  | 19.8  | 29.2  | 21.9  |
| Histidine                    | 11.3   | 10.4  | 9.7   | 13.7  | 8.9   | 10.6  | 11.2  |
| Phenylalanine                | 19.4   | 17.7  | 24.5  | 27.1  | 25.1  | 29.9  | 27.1  |
| Valine                       | 20.7   | 22.4  | 21.2  | 21.5  | 24.5  | 28.5  | 24.3  |
| Arginine                     | 28.4   | 25.5  | 27.0  | 44.4  | 31.2  | 29.8  | 32.3  |
| Threonine                    | 16.0   | 15.9  | 17.1  | 16.6  | 18.8  | 24.9  | 20.8  |
| Tyrosine                     | 12.9   | 15.4  | 17.7  | 17.3  | 18.8  | 24.0  | 20.7  |
| Aspartic acid                | 40.5   | 37.9  | 40.3  | 45.4  | 43.4  | 57.9  | 45.8  |
| Serine                       | 17.8   | 17.1  | 17.6  | 20.5  | 28.6  | 23.7  | 20.4  |
| Glutamic acid                | 76.0   | 69.5  | 71.6  | 92.8  | 78.7  | 82.4  | 78.9  |
| Glycine                      | 21.3   | 21.0  | 22.9  | 23.4  | 30.2  | 27.7  | 25.5  |
| Alanine                      | 21.1   | 23.5  | 26.1  | 21.8  | 24.0  | 29.9  | 28.9  |
| Proline                      | 20.2   | 20.5  | 20.7  | 22.0  | 27.9  | 23.5  | 23.2  |
| Cystine                      | 4.8    | 4.6   | 5.2   | 5.2   | 5.6   | 6.3   | 5.1   |
| Total                        | 391.4  | 378.4 | 409.4 | 459.1 | 462.7 | 528.2 | 469.5 |

CD: control diet; BSFLM: black soldier fly larvae meal; CM: Chlorella vulgaris meal; CPC: cottonseed protein concentrate; TM: Tenebrio molitor meal; CAP: Clostridium autoethanogenum protein; BPM: methanotroph bacterial meal.
3.3. Apparent Digestibility of Amino Acids. The ADC of amino acids (ADC\textsubscript{AA}) of test ingredients is shown in Table 6. The ADC of all amino acids except histidine and methionine in the ADC of other amino acids was lower than that of the CD. The ADC of methionine of CPC was similar to that of CD and that of other amino acids was lower than the CD. The ADC of all amino acids of CM and BSFLM was lower than that of the CD. Compared to the CD, TM exhibited a similar ADC of methionine and the ADC of other amino acids was lower.

4. Discussion

Determining the apparent digestibility of ingredients is an important prerequisite for evaluating the availability of novel protein sources [36]. The assessment of ADC\textsubscript{D} helps to learn the total amount of nutrients being digested, as the components of the feed are not digested by the animal in the same proportions [37]. In the present study, shrimp showed divergent ADC\textsubscript{D} and ADC\textsubscript{P} when fed with different test diets, which were closely related to ingredients. For insect protein sources, shrimp fed with dietary BSFLM and TM showed a significantly lower ADC\textsubscript{P} of diets as well as ADC\textsubscript{D} and ADC\textsubscript{P} of test ingredients than CD. Also, the ADC\textsubscript{D} and ADC\textsubscript{P} of BSFLM were significantly higher than those of TM. The digestive properties of shrimp fed with insect protein are strongly influenced by the nutritional properties of the ingredients. Typically, the crude protein, crude lipid, nitrogen-free extracts, and ash content of insect proteins varied with species and growth stage [38]. Furthermore, insect exoskeletons are usually composed of chitin, which is generally considered to impede the digestive process [39, 40]. Previous studies demonstrated that BSFLM (crude protein 36.4%, crude lipid 11.0%) contained a higher chitin level than TM (crude protein 42.0%, crude lipid 28.3%), and therefore, rainbow trout (Oncorhynchus mykiss) digested TM better than BSFLM [41]. However, no significant differences were found in the apparent digestibility of protein and lipid between two dietary insect proteins (BSFLM, crude protein 36.4%, crude lipid 11.0%; TM, crude protein 38.7%, crude lipid 12.6%) in the Pacific white shrimp [42]. This is probably because 28.0%–35.5% of dietary chitin in insect meals can be digested by shrimp. In the research on
TM (crude protein 55.6%, crude lipid 34.6%), the value of ADC was 45.9% for dry matter and 76.1% for ADC<sub>Ppro</sub>, and the apparent digestibility of essential amino acids ranged from 72.86% to 86.05% [43], which seems different from our results. This could be explained by the different nutrient compositions of ingredients since the defatted TM may contain higher levels of chitin and the difference in the digestibility system in different growth stages of shrimp. Further, it was observed that the insect protein source had the lowest amino acid digestibility compared to other protein sources in the present study. This can be caused by the chitin being bound to the protein by a covalent bond and negatively affecting their being digested by the shrimp [44]. Although chitin is not easily digested by shrimp, it can act as an immune booster and improve the immune capacity of shrimp [45]. TM and BSFLM have received wide attention due to the great potential for aquafeed application, while the cell-ruptured CM did not negatively affect the digestibility of dry matter, protein, and lipid, while the cell-ruptured CM did not negatively affect the ADC<sub>P</sub> and ADC<sub>Ppro</sub> of Atlantic salmon (Salmo salar L.) [20]. Therefore, rupture of SCP cell wall by physical, thermal, or associated with physical treatments may improve the availability of SCP [56].

Table 6: Apparent digestibility coefficients for amino acids of test ingredients in Litopenaeus vannamei (%).

| Index                  | CD       | BSFLM    | CM        | CPC       | TM      | CAP    | BPM    |
|------------------------|----------|----------|-----------|-----------|---------|--------|--------|
| Essential amino acid   |          |          |           |           |         |        |        |
| Lysine                 | 94.78    | 83.08    | 87.11     | 78.18     | 85.91   | 98.26  | 95.79  |
| Methionine             | 87.50    | 69.34    | 87.32     | 87.84     | 87.56   | 98.21  | 98.97  |
| Arginine               | 95.91    | 80.47    | 90.43     | 93.27     | 59.43   | 96.92  | 92.37  |
| Histidine              | 92.48    | 62.69    | 74.50     | 87.65     | 55.20   | 86.94  | 79.38  |
| Valine                 | 93.74    | 74.63    | 81.70     | 80.53     | 46.37   | 95.20  | 84.59  |
| Threonine              | 92.03    | 73.20    | 85.60     | 78.24     | 50.72   | 97.28  | 88.90  |
| Phenylalanine          | 92.36    | 73.88    | 86.28     | 88.46     | 57.43   | 95.23  | 87.63  |
| Isoleucine             | 93.82    | 75.47    | 82.35     | 79.74     | 54.44   | 97.00  | 87.27  |
| Leucine                | 93.99    | 75.88    | 86.75     | 82.86     | 54.66   | 98.05  | 89.61  |
| Nonessential amino acid|          |          |           |           |         |        |        |
| Aspartic acid          | 93.19    | 76.67    | 86.22     | 86.35     | 58.29   | 97.70  | 89.02  |
| Serine                 | 92.43    | 68.83    | 86.09     | 85.83     | 55.44   | 96.99  | 88.81  |
| Glutamic acid          | 94.97    | 80.49    | 88.92     | 91.50     | 62.14   | 96.81  | 89.05  |
| Glycine                | 89.73    | 59.38    | 83.85     | 80.60     | 55.93   | 96.48  | 87.33  |
| Alanine                | 92.44    | 75.47    | 87.10     | 76.01     | 25.25   | 96.51  | 86.06  |
| Cystine                | 92.01    | 69.28    | 81.86     | 80.16     | 74.16   | 98.05  | 81.29  |
| Tyrosine               | 91.73    | 77.01    | 87.96     | 89.35     | 58.85   | 95.42  | 91.36  |
| Proline                | 93.69    | 70.58    | 90.12     | 88.42     | 52.72   | 95.97  | 94.74  |

CD: control diet; BSFLM: black soldier fly larvae meal; CM: Chlorella vulgaris meal; CPC: cottonseed protein concentrate; TM: Tenebrio molitor meal; CAP: Clostridium autoethanogenum protein; BPM: methanotroph bacterial meal.
chemical hydrolysis, or bioenzymatic methods often releases more protein and amino acid profiles and reduces the antinutrition factors, making intracellular nutrients easily available to animals, which has also been used in some plant and animal protein sources [58–60]. Although the CM used in this experiment was not pretreated to break the cell wall, the high apparent digestibility of protein and lipid has proved its potential for application in shrimp feed. Both CAP and BPM are produced from bacteria fermentation and have attracted attention in the aquafeed field in the last decades. Our previous studies have demonstrated the availability of these two SCP in shrimp feed [61, 62]. In the present study, shrimp showed the highest digestibility to CAP, which may be due to the relatively pure composition of CAP. A previous study showed that the ADCD and ADC pro of largemouth bass (*Micropterus salmoides*) increased with the higher dietary CAP and the activity of protease significantly increased in both the stomach and intestine [27]. In addition, the particle size and cell wall fragmentation of SCP may be one of the key factors affecting its application in aquafeeds [63]. The use of further ground and smaller particle size of BPM can replace a higher percentage of FM without affecting the growth performance of Japanese yellowtail (*Seriola quinqueradiata*) [23]. Further studies should be conducted in this aspect.

Plant-based proteins have been widely used in aquafeeds [64], but it is generally considered that they have disadvantages such as a lack of essential amino acids, rich in antinutritional factors, and poor palatability [37, 65]. Cottonseed meal is an inexpensive and highly practical protein source in shrimp feed. Usually, the cell walls of plant proteins are rich in crude fiber and ash and therefore difficult to be digested by shrimp. Previous studies demonstrated that the ADCD of shrimp to cottonseed meal was about 50%–55% and ADC pro was about 57.6%–82.9% [66, 67]. Besides, gossypol is a natural terpenoid found in the glands of cotton and would reduce the intestinal nutrient digestion and absorption of bony fish [68], which is often mediated by triggering intestinal inflammation and disrupting the intestinal structure [69]. The low digestibility and toxic effects have become the main factors limiting the application of cottonseed meals in aquafeeds [70]. CPC is a high-quality protein produced from cottonseed meal after aqueous alcohol extraction to reduce soluble carbohydrates and remove most of the anti-nutritional factors [71]. The replacement of FM with 150 g/kg dietary CPC had no negative effects on the growth performance of the Pacific white shrimp, while the growth would be impaired with the further increase of substitution [67]. In the present study, although shrimp fed with dietary CPC showed lower apparent digestibility coefficients in all indices than those fed the CD, it was still better than the aforementioned results on cottonseed meal. On the other hand, even though CPC is abundant in phosphorus, it has mainly existed as the form of phytate phosphorus, which is difficult to be digested and absorbed by animals [72, 73] and leads to errors in the assay and insufficient samples. Pretreatment of cottonseed meal and other plant proteins with exogenous phytase or supplementation of the feed with phytase can effectively reduce the phytate phosphorus content and thus increase the availability of phosphorus and other micronutrients [74, 75]. Overall, the Pacific white shrimp showed good digestibility of diets and protein to CPC, but when using CPC to replace fishmeal in the feed, it is necessary to supplement the feed with an appropriate phosphorus source.

In conclusion, the apparent digestibility of six novel protein sources was evaluated in *Litopenaeus vannamei*. Results showed that shrimp had the highest apparent digestibility to SCP (CAP, BPM, and CM), followed by insect proteins (BSF and TM). Although the apparent digestibility of shrimp to the dietary CPC was lower than that of the other tested ingredients, it was better than that of the cottonseed meal. The six novel protein sources showed better digestive properties and appeared to be potential alternatives to fishmeal. This study will provide experimental evidence for the development of shrimp feeds containing novel protein sources.

**Data Availability**

The data that support the findings of this study are available on request from the corresponding author.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

Xiaoyue Li and Yongkang Chen contributed equally to this work.

**Acknowledgments**

This study was supported by the fund of the National Key R&D Program of China (2019YFD0900200), the National Natural Science Foundation of China (32002402), the Guangdong Basic and Applied Basic Research Foundation (2019A1515011970 and 2021A1515010428), and the Zhanjiang Science and Technology Bureau (Grant No. 2020A05003).

**References**

[1] V. C. Cummins Jr., S. D. Rawles, K. R. Thompson et al., “Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*),” *Aquaculture*, vol. 473, pp. 337–344, 2017.

[2] E. Amaya, D. A. Davis, and D. B. Rouse, “Alternative diets for the Pacific white shrimp _Litopenaeus vannamei_,” *Aquaculture*, vol. 262, no. 2-4, pp. 419–425, 2007.

[3] L. Gui, H. Mai, S. Chi, W. Zhou, Y. Li, and B. Tan, “Effects of yeast culture on growth performance, hematological parameters, immunity and disease resistance of *Litopenaeus vannamei*,” *Journal of Guangdong Ocean University*, vol. 39, no. 3, pp. 30–37, 2019.

[4] Food and Agriculture Organization of the United Nations, *The state of world fisheries and aquaculture 2018*, Food and Agriculture Organization of the United Nations, 2020.
Agriculture Nutrition

[5] W. Li, L. Li, H. Y. Li et al., "Effects of Clostridium butyricum on growth, antioxidant capacity and non-specific immunology of Litopenaeus vannamei fed with concentrated cottonseed protein replacement of fishmeal," Journal of Guangdong Ocean University, vol. 42, no. 2, pp. 29–37, 2022.

[6] G. Wang, Y. Sun, F. Niu et al, "Effects of exogenous enzyme supplementation on digestive enzyme activity, apparent digestibility and fecal nitrogen and phosphorus content of juvenile yellow catfish," Journal of Guangdong Ocean University, vol. 37, no. 6, pp. 19–25, 2017.

[7] A. Wirtz, C. G. Carter, M. B. Codabaccus, Q. P. Fitzgibbon, A. T. Townsend, and G. G. Smith, "Protein sources influence both apparent digestibility and gastrointestinal evacuation rate in juvenile slipper lobster (Thenus australiensis)," Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, vol. 265, p. 111121, 2022.

[8] A. Van Huis, J. Van Itterbeeck, H. Klunder et al., Edible insects: future prospects for food and feed security no. 171, Food and Agriculture Organization of the United Nations (FAO), 2013.

[9] H. P. S. Makkar, G. Tran, V. Heuzé, and P. Ankers, "State-of-the-art on use of insects as animal feed," Animal Feed Science and Technology, vol. 197, pp. 1–33, 2014.

[10] Y. Xie, K. Peng, J. Hu, and G. Wang, "Review on application of black soldier fly (Hermetia illucens linnaeus) in aquatic feed," Journal of Guangdong Ocean University, vol. 42, no. 1, pp. 144–150, 2022.

[11] A. Dumas, T. Raggi, J. Barkhouse, E. Lewis, and E. Wetzlzen, "The oil fraction and partially defatted meal of black soldier fly larvae (Hermetia illucens) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (Oncorhynchus mykiss)," Aquaculture, vol. 492, pp. 24–34, 2018.

[12] R. Lu, Y. Chen, W. Yu et al., "Defatted black soldier fly (Hermetia illucens) larval meal can replace soybean meal in juvenile grass carp (Ctenopharyngodon idellus) diets," Aquaculture Reports, vol. 18, article 100520, 2020.

[13] A. Bordleau, M. Krzyżaniak, M. J. Stolarzski, S. Czachorowski, and D. Peni, "Will yellow mealworm become a source of safe proteins for Europe?," Agriculture, vol. 10, no. 6, p. 233, 2020.

[14] L. Gasco, M. Henry, G. Piccolo et al., "Tenebrio molitor meal in diets for European sea bass (Dicentrarchus labraxL.) juveniles: growth performance, whole body composition and in vivo apparent digestibility," Animal Feed Science and Technology, vol. 220, pp. 34–45, 2016.

[15] G. Piccolo, V. Iaconisi, S. Marono et al., "Effect of Tenebrio molitor larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (Sparus aurata)," Animal Feed Science and Technology, vol. 226, pp. 12–20, 2017.

[16] M. Saeed, I. Yasmin, M. A. Murtaza, I. Fatima, and S. Saeed, "Single cell proteins: a novel value added food product," Pakistan Journal of Food Sciences, vol. 26, no. 4, pp. 211–217, 2016.

[17] G. Ma, P. Xu, L. Song, and Y. Zhang, "Research progress of ecological factors affecting the synthesis of EPA in microalgae," Journal of Guangdong Ocean University, vol. 34, no. 6, pp. 103–108, 2014.

[18] M. Teimouri, S. Yeganeh, G. R. Mianji, M. Najafi, and S. Mahjoub, "The effect of spirulina platensis meal on antioxidant gene expression, total antioxidant capacity, and lipid peroxidation of rainbow trout (Oncorhynchus mykiss)," Fish Physiology and Biochemistry, vol. 45, no. 3, pp. 977–986, 2019.

[19] A. A. Raji, W. A. Jimoh, N. Bakar et al., "Dietary use of spirulina (Arthospira) and chlorella instead of fish meal on growth and digestibility of nutrients, amino acids and fatty acids by African catfish," Journal of Applied Physiology, vol. 32, no. 3, pp. 1763–1770, 2020.

[20] S. M. Tabbett, J. Mann, and A. Dumas, "Apparent digestibility of nutrients, energy, essential amino acids and fatty acids of juvenile Atlantic salmon (Salmo salar L.) diets containing whole- cell or cell-ruptured Chlorella vulgaris meals at five dietary inclusion levels," Aquaculture, vol. 481, pp. 25–39, 2017.

[21] S. W. Jones, A. Karpol, S. Friedman, B. T. Maru, and B. P. Tracy, "Recent advances in single cell protein use as a feed ingredient in aquaculture," Current Opinion in Biotechnology, vol. 61, pp. 189–197, 2020.

[22] Y. Chen, S. Chi, S. Zhang et al., "Replacement of fish meal with Methanotroph (Methylococcus capsulatus, Bath) bacteria meal in the diets of Pacific white shrimp (Litopenaeus vannamei)," Aquaculture, vol. 541, article 736801, 2021.

[23] A. Biswas, F. Takakuwa, S. Yamada et al., "Methanotroph (Methylococcus capsulatus ,Bath) bacteria meal as an alternative protein source for Japanese yellowtail, Seriola quinqueradiata," Aquaculture, vol. 529, p. 735700, 2020.

[24] J. Zheng, W. Zhang, Z. Dan et al., "Effects of fish meal replaced by methanotroph bacteria meal (Methylococcus capsulatus) on growth, body composition, antioxidant capacity, amino acids transporters and protein metabolism of turbot juveniles (Scophthalmus maximus L.)," Aquaculture, vol. 562, p. 738782, 2023.

[25] H. Wei, H. Yu, X. Chen et al., "Effects of soybean meal replaced by Clostridium autoethanogenum protein on growth performance, plasma biochemical indexes and hepatopancreas and intestinal histopathology of grass carp (Ctenopharyngodon idilis)," Chinese Journal of Animal Nutrition, vol. 30, no. 10, pp. 4190–4201, 2018.

[26] W. Yao, P. Yang, X. Zhang et al., "Effects of replacing dietary fish meal with Clostridium autoethanogenum protein on growth and flesh quality of Pacific white shrimp (Litopenaeus vannamei)," Aquaculture, vol. 549, p. 737770, 2022.

[27] S. Zhu, W. Gao, Z. Wen et al., "Partial substitution of fish meal by Clostridium autoethanogenum protein in the diets of juvenile largemouth bass (Micropterus salmoides)," Aquaculture Reports, vol. 22, p. 100938, 2022.

[28] X. Cui, Q. Ma, M. Duan, H. Xu, M. Liang, and Y. Wei, "Effects of fishmeal replacement by Clostridium autoethanogenum protein on the growth, digestibility, serum free amino acid and gene expression related to protein metabolism of obscure pufferfish (Takifugu obscurus)," Animal Feed Science and Technology, vol. 292, article 115445, 2022.

[29] G. Ye, X. Dong, Q. Yang et al., "Low-gossypol cottonseed protein concentrate used as a replacement of fish meal for juvenile hybrid grouper (Epinephelus fuscoguttatus ♀× Epinephelus lanceolatus ♂): Effects on growth performance, immune responses and intestinal microbiota," Aquaculture, vol. 524, article 735309, 2020.

[30] H. Zhang, X. Pu, Q. Yang et al., "Effects of replacing fish meal with high-protein cottonseed meal on growth performance, non-specific immune index and disease resistance for Litopenaeus vannamei," Journal of Guangdong Ocean University, vol. 38, no. 4, pp. 20–26, 2018.

[31] National Research Council, Nutrient requirements of fish and shrimp, Animal Nutrition Series, National Research Council of the National Academies, 2011.
[32] Association of Official Analytical Chemists, *Official Methods of Analysis*, Association of Official Analytical Chemists, Arlington, VA, USA, 18th edition, 2006.

[33] Y. C. Wu, R. M. Li, G. R. Shen et al., “Effects of dietary small peptides on growth, antioxidant capacity, nonspecific immunity and gut microflora structure of Litopenaeus vannamei,” *Journal of Guangdong Ocean University*, vol. 41, no. 5, pp. 1–9, 2021.

[34] J. Pan, Y. Han, Y. Huo, P. Su, and Z. Jiang, “Effects of dietary alginare oligosaccharide on intestinal morphology, activities of digestive enzymes and apparent digestibility of turbot (Scophthalmus maximus L.),” *Journal of Guangdong Ocean University*, vol. 36, no. 3, pp. 39–44, 2016.

[35] Q. Yang, X. Zhou, Q. Zhou, B. Tan, S. Chi, and X. Dong, “Apparent digestibility of selected feed ingredients for white shrimp Litopenaeus vannamei, Boone,” *Aquaculture Research*, vol. 41, no. 1, pp. 78–86, 2009.

[36] R. Jannathulla, J. S. Dayal, D. Vasanthakumar, K. Ambasankar, and V. B. Meyer-Rochow, “K. Hua, J. M. Cobcroft, A. Cole et al., "The future of aquatic protein: implications for protein sources in aquaculture diets," *One Earth*, vol. 1, no. 3, pp. 316–329, 2019.

[37] R. Jannathulla, J. S. Dayal, D. Vasanthakumar, K. Ambasankar, and M. Muradalhar, “Effect of fungal fermentation on apparent digestibility coefficient for dry matter, crude protein and amino acids of various plant protein sources in Peneaus vannamei,” *Aquaculture Nutrition*, vol. 24, no. 4, pp. 1318–1329, 2018.

[38] K. Hua, J. M. Cobcroft, A. Cole et al., “The future of aquatic protein: implications for protein sources in aquaculture diets,” *One Earth*, vol. 1, no. 3, pp. 316–329, 2019.

[39] V. B. Meyer-Rochow, R. T. Gahukar, S. Ghosh, and C. Jung, “Chemical composition, nutrient quality and acceptability of edible insects are affected by species, developmental stage, gender, diet, and processing method,” *Food*, vol. 10, no. 5, p. 1036, 2021.

[40] M. Woods, N. Goosen, L. Hoffman, and E. Pieterse, “A simple and rapid protocol for measuring the chitin content of Hermetia illucens(L.) (Diptera: Stratiomyidae) larvae,” *Journal of Insects as Food and Feed*, vol. 6, no. 3, pp. 285–290, 2020.

[41] Y. Song, M. Kim, C. Moon et al., “Extraction of chitin and chitosan from larval exuvium and whole body of edible mealworm, Tenebrio molitor,” *Entomological Research*, vol. 48, no. 3, pp. 227–233, 2018.

[42] F. Melenchón, A. M. Larrán, E. De Mercado et al., “Potential use of black soldier fly (Hermetia illucens) and mealworm (Tenebrio molitor) insectmeals in diets for rainbow trout (Oncorhynchus mykiss),” *Aquaculture Nutrition*, vol. 27, no. 2, pp. 491–505, 2021.

[43] J. Shin and K. Lee, “Digestibility of insect meals for pacific white shrimp (Litopenaeus vannamei) and their performance for growth, feed utilization and immune responses,” *PLoS One*, vol. 16, no. 11, p. e0260305, 2021.

[44] R. L. Panini, L. E. L. Freitas, A. M. Guimarães et al., “Potential use of mealworms as an alternative protein source for pacific white shrimp: digestibility and performance,” *Aquaculture*, vol. 473, pp. 115–120, 2017.

[45] T. W. Abun and K. Haetami, “Effect of time processing at steps of bioprocess shrimp waste by three microbes on protein digestibility and metabolizable energy products of native chicken,” *Agrolife Scientific Journal*, vol. 1, no. 5, pp. 209–213, 2016.

[46] A. Cheng, Y. Shiu, S. Chiu, R. Ballantyne, and C. H. Liu, “Effects of chitin from *Daphnia similis* and its derivative, chitosan on the immune response and disease resistance of white shrimp, Litopenaeus vannamei,” *Fish & Shellfish Immunology*, vol. 119, pp. 329–338, 2021.

[47] D. Turck, J. Castenmiller, S. De Henauw et al., “Safety of dried yellow mealworm (Tenebrio molitor larva) as a novel food pursuant to regulation (eu) 2015/2283,” *EFSA Journal*, vol. 19, no. 1, article 6343, 2021.

[48] M. Cullere, G. Tasoniero, V. Giaccone et al., “Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits,” *Animal*, vol. 10, no. 12, pp. 1923–1930, 2016.

[49] Z. Sankian, S. Khosravi, Y. Kim, and S. M. Lee, “Effects of dietary inclusion of yellow mealworm (Tenebrio molitor) meal on growth performance, feed utilization, body composition, plasma biochemical indices, selected immune parameters and antioxidant enzyme activities of mandarin fish (Siniperca scherzeri) juveniles,” *Aquaculture*, vol. 496, pp. 79–87, 2018.

[50] S. Khosravi, E. Kim, Y. Lee, and S. M. Lee, “Dietary inclusion of mealworm (Tenebrio molitor) meal as an alternative protein source in practical diets for juvenile rockfish (Sebastes schlegeli),” *Entomological Research*, vol. 48, no. 3, pp. 214–221, 2018.

[51] C. Motte, A. Rios, T. Lefebvre, H. Do, M. Henry, and O. Jintasataporn, “Replacing fish meal with defatted insect meal (yellow mealworm Tenebrio molitor) improves the growth and immunity of pacific white shrimp (Litopenaeus vannamei),” *Animals*, vol. 9, no. 5, p. 258, 2019.

[52] Y. Chen, S. Chi, S. Zhang et al., “Effect of black soldier fly (Hermetia illucens) larvae meal on lipid and glucose metabolism of pacific white shrimp Litopenaeus vannamei,” *British Journal of Nutrition*, vol. 128, no. 9, pp. 1674–1688, 2021.

[53] S. Errico, A. Spagnolletta, A. Verardi, S. Moliterni, S. Dimatteo, and P. Sangiorgio, “Tenebrio molitor as a source of interesting natural compounds, their recovery processes, biological effects, and safety aspects,” *Comprehensive Reviews in Food Science and Food Safety*, vol. 21, no. 1, pp. 148–197, 2022.

[54] M. Sharif, M. H. Zafar, A. I. Aqib, M. Saeed, M. R. Farag, and M. Alagawany, “Single cell protein: sources, mechanism of production, nutritional value and its uses in aquaculture nutrition,” *Aquaculture*, vol. 531, p. 735885, 2021.

[55] S. Cao, T. Zou, P. Zhang et al., “Effects of dietary fishmeal replacement with Spirulina platensis on the growth, feed utilization, digestion and physiological parameters in juvenile Gibel carp (Carassius auratus gibelio var. Cas iii),” *Aquaculture Research*, vol. 49, no. 3, pp. 1320–1328, 2018.

[56] C. Zhang, Z. Lao, and Y. Liu, “Change of phytoplankton and physicochemical factors in ponds of shrimp Penaeus vannamei with different cultural patterns during late period,” *Journal of Guangdong Ocean University*, vol. 4, pp. 38–44, 2007.

[57] S. Pakravan, A. Akbarzadeh, M. Sajjadi, A. Hajimoradloo, and F. Noori, “Chlorella vulgaris meal improved growth performance, digestive enzyme activities, fatty acid composition and tolerance of hypoxia and ammonia stress in juvenile pacific white shrimp Litopenaeus vannamei,” *Aquaculture Nutrition*, vol. 24, no. 1, pp. 594–604, 2018.

[58] G. C. Maliwat, S. Velasquez, J. L. Robil et al., “Growth and immune response of giant freshwater prawn Macrobrachium rosenbergii (de man) postlarvae fed diets containing Chlorella vulgaris (beijerinck),” *Aquaculture Research*, vol. 48, no. 4, pp. 1666–1676, 2017.

[59] M. J. Sánchez-Muros, P. Renteria, A. Vizcaino, and F. G. Barroso, “Innovative protein sources in shrimp (Litopenaeus vannamei) feeding,” *Reviews in Aquaculture*, vol. 12, no. 1, pp. 186–203, 2020.
[59] X. Zhu, Q. Deng, H. Guo, G. Li, and C. Zhu, “Effects of dietary hydrolyzable tannins on growth performance, antioxidant capacity, intestinal microflora and resistance against Vibrio parahaemolyticus of juvenile Pacific white shrimp, Litopenaeus vannamei (Boone, 1931),” Journal of Guangdong Ocean University, vol. 19, no. 3, pp. 100601–100619, 2021.

[60] A. Wang, Q. Yang, B. Tan et al., “Effects of enzymolytic soybean meal on growth performance, serum biochemical indices, non-specific immunity and disease resistance of juvenile Litopenaeus vannamei,” Journal of Guangdong Ocean University, vol. 38, no. 1, pp. 14–21, 2018.

[61] Y. Chen, S. Chi, S. Zhang et al., “Evaluation of methanotroph (Methylococcus capsulatus, bath) bacteria meal on body composition, lipid metabolism, protein synthesis and muscle metabolites of Pacific white shrimp (Litopenaeus vannamei),” Aquaculture, vol. 547, article 737517, 2022.

[62] C. Zheng, S. Gong, J. Cao et al., “Effects of dietary lipid sources on alleviating the negative impacts induced by the fishmeal replacement with Clostridium autoethanogenen protein in the diet of Pacific white shrimp (Litopenaeus vannamei),” Science, vol. 9, 2022.

[63] M. K. H. Chama, H. Liang, D. Huang et al., “Methanotroph (Methylococcus capsulatus, Bath) as an alternative protein source for genetically improved farmed tilapia (GIFT: Oreochromis niloticus) and its effect on antioxidants and immune response,” Aquaculture Reports, vol. 21, p. 100872, 2021.

[64] X. Mo, C. Huang, S. Jiang et al., “Effects of concentrated dephenolic cottonseed protein instead of fish meal on stress resistance of Penaeus monodon,” The Israeli Journal of Aquaculture-Bamidgeh, vol. 73, 2021.

[65] W. Zhang, B. Tan, A. Pang, J. Deng, Q. Yang, and H. Zhang, “Screening of potential biomarkers for soybean meal induced enteritis in pearl gentian grouper (Epinephelus fuscoguttatus♀×Epinephelus lanceolatus♂),” Journal of Guangdong Ocean University, vol. 42, no. 4, pp. 1–12, 2021.

[66] A. J. Siccardi, C. M. Richardson, M. K. Dowd, T. C. Wedegaertner, and T. M. Samocha, “Digestibility of glandless cottonseed protein in diets for Pacific white shrimp, Litopenaeus vannamei,” Journal of the World Aquaculture Society, vol. 47, no. 1, pp. 97–106, 2016.

[67] M. Wan, P. Yin, W. Fang et al., “The effect of replacement of fishmeal by concentrated dephenolization cottonseed protein on the growth, body composition, haemolymph indexes and haematological enzyme activities of the Pacific white shrimp (Litopenaeus vannamei),” Aquaculture Nutrition, vol. 24, no. 6, pp. 1845–1854, 2018.

[68] K. Wang, W. Jiang, P. Wu et al., “Gossypol reduced the intestinal amino acid absorption capacity of young grass carp (Ctenopharyngodon idella),” Aquaculture, vol. 492, pp. 46–58, 2018.

[69] H. Liu, X. Dong, B. Tan et al., “Effects of fish meal replacement by low-gossypol cottonseed meal on growth performance, digestive enzyme activity, intestine histology and inflammatory gene expression of silver sillage (Sillago shaham Forsskål) (1775),” Aquaculture Nutrition, vol. 26, no. 5, pp. 1724–1735, 2020.

[70] J. Wang, H. Zhang, Q. Yang et al., “Effects of replacing soybean meal with cottonseed meal on growth, feed utilization and non-specific immune enzyme activities for juvenile white shrimp, _Litopenaeus vannamei_,” Aquaculture Reports, vol. 16, p. 100255, 2020.

[71] B. Yin, H. Liu, B. Tan et al., “Cottonseed protein concentrate (CPC) suppresses immune function in different intestinal segments of hybrid grouper _♀ Epinephelus fuscoguttatus_×♂ Epinephelus lanceolatus_ via TLR-2/MyD88 signaling pathways,” Fish & Shellfish Immunology, vol. 81, pp. 318–328, 2018.

[72] D. Lemos and A. G. Tacon, “Use of phytases in fish and shrimp feeds: a review,” Reviews in Aquaculture, vol. 9, no. 3, pp. 266–282, 2017.

[73] W. An, X. Chen, W. Li, X. Dong, B. Tan, and X. Zhao, “Optimum calcium and phosphorus supplemental levels in diets of large size Litopenaeus vannamei,” Journal of Guangdong Ocean University, vol. 38, no. 4, pp. 8–19, 2018.

[74] A. von Danwitz, C. G. van Bussel, S. F. Klatt, and C. Schulz, “Dietary phytase supplementation in rapeseed protein based diets influences growth performance, digestibility and nutrient utilisation in turbot (Psetta maxima L.),” Aquaculture, vol. 450, pp. 405–411, 2016.

[75] L. Li, D. Zhang, T. Su, S. Gong, and S. Jiang, “Isolation and identification of a phytase-producing strain,” Journal of Guangdong Ocean University, vol. 27, no. 4, pp. 89–92, 2007.