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Research Article

Replacement of Commercial Feed with Fresh Black Soldier Fly (*Hermetia illucens*) Larvae in Pacific White Shrimp (*Litopenaeus vannamei*)

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This study evaluated survival, growth performance, digestive enzyme activities, intestinal histology, and antioxidant enzyme activities of the Pacific white shrimp (*Litopenaeus vannamei*), which were fed with five different diets, containing 0%, 25%, 50%, 75%, and 100% fresh black soldier fly larvae (BSFL), respectively, to replace commercial feed at an equal wet weight. The cultured experiment was lasted for 45 days, and the results showed that survival rate (SR), final body weight (FBW), and weight gain (WG) were negatively related with the replacement rate of fresh BSFL in the diet, where the maximum value was found in the BSFL 25% group, while the minimum value appeared in the BSFL 100% group. When BSFL replacement level was equal to or above 75%, the SR, FBW and WG were significantly decreased. However, hepatosomatic index (HSI) was increased with the increasing BSFL replacement level, which was significantly higher in BSFL 75% and BSFL 100% groups than the other groups. No significant differences on hepatopancreatic amylase and lipase activities of the shrimp were observed among all the groups. However, compared with the control group, protease activity in hepatopancreatic of the shrimp was significantly higher when up to 25% of commercial feed was replaced. The histological structure of the intestine gradually changed pathologically, such as tissue disruption, with increasing proportion of fresh BSFL in the diets. A significant reduction in intestinal fold height was found in the BSFL 100% group, and a decreased thickness of intestinal muscular was also observed in all treatment groups. The serum SOD and GSH-PX activities of shrimp in all treatment groups were significantly higher than that of the control group. In conclusion, replacing commercial feed up to 50% with fresh BSFL could be feasible for *L. vannamei* farming when growth performances, digestive enzyme activities, intestinal histology, and antioxidant enzymes were being considered.

1. Introduction

The Pacific white shrimp *Litopenaeus vannamei* is the most important farmed penaeid species worldwide with global production exceeding 3 million tons annually. Currently, over 90% of farmed shrimp relies on high-protein diets containing a high percentage of fish meal (FM), which has been a major protein source in aquatic feed industrial for decades due to its high protein content, balanced amino acid composition, and high palatability [1–3]. However, the supply of fish meal cannot meet the increasing demands of a blooming aquaculture industry due to ever declining wild capture fisheries. It has become a focus in aquaculture industry to find an alternative protein source with high nutrition, easy digestibility, stable supply, and low cost (Tacon & Metian [4]). A variety of renewable plant-based proteins have been evaluated to reduce the proportion of marine fish meal used in shrimp diets, with the feed cost being considered [5].

Generally, it was found that high percentage of plant-based proteins such as lupin meal [6], defatted microalgae meal [7], peanut meal [8], and soybean-based diets [9] reduced feed intake and growth rate in the trailed animals due to an unbalanced amino acid profile, shortage of one or more essential amino acids, or presence of some antinutritional factors comparing with fish meal [10–12].

Insects usually have a biological capacity to convert low-quality organic material into high-quality animal protein at a lower culturing cost of water, land, and so on, with less greenhouse gases emitting (Huis & [13]). The most common insects or their larvae cultured as a protein source include yellow mealworm *Tenebrio molitor* and house fly *Musca domestica*. Compared with those protein sources of plants, insects are characterized by relatively higher protein content, more balanced amino acid, and better palatability. The black soldier fly *Hermetia illucens* was thought as one of the most promising insect species for commercial production [14]. And black soldier fly larvae (BSFL) is abundant in proteins (approximately 42%) with relatively balanced amino acid profile and lipids especially those unsaturated fatty acids [15, 16]. BSFL meal has been found to benefit for growth performance of juvenile carp *Cyprinus carpio* var. Jian [17], shrimp *L. vannamei* [5], and Atlantic salmon*Salmo salar* [18]. Interestingly, BSFL also showed an antimicrobial activity, which could be a potential method to prevent and control the outbreak of diseases and reduce the use of antibiotics in aquaculture [19–21].

Recently, studies have been conducted to evaluate the effects of BSFL meal replacing fish meal in formulated feed on the growth performance of *L. vannamei* [5]. However, this common method has to consume more energy and produce carbon dioxide to obtain insect meal firstly and is unavoidably to destroy bioactivities of some nutritional components. Therefore, it is necessary to assess whether fresh BSFL could be fed to the shrimp *L. vannamei* and to replace commercial feeds at a proper ratio. This could also be a potential way to provide the shrimp with some special nutrients such as vitamins or astaxanthin, which is usually sensitive and vulnerable to high temperature, strong light, etc., during the processing and storing period. In the present work, experiments were performed to investigate the effects of fresh BSFL on survival, growth performance, digestive enzyme activities, intestinal histology, and serum antioxidant enzyme activities of *L. vannamei* in order to evaluate the feasibility of using fresh BSFL as biofeed to replace commercial feed and to provide more feeding strategies for shrimp farming.

2. Materials and Method

2.1. Feeding Regime. In this experiment, commercial feed (crude protein: 42% and crude lipid: 6%) was used as a control feeding regime. Then, different percentages (25%, 50%, 75%, and 100%) of commercial feed were replaced by fresh BSFL. The proximate composition of commercial feed and fresh BSFL is listed in Table 1. Feeding strategy and theoretical proximate composition of ingested diets for each treatment are shown in Table 2.

Table 1: Proximate composition of commercial feed and fresh BSFL (% weight).

Proximate composition	Commercial feed	Fresh BSFL
Crude protein	42	17.8
Crude ash	16	2.2
Crude lipid	4	7.4
Phosphorus	1.2	0.3
Moisture and other volatile	11	69.3

Notes: The eggs of black soldier fly were hatched by our team, and the larvae were fed with bran (bran 3 : water 1) regularly every day. Commercial shrimp feeds were purchased from a local dealer.

2.2. Feeding Experiment. L. vannamei postlarvae (PL5 stage with a body length of 0.4-0.5 cm) were purchased from a commercial hatchery in Hainan province, China, and were cultivated to juveniles with a body length of 3.27 ± 0.23 cm. Then, healthy individuals at a similar size were selected and distributed into 400 L glass fiber tanks filled with 300 L of seawater. The shrimp were acclimated for 7 days in the tanks and then were randomly divided into 15 tanks at a density of 50 individuals in each tank. Three replications were maintained in each group. Shrimp were fed four times a day (6: 00, 12: 00, 18: 00, and 24: 00) and to a visual satiation each time. The remaining feeds and feces were drained out of the tank two times a day through a sewer pipe on the bottom, and then, clean water was filled in with a daily water exchange about 30% of the total volume. The experiment lasted for 45 days. During the trial, the water temperature, salinity, and dissolved oxygen were maintained at 28 ± 3°C, 27 ± 2, and above 5.0 mg/L, respectively.

2.3. Sample Collection of Shrimp. At the end of the experiment, shrimp were starved for 12 h prior to sampling and then were taken out from each tank and individually weighed. Hemolymph from five shrimps in each tank were collected and mixed with anticoagulant solution (1 : 1) and kept at 4°C for 6 h. The separated serum was removed by centrifuging (3000 rpm, 10 min) and was aliquoted immediately and stored at -80°C for the analysis of antioxidant enzyme activities. After that, hepatopancreas of three shrimp from each tank were dissected and pooled into one sterile tube, flash frozen, and stored at -80°C for the subsequent analysis of digestive enzyme activities. Finally, the intestines were collected and fixed in 10% buffered formalin for the histological examination.

2.4. Assay of Digestive Enzyme and Antioxidant Enzyme Activities. Hepatopancreas were homogenized in ice-cold distilled water at a proportion of 1 : 9 (w/v). After centrifugation ($2500 \times g$, 10 min, 4°C), the supernatants were separated and kept at 4°C. The protease, lipase, and amylase activities were analyzed according to the instructions of commercially available kits (Nanjing Jiancheng biotech. Co, Nanjing, China). The protein concentration of the enzyme extracts was measured by using the Bradford method [22].

The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX) in the serum

Table 2: Feeding strategy and proximate composition of the experimental diets.

	Experiment diets				
	BSFL 0%	BSFL 25%	BSFL 50%	BSFL 75%	BSFL 100%
Feeding times (times/day)					
Commercial feed	4	3	2	1	0
Fresh BSFL	0	1	2	3	4
Proximate composition (% weight)					
Crude protein	42.00	35.95	29.90	23.85	17.80
Crude ash	16.00	12.55	9.10	5.65	2.20
Crude fat	4.00	4.85	5.70	6.55	7.40
Phosphorus	1.20	0.98	0.75	0.53	0.30
Moisture and other volatile	11.00	25.58	40.15	54.73	69.30

Notes: BSFL 0% is the control group. The replacement rate was controlled by adjusting the feeding times of fresh BSFL.

were examined by commercial assay kits following manufacturer's instructions (Nanjing Jiancheng biotech. Co, Nanjing, China).

2.5. Histological Examination of the Intestine Structure. The fixed intestine samples were dehydrated in a graded ethanol series, embedded in paraffin, and sectioned. The sections were stained with hematoxylin solution for 3-5 min and rinsed with distilled water and then were treated with hematoxylin differentiation solution and hematoxylin Scott tap bluing, respectively, and rinsed after each step. After that, the sections were dehydrated with 85% ethanol for 5 min and 95% ethanol for 5 min and stained with eosin dye for 5 min. Finally, the samples were photographed with a light microscope (×100), and the degree of variation in the intestinal fold height and muscular thickness was evaluated by CaseViewer 2.1. Five intact folds were selected for each section with their height (μm) and muscular thickness (μm) measured.

2.6. Calculations and Statistical Analysis. The weight gain (WG), hepatosomatic index (HSI), and survival rate (SR) of the shrimp were calculated based on the following formulae:

$$\text{Weight gain (WG, \%)} = 100 \times \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}},$$

$$\text{Hepatosomatic index (HSI, \%)} = \frac{\text{hepatopancreas weight}}{\text{final body weight}} \times 100,$$

$$\text{Survival rate (SR, \%)} = \frac{\text{final amount of shrimp}}{\text{initial amount of shrimp}} \times 100. \quad (1)$$

One-way analysis of variance (ANOVA) was used to test the effects of dietary manipulation. All data were tested for normal distribution and homogeneity of variance. If a significance was detected, LSD-test multiple comparisons were used to compare means between groups. All statistical analyses were performed by using the software SPSS23. The level of significant difference was chosen at $P < 0.05$, and the results were presented as means ± S.E.M. (standard error of the mean).

3. Results

3.1. Survival and Growth Performance. Results in Table 3 showed that the survival rate (SR), final body weight (FBW), and weight gain (WG) were negatively related with the replacement rate of fresh BSFL in the diet, where the maximum value was found in the BSFL 25% group, while the minimum value appeared in the BSFL 100% group. When BSFL replacement level was equal to or above 75%, the SR, FBW and WG were significantly decreased. However, HSI was significantly increased with the increasing BSFL replacement level, which was significantly higher in BSFL 75% and BSFL 100% groups than the control group ($P < 0.05$).

3.2. Digestive Enzyme Activities. The hepatopancreas lipase and amylase activities of the shrimp were not significantly affected by BSFL replacement level ($P > 0.05$). The maximum value of hepatopancreatic protease activity was found in the BSFL 25% group, with significant differences compared to the control groups ($P < 0.05$), and there were no significant differences ($P > 0.05$) in hepatopancreas protease in other BSFL replacement groups (Table 4).

3.3. Intestinal Histology Structure. Histological sections of the shrimp intestine revealed that the intestinal fold height and muscular thickness decreased with BSFL replacement level increasing. When BSFL replacement level was equal to or above 25%, the muscular thickness of intestine was significantly decreased compared with the control group ($P < 0.05$). And the intestinal fold height in the BSFL 100% group was significantly lower than the other groups ($P < 0.05$) (Table 5).

The intestinal histological structure of shrimp was damaged severely in the BSFL 100% group, which appeared with a broken mucosa, disordered folds, and obvious signs of intestinal inflammation, such as an increase in the cytoplasmic vacuolization, injury, and loss of epidermal integrity (Figure 1).

Table 3: Growth performance and survival of *L. vannamei* fed with diets containing different levels of BSFL for 45 days.

Items	Diets BSFL 0%	BSFL 25%	BSFL 50%	BSFL 75%	BSFL 100%
IBW (g)	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02
FBW (g)	5.76 ± 0.98^{a}	6.44 ± 0.54^{a}	4.73 ± 0.87^{ab}	3.41 ± 0.52^{b}	2.93 ± 0.35^{c}
WG (%)	2516.67 ± 444.19^{a}	2826.26 ± 244.95^{a}	2049.75 ± 394.74^{ab}	1447.98 ± 234.43^{b}	1229.80 ± 159.61^{c}
Survival (%)	55.33 ± 0.94^{b}	62.67 ± 2.49^{a}	51.33 ± 2.49^{b}	44.67 ± 2.49^{c}	26.67 ± 0.94^{d}
HSI (%)	4.63 ± 0.47^{c}	5.31 ± 0.23^{bc}	5.36 ± 0.61^{bc}	6.58 ± 0.58^{ab}	7.70 ± 1.00^{a}

Values presented as mean ± SE of samples.. Values of each parameter in the same row with different superscripts are significantly different ($P < 0.05$).

Table 4: Digestive enzyme activities of *L. vannamei* fed with diets containing different levels of BSFL for 45 days.

Enzyme activity	Diets BSFL 0%	BSFL 25%	BSFL 50%	BSFL 75%	BSFL 100%
Amylase (U/mgprot)	0.31 ± 0.13	0.27 ± 0.02	0.23 ± 0.03	0.27 ± 0.08	0.25 ± 0.10
Protease (U/mgprot)	16.32 ± 3.88^{b}	23.97 ± 2.39^{a}	21.93 ± 3.34^{ab}	20.78 ± 1.48^{ab}	22.31 ± 2.36^{ab}
Lipase (U/gprot)	0.72 ± 0.42	0.65 ± 0.37	1.18 ± 0.51	0.87 ± 0.06	0.55 ± 0.38

Values presented as mean ± SE of samples. Values of each parameter in the same row with different superscripts are significantly different ($P < 0.05$).

Table 5: Histology of the intestine of *L. vannamei* fed with diets containing different levels of BSFL for 45 days.

Parameters	Diets BSFL 0%	BSFL 25%	BSFL 50%	BSFL 75%	BSFL 100%
Intestinal fold height (μm)	51.05 ± 1.84^{a}	44.16 ± 5.52^{a}	44.77 ± 6.72^{a}	48.12 ± 14.50^{a}	33.58 ± 13.41^{b}
Muscular thickness (μm)	73.57 ± 16.19^{a}	45.62 ± 15.35^{b}	54.59 ± 16.49^{b}	51.76 ± 12.95^{b}	53.59 ± 21.14^{b}

Values presented as mean ± SE of samples. Values of each parameter in the same row with different superscripts are significantly different ($P < 0.05$).

3.4. Antioxidant Enzyme Activities. Both SOD and GSH-PX activities in the shrimp from the treatment groups were significantly higher than the control group ($P < 0.05$). As BSFL replacement level increased, the serum antioxidant enzyme activities showed a trend of first increasing and then decreasing. The maximum value for SOD activity was observed in the BSFL 25% group, and the maximum value for GSH-PX activity was observed in the BSFL 50% group. The serum CAT activity was not different significantly among all the groups (Table 6).

4. Discussion

4.1. The Effects of Feeding BSFL on Growth Performance of L. vannamei. It showed that fresh BSFL replacement for an appropriate proportion of commercial feed had no significant negative impacts on growth performance and survival rate in *L. vannamei*. Moreover, upon visual observation, it was clear that shrimp in each group moved actively and rapidly and swam towards the BSFL after feeding, suggesting the fresh BSFL was palatable and attractive to the animal. Similar results were found in yellow catfish *Pelteobagrus fulvidraco* [23], crayfish *Cherax cainii* [24], and rainbow trout *Oncorhynchus mykiss* [25], where partial replacement of fish meal by BSFL meal in the diets did not compromise growth performance. However, some studies found that the growth and feed utilization of aquatic animals were significantly reduced when dietary fish meal was replaced by BSFL meal. These results might be due to differences in aquatic animal species, size, or the food and processing method for insects [26]. As to the Pacific white shrimp *L. vannamei*, no apparent effects on the weight gain, feed efficiency, and survival rate were found when dietary fish meal replaced by BSFL meal was no more than 60% [5]. Above all, it would be feasible to use either fresh BSFL or BSFL meal to replace commercial feeds in aquaculture.

In this work, the growth performance and survival rate of the shrimp were significantly reduced as BSFL replacement level was equal to or above 75%. This might be due to insufficient protein intake when the commercial feeds were replaced by BSFL out of an optimal range. Many studies had been performed to investigate protein requirements of *L. vannamei*, and suggesting the lower limit of dietary protein level for this species would be around 30% [27]. The growth performance of shrimp was significantly reduced when dietary protein level was lower than 37.11% [28].

In addition, dietary lipid content can affect the lipid deposition in hepatopancreas of aquatic animals [29]. Fresh BSFL have higher lipid content than commercial feeds, which led to a significantly higher lipid contents in hepatopancreas of shrimp from BSFL 75% and BSFL 100% groups compared with that of the commercial feed group. An increased lipid intake could account for the increasing hepatosomatic index in shrimp as BSFL replacement level increasing. Appropriate lipid level not only enhanced feed efficiency and save protein

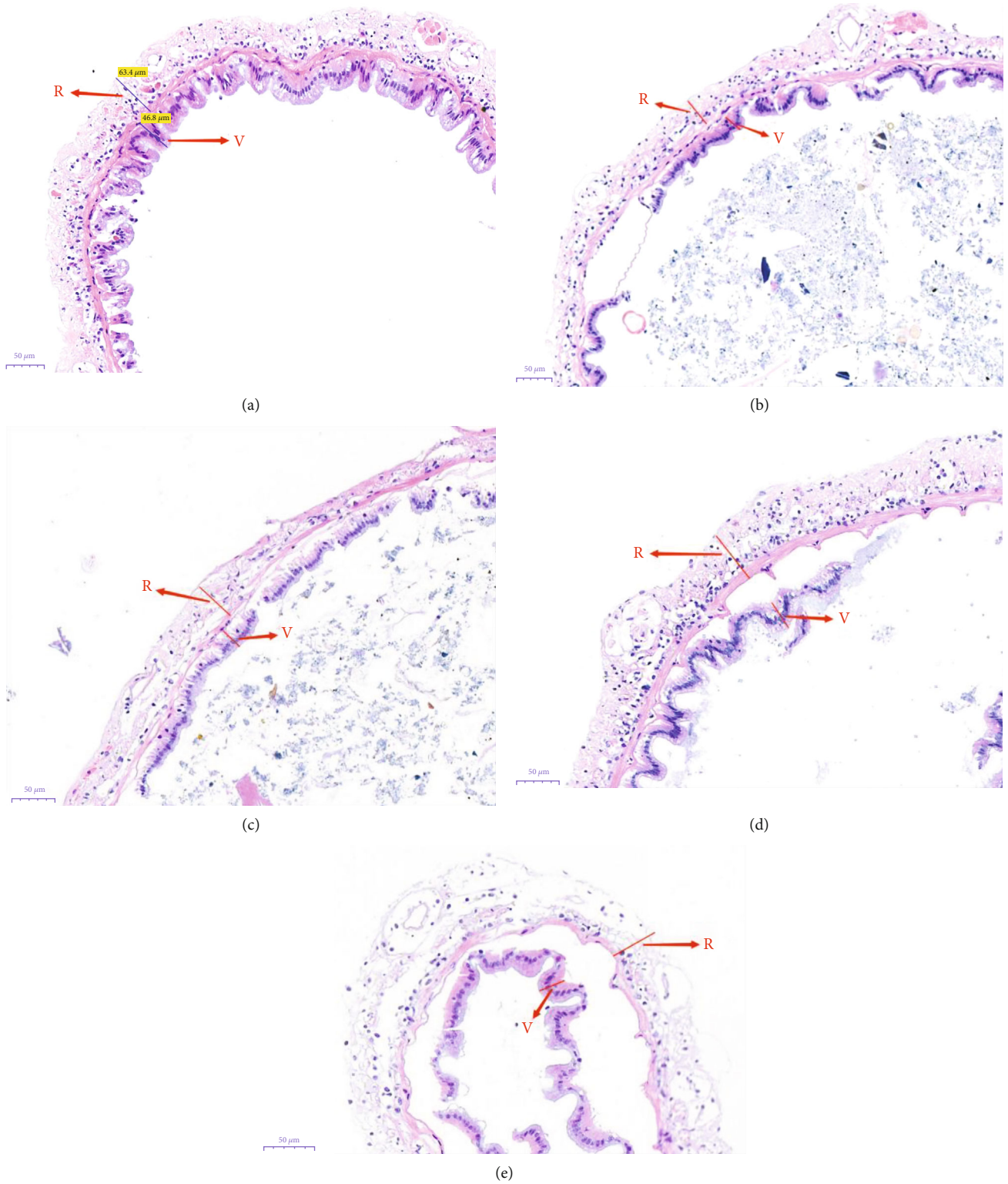

Figure 1: Intestinal segments obtained from shrimp fed with (a) BSFL 0%, (b) BSFL 25%, (c) BSFL 50%, (d) BSFL 75%, and (e) BSFL100 %. R: muscular thickness; V: fold height; measuring stick = 50 μm.

but also improved the immune system of aquatic animals [30–32]. Previous studies found that growth performance of juvenile turbot *Scophthalmus maximus* was significantly improved with an increasing dietary lipid level [33]. A diet with 9.69%-13.75% crude lipid can enhance the CAT and GSH-PX activity of *L. vannamei* [34]. However, growth performance and immune response were suppressed as dietary lipid level exceeding an optimal range [35].

Table 6: Antioxidant enzyme activities of *L. vannamei* fed with diets containing different levels of BSFL for 45 days.

Antioxidant enzyme	Diets				
	BSFL 0%	BSFL 25%	BSFL 50%	BSFL 75%	BSFL 100%
SOD (U/ml)	15.50 ± 3.10^{b}	93.64 ± 11.78^{a}	65.35 ± 2.09^{a}	67.05 ± 13.11^{a}	67.60 ± 5.58^{a}
CAT (U/ml)	9.26 ± 3.25	14.00 ± 3.32	15.96 ± 2.03	14.15 ± 5.90	14.20 ± 2.97
GSH-PX (U)	171.76 ± 16.03^{d}	774.05 ± 27.48^{b}	893.13 ± 22.90^{a}	476.18 ± 47.75^{c}	556.87 ± 30.15^{c}

Values presented as mean ± SE of samples. Values of each parameter in the same row with different superscripts are significantly different ($P < 0.05$).

4.2. The Effects of Feeding BSFL on Digestive Enzyme Activity of L. vannamei. It has been well known that the exoskeleton of insects is rich in chitins. Many studies have shown that chitin promoted the growth performance and digestibility in crustaceans. Rice-field crab *Esanthelphusa dugasti* fed a diet containing 2% chitin showed better growth performance and survival rate [36]. Shrimp fed a diet supplemented with chitin exhibited higher survival rate, growth performance, and stress tolerance abilities [37]. Diet supplemented with 5% chitin improved the protein and lipid digestibility and growth performance of tiger prawn *Penaeus monodon* [38]. Addition of a low dose of chitosan in the diets can effectively enhance the activity of hepatopancreas protease of *L. vannamei* [39]. In the present work, protease activity of the shrimp fed BSFL 50% was significantly higher than that of the control group, which might be attributed to the chitin from the insect's shell. It was worth noting that the daily protein intake of shrimp in the BSFL 50% group was only about 60% of that in the control group, indicating that an elevated protease activity could improve the protein efficiency and give a comparable growth performance in shrimp from the BSFL 50% group and the control group.

4.3. The Effects of Feeding BSFL on Intestinal Histology of L. vannamei. Histological section showed that more fresh BSFL replacement in the diets would damage the intestinal structure of shrimp. As we have known, intestine is one of the main digestive organs and plays an important role in excretory process, which could be a bio-mark to justice healthy degree for a shrimp. Dietary fiber influences movement of food particles along the digestive tract, however, more food fiber might result in food nutrient absorption disorder. In this work, the chitin from BSFL could also slow down the movement of nutrients along the digestive tract and have negative effects on digestion and absorption activities in the animal [38]. Defatted BSFL meal added to the diet also caused intestinal damage to juvenile carp *C. carpio* var. jian [17]. Similar studies showed that growth performance of *L. vannamei* was decreased, and histological structure of intestine was disrupted with increasing BSFL meal in the diets, which could be due to increased deficiency of essential amino acid and imbalanced EAA/NEAA [5]. Previous studies showed that amino acid deficiency in plant-based diets adversely could affect fish intestinal epithelium [40]. Therefore, there could be a complex mechanism under the phenomenon about a histological damage occurred in shrimp intestine by overfeeding with fresh BSFL, which requires further research.

4.4. The Effects of Feeding BSFL on Antioxidant Enzyme Activity of L. vannamei. Oxidative stress occurred when the balance between the reactive oxygen species (ROS) and the antioxidant capacity was disturbed, leading to some damaging effects on the animals [41]. The oxidative stress in aquatic organisms is more reflective during nutritional stress [42]. Antioxidant enzymes played the major cellular protective role against oxidative stress, in which SOD-CAT-GSH-PX enzyme cascade represents the first line of defense against ROS [43, 44]. In the present study, significant higher activities of antioxidant enzymes were detected in the serum of shrimp fed with BSFL diets. This was in accordance with previous study [23], where SOD activity in the serum of yellow catfish *P. fulvidraco* was increased by appropriate level of BSFL meal supplemented in the feed. Furthermore, CAT activity in carp *C. carpio* var. jian was also increased when defatted BSFL meal was added in their diets [17]. Some research works also demonstrated that chitin and its derivatives could exert antioxidant activities and enhance the immune response [45, 46]. Chitin extracted from insects was found to show obvious antioxidant properties and antibacterial activity against pathogenic microorganisms [47]. This could explain the results about the significant higher activities of antioxidant enzymes for shrimp fed with BSFL diets during former period of the experiment. However, some research showed that shrimp fed diets with lipid level above 14% produced more MDA in hemolymph lead to negative effects on the immune system [34]. In this work, the daily lipid intake of the shrimp elevated as BSFL replacement level in the diets increasing, which could produce peroxidation of lipid and lead to a downward trend of the serum antioxidant activity.

Fortunately, most insect larvae have the characteristics of nutrition fortification, through which their nutrient composition would be adjusted upon changing the culturing substrates [48]. Meneguz et al. [49] found that the proximate composition of the fresh BSFL was significantly changed by using agro-industrial by-products instead of organic waste. Thus, it would be necessary to develop nutrition enhancement strategies to produce high-quality insect larvae with more comparable nutrients in the future.

5. Conclusion

This study demonstrated that at least 50% of commercial feed could be replaced with fresh BSFL without significant adverse effects on growth performance and survival rate, digestive enzyme activities, and antioxidant enzyme

activities of *L. vannamei*. However, the intestinal health of shrimp was compromised with an increasing proportion of BSFL over 50% in the daily diet, which could limit a higher replacement on the fish meal. A nutrition enhancement for the live BSFL before feeding could benefit to intestinal health and contribute to a sustainable shrimp farming industry by reducing fish meal consumption.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Yuhang He designed and performed the whole experiment under the help of Yusheng Jiang. Yuhang He drafted the manuscript. Xin Liu and Naida Zhang measured the activity of digestive and antioxidant enzymes. Sizhe Wang and Aolin Wang performed the statistical analysis of the original data. Dr. Rantao Zuo revised the manuscript.

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