Original Research Articles

Effects of replacing dietary fishmeal with zymolytic black soldier fly larvae on the growth performance of the mud crab (*scylla paramamosain*) larvae

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Black soldier fly have been shown to be one of the optimal alternatives to fishmeal, but there are few reports on the effects of zymolytic black soldier fly larvae (ZBSFL) on the growth and digestion of crustaceans. An 8-week feeding trial was conducted to evaluate the effects of different replacement levels of ZBFLS on growth performance, body composition, and digestive enzyme activity of the mud crab larvae. Four diets were formulated by replacing fishmeal with 0%, 5%, 10% and 15% ZBSFL in the basal diet. Crab larvae were randomly divided into four groups of three replicates each and fed twice daily. The results showed that the SR of crab larvae was higher than that of the no-substitution group when the substitution rate reached 5% ($P < 0.05$). There was no significant change in SR when the substitution rate was further increased. Weight growth rate and Specific growth rate were similar, both highest at 10% substitution ratio. The crude protein content of whole crab larvae gradually increased as the proportion of FM substituted by ZBSFL increased. The lipid content of whole crab larvae in the 5% substitution ratio group was significantly higher than that in all other groups ($P < 0.05$). Meanwhile. The activities of amylase, protease and lipase gradually increased. In this experiment, when the percentage of ZBSFL substitution for FM reached 10%, its growth performance was optimal, with higher SR, less negative effects and more balanced indicators in all aspects. When the substitution rate was further increased, it might increase the digestive burden of the crab and negatively affect its growth.

INTRODUCTION

The mud crab (*Scylla paramamosain*), is a crustacean with important economic value.[1] With various advantages such as fast growth, large size and delicious taste, it is favored by the aquaculture industry and consumers. Therefore its culture scale is expanding, becoming a major aquaculture species in coastal areas of Southeast Asia.[2] In 2020, the farming production of the mud crab in China exceeded 150,000 tons.[3] However, crab farming is still deeply dependent on fishmeal (FM). In recent years, the limited production of FM has led to its high price, which has increased by 40-100 USD/ton compared to previous years, greatly increasing the cost of crab farming.[4] The pursuit of increased FM production is not conducive to the sustainable development of aquaculture and the marine environment.[5] In related studies in the field of aquatic feeds, the use of plant protein sources like soybean meal, cottonseed meal, single-celled algae and soybean protein concentrate can replace FM in some aquatic animal feeds to some extent and will not have negative effects on aquatic animals' growth, feed utilization, nutritional quality and immunity.[6-9] However, there are many problems with plant protein raw materials, for example, containing different kinds of antinutritional

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factors, high fiber content, amino acid imbalance, and its replacement of FM ratio above a certain level will affect the growth and health of aquatic animals to varying degrees, especially for carnivorous fish.[10,11] And the increased use of plant protein raw materials will lead to increased competition between feed production and human food supply.[12] There are still some controversies about the application of plant protein ingredients to replace FM in aquatic animal farming. It is necessary to seek other alternatives with similar nutritional value to FM.

As a natural renewable resource with rich protein content, black soldier fly, *Diptera*, *Stratiomyidae*, is not a resonable option. Its larvae is saprophagous and have a high rate of absorption and conversion of livestock manure and household waste.[13] The protein content of black soldier fly larvae is about 40% of dry weight and is more similar to the amino acid composition of FM than alternatives from plant sources, even with higher levels of some essential amino acids, such as valine, than FM, making it one of the suitable protein sources to replace FM.[14] However, it has its own limitations, as black soldier fly larvae have a higher content of lauric acid compared to FM. Therefore, once the optimal replacement rate is exceeded, there may be adverse effects on fish production or health. In recent years, experiments using black soldier fly as a feed ingredient or additive have successfully demonstrated that it can completely or partially replace FM. Black soldier fly larvae meal can almost completely replace FM and has no significant negative effects on Atlantic salmon (*Salmo salar*).[15] Another study has shown that black soldier fly larval oil can be a completely safe substitute for fish oil in diets of juvenile carp (*Cyprinus carpio*).[16] Such a good substitution effect is not common, in most of the studied species, black soldier fly products can only partially replace FM, such as pikeperch (*Sander lucioperca*),[17] rice field eel (*Monopterus albus*),[14] rainbow trout (*Oncorhynchus mykiss*),[18] red sea bream (*Pagrus major*)[19] and *Totoaba macdonaldi*[20] etc.

Since there are few related studies in crustaceans, in this experiment, we selected a more easily absorbed zymolytic black soldier fly larvae (ZBSFL) to investigate its effect on growth performance, body composition and digestive enzyme activity of the mud crab larvae, in order to evaluate its practical application and lay the foundation for nutritional management and cost saving in culture of the mud crab and even crustaceans.

MATERIALS AND METHODS

EXPERIMENTAL DIETS

The formulation and proximate composition of the experimental diets were shown in **Table 1**. ZBSFL was provided by Guangzhou Fuxing Biotechnology Co., Ltd (Guangzhou, China). ZBSFL was used to replace FM at 0%, 5%, 10% and 15%, respectively. The crude protein content of the experimental diets gradually decreased and the lipid content gradually increased as the proportion of FM substituted by ZBSFL increased (**Table 1**). All diets were extruded into 2 mm pellets using a Valva-60D-III extruder (Valva Machinery & Equipment Co., Ltd., Guangzhou, China), dried overnight at 55℃ and stored at -20℃ until use. The crude protein content in diets was determined using Kjeldahl method. The lipid content in diets was determined using Soxhlet method.[21] The gross energy of the diets was determined using an oxygen bomb calorimeter (Parr 6100, USA).

FEEDING EXPERIMENT

Sanya Tropical Fisheries Research Institute provided 1200 crab larvae of similar size and vigor (initial weight 1.43 ± 0.15 g). The crab larvae were kept in 300 L plastic buckets with aerated filtered saltwater, separated into four groups of three replicates each at random (natural light, temperature 26-29℃, salinity 29-30‰, dissolved oxygen content > 6.0 mg/l, ammonia-nitrogen content below 0.05 mg/L). The crab larvae were fed daily at 9:30 and 17:30 during an eight-week experiment. Mortality was recorded daily during the experiment.

GROWTH AND SURVIVAL INDICES

After eight weeks of culture, the feeding of crab larvae was suspended for 24 h, and the larvae were collected and survival was determined. The weight of crab larvae was accurately weighed using an electronic balance (accuracy 0.1 g), and the carapace data was measured using vernier caliper (accuracy 0.01 cm). For the analysis of digestive enzyme activity and body composition, crab larvae from each sample group were separated into two halves and kept in a -80°C freezer until enzyme assay. The following are the equations used in this experiment.

Survival rate (SR, %) = 100 × (final number of larvae)/(initial number of larvae).

Weight growth rate (WGR, %) = 100 × (final body weight (g) − initial body weight (g))/initial body weight (g).

Specific growth rate (SGR, %/d) = 100 × (ln final body weight (g) − ln initial body weight (g))/days.

Shell height growth rate (SHGR, %) = 100 × (final shell height (mm) − initial shell height (mm))/initial shell height (mm).

Specific shell height growth rate (SGR_{SH}, %/d) = 100 × (ln final shell height (mm) − ln initial shell height (mm))/days.

Shell width growth rate (SWGR, %) = 100 × (final shell width (mm) − initial shell width (mm))/initial shell width (mm).

Specific shell width growth rate (SGR_{SW}, %/d) = 100 × (ln final shell width (mm) − ln initial shell width (mm))/days.

The crab body composition was determined using the following indicators. The crude protein content and lipid content were determined as in 2.1. The moisture content was identified by drying the materials to a constant weight at 105°C. Ash content was determined by continuously searing at 550°C for 6 hours.[21]

Six crabs were randomly selected from each group, and the hepatopancreas, stomach and intestine were taken. The tissues of each crab were homogenized separately in pre-cooled double-distilled water and centrifuged at 9000 r/min for 30 min at 4℃, and the supernatant was extracted. Protease, amylase and lipase activities were determined using the kit method (Nanjing Jiancheng Bioengineering In-

Table 1. Formulation and nutrients of the experimental diets.

Ingredients (%)	*Control*	*5%*	*10%*	*15%*
Fish meal	40	35	30	25
Zymolytic black soldier fly larvae	0	5	10	15
Soybean meal	38	38	38	38
Soybean oil	2	2	2	2
Rice bran	4	4	4	4
Wheat bran	4	4	4	4
Corn flour	4	4	4	4
Vitamin premix	1	1	1	1
Mineral premix	1	1	1	1
Salt	1	1	1	1
$Ca(H_2PO_4)_2$	2	2	2	2
α-starch	3	3	3	3
Proximate composition (%)				
Crude protein	44.60	44.23	43.15	42.42
Lipid	5.16	6.33	8.24	9.78
Moisture	2.83	3.08	4.05	4.64
Ash	13.71	13.37	13.03	12.69
Gross energy ($kJ·g^{-1}$)	18.89	18.92	18.96	18.99
P/E ($mg·kJ^{-1}$)	22.64	22.94	23.33	23.51

[1] Zymolytic black soldier fly larvae (40% moisture, 28% crude protein, 10% crude fiber, 10% crude ash, 5% crude fat)

stitute, Nanjing, China). Protease activity was defined as 1 U of enzyme activity per milligram of protein per minute change in absorbance of 0.0030 at pH 8.0 at 37°C. Amylase activity is defined as 1 U of enzyme activity per mg of protein interacting with the substrate for 30 min at 37 °C for every 10 mg of hydrolyzed starch. Lipase activity is 1 U of enzyme activity for every 1 μmol of substrate consumed per milligram of protein interacting with the substrate for 1 min.

STATISTICAL ANALYSIS

The data of this experiment met the requirements of one-way ANOVA, so one-way ANOVA was performed on the data using SPSS 26.0 software, and Duncan's multiple range test was used to test the significance of differences. The experimental results were expressed as mean ± SE, and $P < 0.05$ indicated significant differences.

RESULTS

GROWTH PERFORMANCE AND SURVIVAL

The SR of crab larvae was considerably greater than that of the no substitution group ($P < 0.05$), when the substitution ratio reached 5% (**Table 2**). SR did not change significantly when the substitution ratio was further increased. WGR and SGR were similar, both being highest at 10% substitution ratio. The different substitution ratios had almost no significant effect on SHGR and SGR_{SH}, and only SHGR of the 5% substitution ratio group was significantly higher than that of the no substitution group ($P < 0.05$). SWGR and SGR_{SW} gradually increased with the increase of substitution ratio.

PROXIMATE COMPOSITIONS OF CRAB LARVAE

When more ZBSFL was used to replace FM, the crude protein content of the entire crab larvae steadily rose (**Table 3**). Compared to the other groups, the 5% replacement percentage group's entire crab larvae had a considerably greater lipid content ($P < 0.05$). The moisture and ash content of whole crab larvae in each group did not change significantly ($P < 0.05$).

DIGESTIVE ENZYME ACTIVITY

As shown in **Figure 1**, the amylase, protease, and lipase activities gradually increased with the percentage of ZBSFL substitution for FM. The amylase activity was significantly higher in the 15% substitution proportion group compared to the no substitution group ($P < 0.05$). And it had significantly stronger protease and lipase activities than all other groups ($P < 0.05$).

DISCUSSION

The production model of FM as the main protein source of aquafeed is unsustainable.[22] In the long run, fishery resources will be overexploited and depleted, which is not what aquaculture practitioners want to see. In such a situation, the value of edible insects, as a natural and renewable resource, is of interest. Black soldier fly is one of them.[23] Black soldier fly larval meal has a wide range of potential

Table 2. Effect of different levels of ZBSFL to replace FM in the diet on the growth performance of crab larvae.

Measurement indexes	***Control***	***5%***	***10%***	***15%***
SR (%)	76.17 ± 0.40[a]	80.49 ± 0.23[b]	80.41 ± 0.72[b]	81.01 ± 0.37[b]
WGR (%)	560.60 ± 13.88[a]	616.05 ± 5.77[b]	654.09 ± 8.28[c]	635.26 ± 12.75[bc]
SGR (%/d)	3.37 ± 0.04[a]	3.51 ± 0.02[b]	3.61 ± 0.02[c]	3.56 ± 0.03b[c]
SHGR (%)	193.26 ± 27.37[a]	244.87 ± 15.20[b]	202.54 ± 12.29[ab]	223.18 ± 29.98[ab]
SGR_{SH} (%/d)	1.88 ± 0.16[a]	2.14 ± 0.08[a]	1.93 ± 0.10[a]	2.05 ± 0.17[a]
SWGR (%)	117.71 ± 14.00[a]	123.42 ± 11.06[ab]	145.21 ± 20.14[ab]	153.91 ± 17.66[b]
SGR_{SW} (%/d)	1.31 ± 0.12[a]	1.38 ± 0.11[a]	1.53 ± 0.12[ab]	1.62 ± 0.12[b]

[1] Different letters marked in the same row indicate significant differences between the data of different groups ($P < 0.05$).
[2] SR, Survival rate; WGR, Weight growth rate; SGR, Specific growth rate; SHGR, Shell height growth rate; SGR_{SH}, Specific shell height growth rate; SWGR, Shell width growth rate; SGRSW, Specific shell width growth rate.

Table 3. Effect of different levels of ZBSFL to replace FM in the diet on the proximate composition of crab larvae.

Proximate composition (%)	***Control***	***5%***	***10%***	***15%***
Crude protein	32.99 ± 0.70[a]	33.33 ± 0.26[a]	35.13 ± 0.72[b]	36.63 ± 0.36[c]
Lipid	9.20 ± 0.48[a]	10.73 ± 0.20[b]	9.35 ± 0.55[a]	9.24 ± 0.58[a]
Moisture	77.72 ± 0.10[a]	77.60 ± 0.24[a]	77.74 ± 0.03[a]	77.73 ± 0.01[a]
Ash	43.48 ± 0.99[a]	44.38 ± 0.61[a]	43.65 ± 0.69[a]	43.26 ± 0.56[a]

1 Different letter marked in the same row indicate significant differences between the data of different groups ($P < 0.05$).

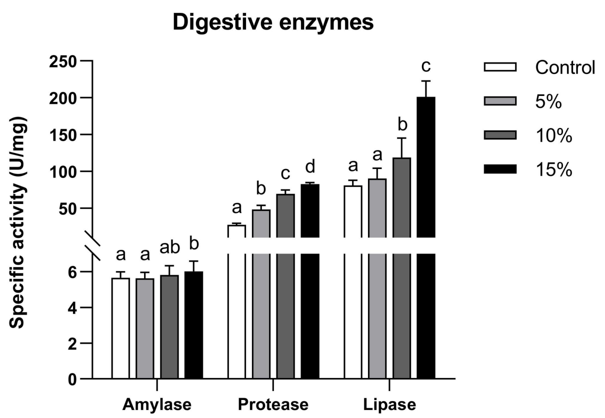

Figure 1. Effect of different substitution levels of ZBSFL on the digestive enzyme activity of *S. paramamosain* larvae.

The data are presented as mean ± SD (n = 3). Different letters denote significant differences ($P < 0.05$).

applications due to its high protein content, full amino acid profile, and abundance in unsaturated fatty acids. It may be utilized as a high quality protein source to substitute FM in aquatic diets.[17] Largemouth bass can grow more quickly when black soldier fly pulp is moderately added, and the hydrolyzed protein possesses bioactive peptides that make it easier to absorb and use. This has been demonstrated in prior experiments.[24] In order to further utilize the black gadfly larvae effectively and maximize the utilization value, ZBSFL was used as an alternative raw material to FM in this experiment.

In this study, there was no statistically significant difference between the three replacement groups, however the SR of crab larvae in the three groups was considerably greater than that of the non-substitution group. This result indicates that ZBSFL is palatable for the mud crab larvae, and within the range of addition in this experiment, it proved to improve the early SR of crab larvae, and there was no corresponding increase in SR when the substitution ratio was increased. There are fewer studies on ZBSFL, and they are more based on primary processing products such as black soldier fly larvae meal. Huang et al. found that when 50% of FM was substituted with black soldier fly larvae meal, the SR of the mud crab was improved, which was similar to the results of this study.[25] Presumably, since the maximum substitution rate in this study was only 15%, there was no suppression of the crab larvae survival.

Research on crustaceans is still scarce, but previous studies on fish have shown that black soldier fly larvae meal can partially or completely substitute FM without significant health effects.[4,14,15,17-20] When ZBSFL replacement for FM reached 10%, the WGR and SGR of crab larvae were most significantly higher than those of the control group ($P < 0.05$). According to previous reports, most of the insects had high protein proportions, between 40% and 60%, similar to the level of soybean meal and lower than FM. The crude protein content of the Orthoptera insects was most similar to that of FM, especially black soldier fly, whose crude protein content was closest to that of FM. This supports, to some extent, the usage of black soldier fly as one of the optimal substitutes for FM. Its composition of essential amino acids was similar but superior to that of soybean meal. Compared to FM, insect meal had lower amino acid content of histidine, lysine and threonine and higher content of tyrosine and valine. Insect meal has a same ratio of saturated fatty acids to FM. The monounsaturated fatty acids were almost twice as high as those of fish meal and soybean meal. Meanwhile, FM contained only 2.5% of total n-6, soybean meal contained 55.4%, and insect meal was between the two at about 15%.[22] This suggests that as the proportion of FM substituted by black soldier fly meal increases, the gap between essential amino acids, polyunsaturated fatty acids, DHA and EPA, which are critical for growth and development, in the feed will become larger and larger. This will lead to beyond a certain substitution range, and it will have an impact on the growth of crab larvae, thus affecting WGR and SGR. In this experiment, 10% ZBSFL was an appropriate substitution level.

The lipid concentration of the diets increased with the increase of the ZBSFL. The ZBSFL had a high fat content than the FM and a very different fatty acid profile, especially high levels of lauric acid.[26] Only in the 5% replacement group compared to the no substitution group did the lipid content in the body composition of crab larvae differ considerably. Previous studies have shown that the addition of full-fat or defatted black soldier fly larvae meal to the diets did not effect on how much lipid the fish deposited.[27] Yi et al. reported that greater consumption of black soldier fly larval oil caused yellow catfish to accumulate more saturated and monounsaturated fatty acids in yellow catfish (*P. fulvidraco*).[28] Such wildly divergent results could be a result of variances in diet composition or variations in animal species. Meanwhile, the crude protein content in the diets gradually decreased, but the crude protein content in the body composition of crab larvae increased instead, showing an opposite trend. This may be due to the fact that black soldier flies contain much higher levels of valine than FM, which is nutritionally regulated for different species of aquatic animals and is the first limiting amino acid for crustaceans.[29,30] Valine is an indispensable amino acid for the development of embryos and juveniles of Atlantic salmon in captivity and in the wild.[31] The ash levels in the four groups of diets were relatively close and there was no significant difference in the content of other body components between the experimental groups. In summary, we speculate that valine, which is abundant in ZBSFL, positively regulated the growth of crabs in this experiment.

Digestive enzyme activity is a key indicator for evaluating the nutritional value and utilization of diets for aquatic animals, and the composition of diets has a significant effect on the activity of digestive enzymes in crabs. The activity of the three digestive enzymes, especially lipase activity, increased progressively with the increase in the substitution ratio. Fatty acids are important signaling molecules that regulate lipid metabolism, and the prominent fatty acid content of ZBSFL prompted the need for crabs to secrete more lipase to aid digestion.[32] In addition, the chitin in ZBSFL can bind to proteins through covalent bonds, which can negatively affect the absorption and utilization of proteins by crabs.[33] However, high levels of ZBSFL in place of FM may add to the digestive burden of the crab due to the increase in components that are unfavorable to digestion and absorption. When the level of digestive enzymes reaches an extreme value, it does not increase with the increase of feed ingredients and even decreases instead, reducing the utilization of nutrients. In this experiment, no decrease in digestive enzyme activity was observed due to the small percentage of substitution. However, the degree of change in amylase activity was smaller than that of the remaining two enzymes, indirectly indicating that the absorption and utilization of proteins by crustaceans is preferred to that of starch. It has been demonstrated that *Argyrosomus regius*'s gut microbiota diversity and humoral immunological state are somewhat impacted by the inclusion of black soldier fly in the diet. Slight alterations in gut histomorphology are induced, which may impair health status, diet digestibility and growth performance.[34] The effect on digestive enzyme activity is an important factor governing the substitution ratio, and the specific effect of ZBSFL on crab digestion needs to be further investigated.

In a comprehensive view, in this experiment, when the proportion of ZBSFL replacing FM reached 10%, its growth performance was optimal, the SR was relatively high, the negative impact was relatively small, and all aspects of indicators were more balanced. However, when the substitution ratio was further increased, it might increase the digestive burden of crabs and negatively affect their growth.

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