Effects of Dietary Lipid Level on Growth Performance, Body Composition, and Digestive Enzyme Activity of Juvenile Scylla paramamosain Postprint

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Abstract

This experiment was conducted with juvenile mud crabs (Scylla paramamosain) with an initial body weight of (0.042(0.002) g, using fish oil and soybean oil (1:1) as the lipid source to formulate seven iso-nitrogenous and iso-energetic experimental diets with lipid levels of 1.93%, 3.95%, 6.35%, 8.14%, 10.54%, 12.30%, and 14.22% (measured values). The feeding trial lasted for 3 weeks to investigate the effects of dietary lipid level on growth performance, body composition, and digestive enzyme activities of juvenile mud crabs. One replicate consisted of 100 juvenile mud crabs, and three replicates were fed each experimental diet. The results showed: 1) Dietary lipid level had a significant effect on weight gain rate and specific growth rate of juvenile mud crabs ($P<0.05$). With increasing dietary lipid level, both weight gain rate and specific growth rate showed a trend of first increasing and then decreasing, reaching maximum values at a dietary lipid level of 8.14%. Quadratic regression analysis determined that the dietary lipid level was 7.52% when weight gain rate reached its maximum. The survival rate of juvenile mud crabs was lowest in the group with a dietary lipid level of 14.22%, which was significantly lower than the other groups ($P<0.05$). 2) Dietary lipid level had a significant effect on crude protein and crude lipid contents in whole crabs ($P<0.05$). Crude protein content in whole crabs first increased and then decreased with increasing dietary lipid level, reaching the highest value in the group with a dietary lipid level of 8.14%; crude lipid content in whole crabs continuously increased with increasing dietary lipid level, reaching the highest value in the group with a dietary lipid level of 14.22%. Dietary lipid level had no significant effect on moisture and crude ash contents in whole crabs ($P>0.05$). 3) With increasing dietary lipid level, the activities of protease, amylase, and lipase in juvenile mud crabs all showed a trend of

first increasing and then decreasing, with the maximum activities of all three enzymes appearing in the group with a dietary lipid level of 8.14%. Therefore, based on weight gain rate as the evaluation index, the optimal dietary lipid level for juvenile mud crabs was 7.52%.

Full Text

Effects of Dietary Lipid Level on Growth Performance, Body Composition and Digestive Enzyme Activities of Juvenile *Scylla paramamosain*

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Abstract

This study investigated the effects of dietary lipid level on growth performance, body composition, and digestive enzyme activities of juvenile *Scylla paramamosain* with an initial body weight of (0.042$±$0.002) g. Seven isonitrogenous and isoenergetic experimental diets were formulated with fish oil and soybean oil (1:1) as lipid sources, containing measured lipid levels of 1.93%, 3.95%, 6.35%, 8.14%, 10.54%, 12.30%, and 14.22%. The feeding trial lasted for 3 weeks. Each experimental diet was fed to three replicates of 100 juvenile crabs. The results showed that: (1) Dietary lipid level significantly affected weight gain rate (WGR) and specific growth rate (SGR) ($P<0.05$). Both WGR and SGR increased initially and then decreased with increasing dietary lipid level, reaching maximum values at 8.14% dietary lipid. Quadratic curvilinear regression analysis determined that the optimal dietary lipid level for maximum WGR was 7.52%. Survival rate was lowest in the 14.22% lipid group, significantly lower than all other groups ($P<0.05$). (2) Dietary lipid level significantly affected whole-body crude protein and ether extract contents ($P<0.05$). Whole-body crude protein content increased initially and then decreased with increasing dietary lipid level, peaking at 8.14% lipid. Whole-body ether extract content increased continuously with dietary lipid level, reaching its highest value at 14.22% lipid. Dietary lipid level had no significant effects on moisture or ash contents ($P>0.05$). (3) Protease, amylase, and lipase activities all increased initially and then decreased with increasing dietary lipid level, with maximum activities observed in the 8.14% lipid group. In conclusion, based on WGR, the optimal dietary lipid level for juvenile *Scylla paramamosain* is 7.52%.

Keywords: juvenile *Scylla paramamosain*; growth performance; body composition; digestive enzyme activities

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Introduction

Scylla paramamosain is a carnivorous crustacean that primarily feeds on small fish, shrimp, and shellfish. It is a euryhaline and eurythermal marine crab species with advantages including rapid growth, short culture period, and high economic value. The species is also prized for its delicious taste, high edible yield, and rich content of all eight essential amino acids required by humans, as well as abundant unsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Consequently, it is highly favored by farmers and consumers in the Beibu Gulf region [1]. As market demand has increased, the aquaculture area for *S. paramamosain* in the Beibu Gulf has gradually expanded [2].

Currently, *S. paramamosain* aquaculture primarily relies on extensive pond culture models with low-value shellfish and small fish/shrimp as feed. This farming model involves high input costs, causes significant environmental pollution, and is not conducive to large-scale promotion [3]. Therefore, investigating the nutritional requirements of *S. paramamosain* and developing an economical, environmentally friendly, and efficient artificial compound feed represents a key step toward intensified aquaculture of this species.

In aquaculture feeds, lipids primarily serve as an energy source for cultured animals, provide essential fatty acids for growth and development, and act as solvents to promote the absorption and transport of fat-soluble vitamins (such as vitamin A). Lipids also serve as raw materials for the synthesis of certain hormones and vitamins [4]. In fish feeds, lipid supplementation is one of the main factors affecting feed quality and fish growth. Research has shown that providing energy through dietary lipids and starch can effectively improve the utilization efficiency of digestible protein, thereby achieving protein-sparing effects [5]. Additionally, appropriate dietary lipid supplementation can improve feed palatability, reduce feed conversion ratio, and lower aquaculture costs [6]. Therefore, investigating optimal dietary lipid levels is of great significance.

Nutrition researchers have conducted numerous studies on optimal dietary lipid levels for crabs and other crustaceans. For example, optimal dietary lipid levels of 6.61%~9.96% have been reported for juvenile Chinese mitten crab (*Eriocheir sinensis*) [7], 9% for swimming crab (*Portunus trituberculatus*) [8], 5%~7% for ridgetail white prawn (*Exopalaemon carinicauda*) [9], and 9% for giant freshwater prawn (*Macrobrachium rosenbergii*) [10].

This study used fish oil and soybean oil as lipid sources to investigate the effects of dietary lipid level on growth performance, body composition, and digestive enzyme activities of juvenile *S. paramamosain*, aiming to provide important theoretical support for the development of artificial compound feeds for intensive culture of this species.

1.1 Experimental Diets

Prior to diet preparation, the crude protein, ether extract, and energy content of each ingredient were determined to guide diet formulation and adjustment. Seven isonitrogenous and isoenergetic experimental diets were formulated with fish meal, casein, and gelatin as protein sources; imported fish oil and Jinlongyu soybean oil (1:1) as lipid sources; and dextrin and microcrystalline cellulose as supplementary energy sources to balance energy levels. The target lipid levels were 0, 2%, 4%, 6%, 8%, 10%, and 12%, with measured values of 1.93%, 3.95%, 6.36%, 8.14%, 10.54%, 12.30%, and 14.22%, respectively . All ingredients were ground to pass through a 100-mesh sieve, weighed in ascending order of inclusion level, and mixed thoroughly. The mixture was then blended completely with fish oil, soybean oil, and water. Following the micro-bound diet processing method described by Blair et al. [11], the diets were extruded through a 2-mm die, labeled, bagged, and stored at 4°C until use.

1.2 Experimental Animals and Rearing Conditions

The feeding trial was conducted from August 1 to August 21, 2015, at the Mariculture Experimental Base of Guangxi Institute of Oceanology. Juvenile *S. paramamosain* used in the trial were artificially hatched from the same batch of fertilized eggs. The crabs were uniform dark brown, undamaged, vigorous, and healthy stage I juveniles with specifications of $(0.042\$\pm0.002)gbodyweight, (0.41\pm0.07)cmcarapacewidth, and(0.33\pm\$0.04)$ cm carapace length. Due to the aggressive nature and severe cannibalistic behavior of *S. paramamosain*, juveniles were cultured individually in this experiment, with 100 crabs per replicate and three replicates per dietary treatment.

The culture containers were plastic buckets (20 cm diameter × 25 cm height) with a thin layer (1–2 cm) of fine sand on the bottom and a tile (~5 cm^2) placed at the bottom edge to provide shelter. Crabs were fed twice daily at 07:00 and 18:00 to slight satiation, with uneaten feed removed before each feeding. Water was exchanged every two days, and culture buckets were thoroughly cleaned weekly. The experiment was conducted under natural photoperiod without aeration, with water temperature maintained at 26–30°C and salinity at 18‰–22‰.

1.3.1 Growth Performance

At the end of the feeding trial, juvenile crabs in each replicate were counted and weighed to calculate survival rate (SR), weight gain rate (WGR), and specific growth rate (SGR) using the following formulas:

$$\text{SR } (\%) = 100 \times \frac{N_t}{N_0}$$

$$\text{WGR } (\%) = 100 \times \frac{W_t - W_0}{W_0}$$

$$\text{SGR } (\%/\text{d}) = 100 \times \frac{1nW_t - lnW_0}{t}$$

where W_0 is the initial body weight, W_t is the final body weight, t is the experimental duration in days, N_0 is the initial number of crabs, and N_t is the final number of crabs.

1.3.2 Proximate Composition

Proximate composition of diets and whole crabs was analyzed according to AOAC (1995) [12] methods. Moisture content was determined by drying to constant weight in an oven at 105°C. Dried samples were ground using a high-speed grinder for determination of crude protein (Kjeldahl method), ether extract (Soxhlet extraction method), and ash (550°C muffle furnace combustion method).

1.3.3 Digestive Enzyme Activities

Whole crab samples were thawed at 4°C and homogenized in a glass homogenizer (immersed in ice-water mixture) with phosphate buffer solution (pH=7) added at twice the sample weight. The homogenate was centrifuged at 4°C and 4,000 r/min for 20 min, and the supernatant was used for digestive enzyme activity assays. Protease activity was determined using the Folin-phenol method. Lipase and amylase activities were measured using assay kits from Nanjing Jiancheng Bioengineering Institute. Protein content in the enzyme solution was determined using the Coomassie brilliant blue method with bovine serum albumin as the standard. All digestive enzyme activities were expressed as specific activity (U/mg prot).

1.4 Statistical Analysis

Data were analyzed using one-way ANOVA with SPSS 19.0 statistical software. If significant differences were detected, Tukey' s multiple comparison test was performed with a significance level of $P<0.05$. Results are presented as "mean ± standard error."

Results

2.1 Effects on Growth Performance

As shown in Table 2 , dietary lipid level significantly affected WGR and SGR of juvenile *S. paramamosain* ($P<0.05$). With 8.14% dietary lipid as the inflection point, WGR and SGR increased significantly as dietary lipid level increased

below this level (P<0.05), but decreased significantly when dietary lipid level exceeded 8.14% (P<0.05). Survival rate was lowest in the 14.22% lipid group, significantly lower than all other groups (P<0.05), while no significant differences were observed among the remaining groups (P>0.05).

As illustrated in Figure 1 [Figure 1: see original paper], quadratic regression analysis between WGR (Y) and dietary lipid level (X) yielded the equation:

$$Y = -7.3928X^2 + 111.22X + 17.438 \quad (R^2 = 0.9881)$$

From this equation, the maximum WGR of 435.75% was achieved at a dietary lipid level of 7.52%.

2.2 Effects on Body Composition

As shown in Table 3 , dietary lipid level significantly affected whole-body crude protein and ether extract contents (P<0.05). Whole-body crude protein content was highest (36.33%) in the 8.14% lipid group, significantly higher than all other groups (P<0.05). The lowest crude protein content (31.67%) was observed in the 14.22% lipid group, significantly lower than the 6.36% and 8.14% lipid groups (P<0.05). Whole-body ether extract content increased continuously with dietary lipid level, with the 1.93% lipid group significantly lower than all other groups (P<0.05), while the 12.30% and 14.22% lipid groups were significantly higher than the remaining groups (P<0.05). Dietary lipid level had no significant effects on moisture or ash contents (P>0.05).

2.3 Effects on Digestive Enzyme Activities

As shown in Table 4 , dietary lipid level significantly affected protease, amylase, and lipase activities (P<0.05), with all three enzymes reaching maximum activities in the 8.14% lipid group. Protease activity was lowest in the 14.22% lipid group, significantly lower than the 8.14% lipid group (P<0.05), with no significant differences among other groups (P>0.05). Amylase activity was also lowest in the 14.22% lipid group (0.50 U/mg prot), significantly lower than all other groups (P<0.05). No significant difference in amylase activity was observed between the 1.93% and 3.95% lipid groups, both of which were significantly lower than groups with 3.95%-12.30% lipid (P<0.05). Lipase activity in the 8.14% and 10.54% lipid groups was significantly higher than in all other groups (P<0.05), with the 8.14% lipid group significantly higher than the 10.54% lipid group (P<0.05). No significant differences in lipase activity were found among the 1.93%-6.36% lipid groups or among the 12.30%-14.22% lipid groups (P>0.05).

Discussion

3.1 Effects on Growth Performance

Lipids are an important source of energy and essential fatty acids for aquatic animals. Among the three major nutrients, lipids contain the highest energy content. Appropriate dietary lipid supplementation can effectively improve protein efficiency, achieve protein-sparing effects, reduce feed costs, and enhance aquaculture efficiency [5-6]. Dietary lipids not only serve as solvents for fat-soluble nutrients (vitamins A, K, carotenoids, etc.) to promote their digestion and absorption, but also provide lipids such as phospholipids and cholesterol that are fundamental components of cell membranes and essential for tissue repair and synthesis [13]. Additionally, appropriate lipid supplementation in aquafeeds can improve palatability, increase weight gain and feed conversion efficiency, and reduce water pollution during culture [6]. Consequently, research on optimal dietary lipid levels has received increasing attention in feed formulation and nutrition studies.

Different crustaceans exhibit varying capacities to utilize dietary lipids, resulting in different optimal lipid requirements. In this study, quadratic regression analysis of dietary lipid level against WGR indicated that the optimal dietary lipid level for juvenile *S. paramamosain* was 7.52%. Previous studies have reported optimal dietary lipid levels of 6.61%–9.96% for juvenile Chinese mitten crab [7], 9% for swimming crab [8], 5%–7% for ridgetail white prawn [9], and 9% for giant freshwater prawn [10].

Previous studies on crustaceans have shown similar trends. Wang et al. [14] reported that in *Litopenaeus vannamei*, WGR, SGR, and protein efficiency increased initially and then decreased with increasing dietary lipid level, while feed conversion ratio showed the opposite trend, with optimal lipid levels of 4.83%–5.98% for juvenile shrimp and 5.57%–7.86% for larger shrimp. Sun et al. [15] found that dietary lipid level significantly affected WGR in *M. rosenbergii*, with WGR increasing initially and then decreasing as lipid level increased from 6% to 12%, suggesting an optimal lipid level of 9% based on growth and feed utilization. Duan et al. [8] observed a similar trend in swimming crab, with WGR increasing initially and then decreasing with dietary lipid level, identifying 9% as the optimal lipid level when dietary protein was 40%. In the present study, WGR and SGR of juvenile *S. paramamosain* peaked in the 8.14% lipid group, significantly higher than all other groups. When dietary lipid was below 8.14%, WGR and SGR increased with lipid level; beyond 8.14%, they decreased. These results are consistent with findings from other crustacean studies. The negative effects of both low and high dietary lipid levels may be explained as follows: (1) Insufficient dietary lipid fails to provide adequate energy, essential fatty acids, and fat-soluble vitamins for growth, impairing development [13]; (2) When lipid energy is inadequate, dietary protein is utilized as an energy source, preventing protein requirements from being met [9]; (3) Although high lipid levels can spare protein, excessive lipid accumulation in muscle tissue af-

fects product appearance and quality, as demonstrated in red swamp crayfish (*Procambarus clarkii*) [16] and oriental river prawn (*Macrobrachium nipponensis*) [17]; (4) Excessive dietary lipid reduces feed palatability and may cause oxidative deterioration, further decreasing palatability and growth [9].

Dietary lipid levels from 1.93% to 12.30% did not significantly affect SR, consistent with studies on *L. vannamei* [14], *P. clarkii* [16], *M. nipponensis* [17], and kuruma shrimp (*Penaeus japonicus*) [18]. In this trial, the 14.22% lipid group showed greater feed residue at the same feeding rate, suggesting that high lipid levels reduced feed intake due to decreased palatability, resulting in insufficient nutrient intake for growth and development and consequently lower SR.

3.2 Effects on Body Composition

Si et al. [17] reported that dietary lipid level significantly affected whole-body ether extract and dry matter contents, muscle ether extract content, and hepatopancreas dry matter content in *M. nipponensis*. Xu et al. [16] found that dietary lipid level significantly affected whole-body ether extract, crude protein, dry matter, and ash contents in *P. clarkii*, while in muscle, only ether extract content was significantly affected, and in hepatopancreas, dry matter and ether extract contents were significantly affected. Wang et al. [14] observed that muscle crude protein content in *L. vannamei* increased initially and then decreased with dietary lipid level, peaking at 10.13% lipid, while dietary lipid had no significant effects on moisture or ash contents in whole shrimp or muscle of larger shrimp.

In this study, dietary lipid levels ranging from 1.93% to 14.22% significantly affected whole-body crude protein and ether extract contents but had no significant effects on moisture or ash contents. Whole-body crude protein content increased with dietary lipid level from 1.93% to 8.14%, likely because dietary lipids exerted a protein-sparing effect. As dietary lipid increased, sufficient energy was provided by lipids, improving protein utilization and promoting growth. However, when dietary lipid increased from 8.14% to 14.22%, high lipid levels reduced whole-body crude protein content, possibly because high-energy diets enhanced satiety signals to the central nervous system, reducing feed intake and consequently decreasing protein and other nutrient intake, thereby affecting growth [19-20].

3.3 Effects on Digestive Enzyme Activities

Research has demonstrated that digestive enzyme activities in crustaceans depend on their physiological digestive characteristics and dietary composition. These activities reflect adaptability to dietary nutrients and digestive capacity, ultimately determining growth performance [21]. Numerous studies have investigated the effects of feed composition on digestive enzyme activities in aquatic animals, with the general pattern that increased dietary components enhance the activity of corresponding digestive enzymes while also affecting

other enzyme activities [22]. In this study, the three digestive enzyme activities in juvenile *S. paramamosain* were relatively low, with protease activity ranging from 1.42–4.27 U/mg prot, amylase from 0.50–0.91 U/mg prot, and lipase from (1.91–10.29)$×10^{-2}$ U/mg prot, similar to results reported for swimming crab [23], Chinese mitten crab [24], and mud crab (*Scylla serrata*) [25].

The effects of dietary lipid level on digestive enzyme activities vary among crustacean species. In *P. clarkii*, dietary lipid level significantly affected lipase activity in the intestine and hepatopancreas, with both tissues showing significantly increased lipase activity at 10% lipid, while amylase activity was not significantly affected [16]. In *M. nipponensis*, dietary lipid level significantly affected both protease and lipase activities in hepatopancreas, with activities increasing significantly as lipid level increased from 5% to 11% [17]. The present results are generally consistent with these findings, showing that dietary lipid level significantly affected protease, amylase, and lipase activities in juvenile *S. paramamosain*, with all three enzymes following a pattern of initial increase followed by decrease. As dietary lipid level increased, sufficient lipid energy improved protein utilization, potentially leading to increased synthesis of adaptive enzymes [26]. Additionally, different dietary nutrient compositions result in varying nutrient intake, requiring physiological adaptation to enhance nutrient absorption and conversion [16]. However, excessive dietary lipid may inhibit lipase activity [27-28]. Furthermore, high-lipid diets contain high total energy, reducing the need for protein and carbohydrates as energy sources and consequently decreasing the secretion of corresponding digestive enzymes. Lipid oxidation products may also be toxic, further reducing protein and carbohydrate utilization [22].

Conclusion

Both excessively high and low dietary lipid levels negatively affect the growth of juvenile *S. paramamosain*. Based on WGR as the evaluation index, quadratic regression analysis determined that the optimal dietary lipid level for juvenile *S. paramamosain* is 7.52%.

References

[1] Tan D F, Wu G X, Lin Y X, et al. Nutritional composition analysis of *Scylla serrata*[J]. Journal of Fujian Normal University (Natural Science Edition), 2000, 16(4): 79–84.

[2] Chen F C. Pollution-free culture technology of *Scylla serrata*[J]. Journal of Guangxi Academy of Sciences, 2012, 28(2): 120–121, 130.

[3] Han Y L. Study on healthy culture model of *Scylla paramamosain*[D]. Master' s thesis. Shantou: Shantou University, 2011: 7–10.

[4] ZHOU Q C, ZHOU J B, CHI S Y, et al. Effect of dietary lipid level on growth performance, feed utilization digestive enzyme juvenile ivory shell, *Babylonia*

areolate[J]. *Aquaculture*, 2007, 272(1/2/3/4): 535-540.

[5] Chen S C. Effects of dietary lipid and different lipid sources on fish[J]. Modern Fisheries Information, 2008, 23(10): 7-11.

[6] Han C Y, Zheng Q M, Huang X H. Effects of dietary lipid level on growth, feed utilization and blood indices of hybrid tilapia (*Oreochromis niloticus* × *O. aureus*)[J]. China Feed, 2013(8): 36-39.

[7] Wang L Q, Hu W, Li H Y, et al. Effects of dietary lipid level on growth and feed utilization of juvenile crabs[J]. Journal of Shanghai Fisheries University, 2003, 12(1): 19-23.

[8] Duan Q Y, Mai K S, Shentu J K, et al. Effects of different protein and lipid levels on growth and ovarian pigment deposition of swimming crab (*Portunus trituberculatus*)[J]. Journal of Fishery Sciences of China, 2011, 18(4): 809-818.

[9] Suo S, Li J, Li J T, et al. Effects of protein and lipid levels on growth, non-specific immunity and disease resistance of ridgetail white prawn (*Exopalaemon carinicauda*)[J]. China Fishery Quality and Standards, 2015, 5(4): 40-47.

[10] Sun L S, Wang H, Wei K, et al. Effects of different dietary lipid levels on growth and tissue composition of giant freshwater prawn (*Macrobrachium rosenbergii*)[J]. Feed Industry, 2012, 33(16): 28-32.

[11] BLAIR T, CASTELL J, NEIL S, et al. Evaluation of microdiets versus live feeds on growth, survival and fatty acid composition of larval haddock (*Melanogrammus aeglefinus*)[J]. *Aquaculture*, 2003, 225(1/2/3/4): 451-461.

[12] AOAC. Official methods of analysis association of official analytical chemists[S]. 16th ed. Arlington, VA: Association of Official Analytical Chemists International, 1995.

[13] Li A J. Aquatic Animal Nutrition and Feed Science[M]. Beijing: China Agriculture Press, 1996: 36-46.

[14] Wang M Q, Zheng C Q, Jin M, et al. Effects of crude lipid level on growth performance, feed utilization and non-specific immunity of two sizes of Pacific white shrimp (*Litopenaeus vannamei*)[J]. Chinese Journal of Animal Nutrition, 2014, 26(9): 2687-2697.

[15] Sun L S, Wang H. Effects of oil supplementation level on growth performance, blood biochemical indices and tissue fatty acid composition of giant freshwater prawn (*Macrobrachium rosenbergii*)[J]. Chongqing Fisheries, 2010(3): 9-16.

[16] Xu W N, Liu W B, Shen M F. Effects of different dietary protein and lipid levels on growth performance, body composition and digestive enzyme activities of red swamp crayfish (*Procambarus clarkii*)[J]. Oceanologia et Limnologia Sinica, 2011, 42(4): 521-529.

[17] Si L G, Zou L C, Shentu J K, et al. Effects of different dietary lipid and protein levels on growth performance, body composition and digestive enzyme activities of oriental river prawn (*Macrobrachium nipponensis*)[J]. Oceanologia et Limnologia Sinica, 2014, 45(2): 400-408.

[18] Teshima S, Kanazawa A. Effects of protein, lipid and carbohydrate contents in compound feed on growth and survival of shrimp larvae[J]. Fisheries Science, 1986, 5(3): 48-51.

[19] Huang W J, Zhou M Y, Niu J J, et al. Food intake and energy balance[J]. Journal of Liaoning University of Traditional Chinese Medicine, 2014, 16(10): 79-84.

[20] Song Q C, Qi Z L. Aquatic Animal Nutrition and Compound Feed Science[M]. Beijing: China Agricultural University Press, 2010: 57-59.

[21] Yang Z G, Que Y Q, Ji L Y, et al. Effects of replacing trash fish with compound feed on growth and digestive enzyme activities of Chinese mitten crab (*Eriocheir sinensis*)[J]. Journal of Dalian Ocean University, 2013, 28(3): 293-297.

[22] GANGADHARA B N, EESHA M C, VARGHESE T J, et al. Effect of varying protein and lipid levels on the growth of rohu, *Labeo rohita*[J]. *Asian Fisheries Society*, 1997, 10(2): 139-147.

[23] Ding X Y, He Z Y, Qiu X L, et al. Study on digestive enzyme activities at different growth stages of swimming crab (*Portunus trituberculatus*) and effects of compound feed[J]. Chinese Journal of Animal Nutrition, 2010, 22(2): 492-497.

[24] Sun J H, Sun J F, Sun X L, et al. Effects of different dietary protein contents on growth performance and digestive enzyme activities of Chinese mitten crab (*Eriocheir sinensis*)[J]. China Feed, 2012(23): 25-27.

[25] Tang H, Li S J, Wang G Z, et al. Digestive enzyme activities of *Scylla serrata* larvae[J]. Journal of Xiamen University (Natural Science Edition), 1995, 34(1): 88-93.

[26] Tang L, Wang J Q, Cheng J C, et al. Research on digestive enzymes of aquatic animals[J]. Feed Industry, 2007, 28(2): 28-31.

[27] Han G M, Wang A M, Xu P, et al. Effects of dietary lipid level on survival, muscle composition and digestive enzyme activities of juvenile GIFT tilapia (*Oreochromis niloticus*)[J]. Journal of Shanghai Ocean University, 2010, 19(4): 469-474.

[28] Xiang X, Zhou X H, Chen J, et al. Effects of dietary lipid content on digestive enzyme activities of juvenile topmouth culter (*Culter alburnus*)[J]. Beijing Fisheries, 2008(5): 35-38.

Note: Figure translations are in progress. See original paper for figures.