Effects of Phospholipids on the Dietary Lipid Requirement of Macrobrachium nipponense (Postprint)

Authors: Wang Zhi, Cui Guangtong, Cai Chunfang, Ren Shengjie, Qiu Xiaoliang, Ding Huiming, Zhang Weiye, Ye Yuantu

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Abstract

This experiment aimed to investigate the effect of dietary phospholipids on the lipid requirement of Macrobrachium nipponense. Juvenile Macrobrachium nipponense with an initial body weight of (0.075±0.001) g were selected as experimental subjects and subjected to an 8-week growth trial in indoor culture tanks.

Fish oil and soybean oil were mixed in equal proportions, then blended with soybean phospholipid at ratios of 2:1 and 1:2, resulting in soybean phospholipid comprising 33.3% (low phospholipid group) and 66.7% (high phospholipid group) of the lipid mixture, respectively. The two lipid mixtures were added to the diets at proportions of 3% (low lipid group), 6% (medium lipid group), and 9% (high lipid group) to formulate six isonitrogenous and isoenergetic experimental diets. Based on the addition levels of the lipid mixture and soybean phospholipid in the experimental diets, the six diets were designated as L3P1, L3P2, L6P2, L6P4, L9P3, and L9P6, with measured dietary lipid levels of 7%, 7%, 10%, 10%, 13%, and 13%, respectively.

Each experimental diet was fed to four tanks of shrimp, with 150 individuals stocked per tank. The results showed that survival rates of Macrobrachium nipponense among the six groups ranged from 47.8% to 66.9%, with the L6P4 group exhibiting the highest survival rate, followed by the L3P2 group, and the L9P3 group showing the poorest survival.

The high lipid group exhibited significantly lower final body weight, weight gain rate, specific growth rate, and survival rate compared with the medium and low lipid groups ($P<0.05$), while feed conversion ratio was significantly higher ($P<0.05$). The high phospholipid group demonstrated significantly higher final body weight, weight gain rate, specific growth rate, and survival rate than the

low phospholipid group (P<0.05), with a significantly lower feed conversion ratio (P<0.05).

The addition level of lipid mixture and the proportion of soybean phospholipid showed significant interactive effects on all growth performance indicators (P<0.05).

Body crude lipid content increased significantly with increasing dietary lipid levels (P<0.05); however, the high phospholipid group exhibited significantly lower body crude lipid content than the low phospholipid group (P<0.05).

The high lipid group showed significantly lower hepatopancreatic trypsin-like enzyme and lipase activities compared with the medium and low lipid groups (P<0.05); hepatopancreatic lipase activity in the high phospholipid group was significantly higher than that in the low phospholipid group (P<0.05).

The addition level of lipid mixture and the proportion of soybean phospholipid exhibited significant interactive effects on hepatopancreatic lipase activity (P<0.05).

The addition level of lipid mixture had significant effects on hepatopancreatic superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) (P<0.05), whereas the proportion of soybean phospholipid showed no significant effects on either parameter (P>0.05); however, the addition level of lipid mixture and the proportion of soybean phospholipid demonstrated significant interactive effects on hepatopancreatic T-AOC (P<0.05).

These results suggest that dietary phospholipids should maintain a certain proportion relative to lipids in Macrobrachium nipponense feeds. Based on the findings of this experiment, a dietary lipid level of 10% with a soybean phospholipid supplementation level of 4% appears to be appropriate for Macrobrachium nipponense.

Full Text

Phospholipid Affects Dietary Lipid Requirement of Oriental River Prawn (Macrobrachium nipponensis)

WANG Zhi[1], CUI Guangtong[1], CAI Chunfang[1]*, REN Shengjie[1], QIU Xiaoliang[1], DING Huiming[2], ZHANG Weiye[2], YE Yuantu[1]

[1]Key Laboratory of Aquatic Nutrition of Jiangsu Province, School of Biology and Basic Medical Sciences, Soochow University, Suzhou 215123, China
[2]Suzhou Yangchenghu National Modern Agricultural Industrial Park Co., Ltd., Suzhou 215123, China

Abstract

This experiment investigated the effects of dietary phospholipids on the lipid requirement of oriental river prawn (*Macrobrachium nipponense*). Juvenile prawns

with an initial body weight of (0.075±0.001) g were cultured in indoor tanks and fed experimental diets for 8 weeks. Fish oil and soybean oil were first mixed at a 1:1 ratio, then blended with soybean phospholipid at ratios of 2:1 and 1:2, resulting in soybean phospholipid comprising 33.3% (low-phospholipid group) and 66.7% (high-phospholipid group) of the lipid mixture, respectively. Each lipid mixture was then added to diets at 3% (low-lipid group), 6% (medium-lipid group), and 9% (high-lipid group) to formulate six isonitrogenous and isoenergetic experimental diets. Based on the inclusion levels of the lipid mixture and soybean phospholipid, the six diets were designated L3P1, L3P2, L6P2, L6P4, L9P3, and L9P6, with measured dietary lipid levels of 7%, 7%, 10%, 10%, 13%, and 13%, respectively. Each diet was fed to four replicate tanks, with each tank stocked with 150 prawns.

The results showed that survival rates across the six groups ranged from 47.8% to 66.9%, with the highest observed in the L6P4 group, followed by L3P2, and the lowest in L9P3. The high-lipid group exhibited significantly lower final body weight, weight gain rate, specific growth rate, and survival rate compared to the medium- and low-lipid groups ($P<0.05$), while showing a significantly higher feed conversion ratio ($P<0.05$). The high-phospholipid group demonstrated significantly higher final body weight, weight gain rate, specific growth rate, and survival rate than the low-phospholipid group ($P<0.05$), with a significantly lower feed conversion ratio ($P<0.05$). Significant interactions between lipid mixture inclusion and soybean phospholipid proportion were observed for all growth performance indices ($P<0.05$). Body crude lipid content increased significantly with dietary lipid level ($P<0.05$) but was significantly lower in the high-phospholipid group compared to the low-phospholipid group ($P<0.05$). Hepatopancreatic trypsin and lipase activities in the high-lipid group were significantly lower than those in the medium- and low-lipid groups ($P<0.05$), while hepatopancreatic lipase activity in the high-phospholipid group was significantly higher than in the low-phospholipid group ($P<0.05$). A significant interaction between lipid mixture inclusion and soybean phospholipid proportion was detected for hepatopancreatic lipase activity ($P<0.05$). Lipid mixture inclusion significantly affected hepatopancreatic superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) ($P<0.05$), whereas soybean phospholipid proportion showed no significant effect on these parameters ($P>0.05$). However, a significant interaction between lipid mixture inclusion and soybean phospholipid proportion was observed for hepatopancreatic T-AOC ($P<0.05$). These results indicate that dietary phospholipid should be maintained in an appropriate proportion relative to lipid. Based on the findings, a dietary lipid level of 10% with 4% soybean phospholipid supplementation appears optimal for oriental river prawn.

Keywords: oriental river prawn (*Macrobrachium nipponense*); soybean phospholipid; lipid; growth performance; digestive enzymes; antioxidant capacity

Introduction

Oriental river prawn (*Macrobrachium nipponense*), commonly known as "qingxia," is an important freshwater aquaculture species in China, valued for its high nutritional quality, omnivorous feeding habits, rapid growth, and strong reproductive capacity. In 2016 alone, production reached 272,600 tonnes, representing a 2.8% increase from the previous year [1]. Previous studies have investigated the nutritional requirements of this species, establishing optimal dietary protein levels of 38.0–41.5% [2-3], lipid requirements of 6–12% [4-5], vitamin E requirements of 66.07–212.68 mg/kg [6], and vitamin B6 requirements of 80 mg/kg [7]. However, research on the effects of dietary phospholipid levels in *M. nipponense* remains limited, with most related studies focusing on comparisons of different lipid sources [2,8].

Crustaceans exhibit substantial differences in digestive system morphology and structure compared to vertebrates, most notably the absence of a gallbladder. This absence suggests that lipid emulsification in crustaceans may be less efficient than in vertebrates. Phospholipids are amphiphilic bioactive compounds that enhance lipid emulsification in food and facilitate the absorption and transport of triglycerides, cholesterol, and fat-soluble nutrients [9]. Numerous studies have confirmed that phospholipids are essential for growth and survival in shrimp species [10-13]. However, whether phospholipids can promote growth, survival, and lipid utilization in oriental river prawn remains unclear. Therefore, this study examined the effects of dietary phospholipid levels on growth and physiology of *M. nipponense* at different dietary lipid levels, aiming to elucidate how phospholipid influences lipid requirements and provide scientific evidence for advancing lipid nutrition theory in crustaceans.

1.1 Diet Preparation

Soybean phospholipid, fish oil, and soybean oil served as lipid sources. Soybean phospholipid (containing 15% phosphatidylcholine, 13% phosphatidylethanolamine, and 9% phosphatidylinositol) was provided by Suzhou Xinyu Feed Co., Ltd. Fish oil was derived from deep-sea anchovy oil, and soybean oil was commercial Jinlongyu brand. Fish oil and soybean oil were first mixed at a 1:1 ratio, then blended with soybean phospholipid at ratios of 2:1 and 1:2, resulting in soybean phospholipid comprising 33.3% (low-phospholipid group) and 66.7% (high-phospholipid group) of the lipid mixture, respectively. Each lipid mixture was then incorporated into diets at 3% (low-lipid group), 6% (medium-lipid group), and 9% (high-lipid group) to formulate six isonitrogenous and isoenergetic experimental diets. Based on the inclusion levels of lipid mixture and soybean phospholipid, the six diets were designated L3P1, L3P2, L6P2, L6P4, L9P3, and L9P6, with measured dietary lipid levels of 7%, 7%, 10%, 10%, 13%, and 13%, respectively. Dietary formulations and proximate composition are presented in Table 1 .

During pellet preparation, solid ingredients were ground using a pulverizer

(Model 1500, Yongkang Zhaoshen Electric Appliance Co., Ltd.), passed through an 80-mesh sieve, and thoroughly mixed using a mixer (Model B-20, Guangzhou Panyu Lifeng Food Machinery Factory). Micro-ingredients such as vitamins and minerals were incorporated using the progressive expansion method. The lipid mixture was then added and manually kneaded, followed by addition of 40% distilled water and homogenization. Pellets (1.0 mm diameter) were extruded using a twin-screw extruder (self-developed equipment), air-dried, and stored at -20°C until use.

1.2 Experimental Animals and Culture Management

The experiment was conducted at the Graduate Student Workstation of the Distribution Center, Yangchenghu Modern Agricultural Industrial Park Co., Ltd., Xiangcheng District, Suzhou. Juvenile prawns were obtained from a local hatchery, with an initial body weight of (0.075±0.001) g, and were healthy and uniform in size. Prawns were acclimated in culture tanks for 15 days, then fasted for 24 h before being randomly distributed into 24 plastic tanks (100 cm × 100 cm × 100 cm) at a density of 150 individuals per tank. Each tank contained approximately 300 L of water and was equipped with biological and non-biological substrates for attachment. Each experimental diet was randomly assigned to four replicate tanks. Prawns were fed to satiation twice daily at 07:30 and 18:30. Dead individuals were removed and weighed when observed. Culture water consisted of lake water filtered through a mesh screen and settled for 24 h.

During the experimental period, water pH ranged from 7.5 to 8.1, ammonia nitrogen concentration remained below 0.05 mg/L, and dissolved oxygen concentration was maintained at 6.2–7.1 mg/L through continuous aeration. Tanks were cleaned daily, with one-third water exchange every other day. Water temperature was maintained at 24–28°C. Prawns were weighed after 4 weeks, and sampling was conducted after 8 weeks following a 24-hour fast.

1.3 Sample Collection and Analysis

1.3.1 Growth Performance Assessment Total weight and number of prawns in each tank were recorded to calculate weight gain rate (WGR), survival rate (SR), specific growth rate (SGR), and feed conversion ratio (FCR) using the following formulas:

- Weight gain rate (%) = 100 × (final body weight - initial body weight) / initial body weight
- Survival rate (%) = 100 × final number of prawns / initial number of prawns
- Specific growth rate (%/d) = 100 × (ln final body weight - ln initial body weight) / feeding days
- Feed conversion ratio = dry matter intake / body weight gain

1.3.2 Hepatopancreatic Digestive Enzyme and Antioxidant Indices Analysis Eight prawns were randomly selected from each tank, briefly anesthetized on ice, rinsed slowly with phosphate-buffered saline (PBS), and blotted dry. Hepatopancreas was dissected under aseptic conditions (XW-80 sterile operation box, Taizhou Gaogang Xinwei Test Equipment Factory), minced and homogenized. Approximately 0.5 g of tissue was mixed with 9 volumes of 0.8% physiological saline to prepare 10% tissue homogenate, centrifuged at 3,000 r/min for 10 min, and the supernatant was collected for analysis. Activities of amylase, trypsin, lipase, and superoxide dismutase (SOD), as well as total antioxidant capacity (T-AOC) and total protein content, were measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.3.3 Hepatopancreas Histological Observation Frozen sections were prepared for histological observation following the method of Zhou et al. [14] with minor modifications. Three prawns from each tank were anesthetized on ice, and their hepatopancreas was dissected, embedded in OCT compound, and sectioned at 10 m thickness using a Leica CM1900 cryostat (Leica, Germany). Sections were mounted on slides, stained with Sudan III solution, washed with 70% ethanol to remove excess stain, and sealed with glycerol gelatin. Hepatopancreas morphology was observed and photographed using an OLYMPUS BX 51 microscope imaging system (Olympus, Japan).

1.3.4 Proximate Composition Analysis Diet samples were collected for proximate analysis, and remaining prawns were used for whole-body composition analysis. Moisture content was determined by freeze-drying (LJB-18 freeze dryer, Beijing Sihuan Scientific Instrument Co., Ltd.). Crude protein content was measured by the Kjeldahl method (GB 5009.5-2010) using a digestion unit (LNK-87, Jiangsu Yixing Scientific and Educational Instrument Research Institute) and distillation apparatus (KN-520, Jinan Alva Instrument Co., Ltd.). Crude lipid content was determined by Soxhlet extraction (GB/T 14772-2008). Gross energy was measured using an automatic oxygen bomb calorimeter (PARR 6300, Shanghai Changji Geological Instrument Co., Ltd.). Crude ash content was determined according to GB 5009.4-2010 by carbonizing samples at 200°C until smoke-free, followed by ashing at 550°C in a ceramic fiber muffle furnace (8-10TP, Shanghai Huitai Instrument Manufacturing Co., Ltd.) to constant weight.

1.4 Statistical Analysis

All data are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS v.11 software. Data were analyzed by one-way ANOVA and two-way ANOVA, with Duncan' s multiple comparison test applied when significant differences were detected. The significance level was set at $P<0.05$.

Results

2.1 Growth Performance

As shown in Table 2 , dietary lipid level (lipid mixture inclusion) and soybean phospholipid proportion significantly affected final body weight, weight gain rate, specific growth rate, feed conversion ratio, and survival rate of oriental river prawn ($P<0.05$), with significant interactions between these factors for final body weight, weight gain rate, and specific growth rate ($P<0.05$). Survival rates ranged from 47.8% to 66.9% across all groups, with the highest observed in L6P4. Two-way ANOVA revealed that survival rates in the low- and medium-lipid groups were higher than in the high-lipid group ($P<0.05$), while the high-phospholipid group showed significantly higher survival than the low-phospholipid group ($P<0.05$). Weight gain rate and specific growth rate were highest in L6P4, followed by L3P2, both significantly exceeding other groups ($P<0.05$). These parameters decreased progressively with increasing dietary lipid level, while the high-phospholipid group showed significantly higher values than the low-phospholipid group ($P<0.05$). Feed conversion ratio showed opposite trends to weight gain rate and specific growth rate.

2.2 Body Composition

As shown in Table 3 , dietary lipid mixture inclusion and soybean phospholipid proportion significantly affected whole-body crude lipid content ($P<0.05$) but had no significant effect on crude protein or moisture content ($P>0.05$). No significant interactions were detected for crude lipid, crude protein, or moisture content ($P>0.05$). Whole-body crude lipid content was highest in L9P3, significantly exceeding all other groups ($P<0.05$), and increased significantly with dietary lipid level ($P<0.05$). The high-phospholipid group exhibited significantly lower body crude lipid content than the low-phospholipid group ($P<0.05$).

2.3 Hepatopancreatic Digestive Enzyme Activity and Antioxidant Indices

As shown in Table 3 , dietary lipid mixture inclusion and soybean phospholipid proportion had no significant effect on hepatopancreatic amylase activity ($P>0.05$). Both trypsin and lipase activities were significantly affected by lipid mixture inclusion ($P<0.05$), with activities in the high-lipid group significantly lower than in the medium- and low-lipid groups ($P<0.05$). Lipase activity was also significantly affected by soybean phospholipid proportion ($P<0.05$), with the high-phospholipid group showing significantly higher activity than the low-phospholipid group ($P<0.05$). A significant interaction between lipid mixture inclusion and soybean phospholipid proportion was observed for lipase activity ($P<0.05$).

Hepatopancreatic SOD activity and T-AOC were significantly influenced by lipid mixture inclusion ($P<0.05$), while soybean phospholipid proportion had no significant effect on these parameters ($P>0.05$). SOD activity increased initially

then decreased with rising dietary lipid level, whereas T-AOC increased progressively. A significant interaction between lipid mixture inclusion and soybean phospholipid proportion was detected for hepatopancreatic T-AOC ($P<0.05$).

2.4 Hepatopancreas Histological Observation

Microscopic examination revealed that hepatopancreatic lipid droplets in L3P1 and L3P2 groups were small and sparse, while L6P2 and L6P4 groups exhibited larger lipid granules. In the two high-lipid groups (L9P3 and L9P6), hepatopancreatic lipid droplets were dense but small, with L9P3 showing more numerous lipid granules than L9P6 [Figure 1: see original paper].

Discussion

Growth and survival of shrimp are susceptible to various environmental stressors, including ammonia concentration [15], nitrite levels [16], pH [17], and dissolved oxygen [18] in the water. Additionally, *M. nipponense* exhibits strong substrate attachment behavior, and the quantity and quality of attachment substrates affect its growth and survival. In this experiment, filtered and settled Yangcheng Lake water was used, with potted *Elodea* provided as climbing substrate and fine-bubble nanotube aeration maintaining high dissolved oxygen levels. All monitored water quality parameters remained within safe thresholds for prawn growth, creating suitable conditions for molting and attachment. The 8-week growth trial yielded survival rates of 47.8–66.8%, weight gain rates of 173.67–272.02%, and feed conversion ratios of 1.31–1.70, indicating good growth performance and adequate reflection of dietary differences.

Two-way ANOVA revealed that the 6% lipid mixture inclusion (10% dietary lipid) produced the highest survival rate, though weight gain rate was significantly lower than at 3% lipid mixture inclusion (7% dietary lipid). However, specific growth rate and feed conversion ratio did not differ significantly between these groups, and one-way ANOVA showed that L6P4 (10% dietary lipid) achieved the highest specific growth rate. *Macrobrachium nipponense* exhibits cannibalistic behavior, making crowding stress a significant factor. Previous studies by our research group have demonstrated that high stocking densities typically result in lower survival rates, while lower survival rates at equivalent densities often correspond to higher individual weight gain rates. Considering the crowding stress effects associated with high survival rates, a dietary lipid level of 10% appears to approximate the optimal requirement for *M. nipponense*.

Hari et al. [19] reported that giant freshwater prawn (*M. rosenbergii*) exhibited optimal growth at dietary lipid levels of 6–8%, with negative effects observed at levels exceeding 12%. Huang et al. [20] found that Pacific white shrimp (*Litopenaeus vannamei*) achieved maximum weight gain and survival at 8.47% dietary lipid, with growth inhibition occurring at both higher and lower levels. Our results showing a substantial decline in specific growth rate when dietary lipid increased from 10% to 13% support these findings. Additionally, studies

have indicated that lipase activity can be induced by dietary lipid level and correlates with lipid intake [21]. In this experiment, digestive enzyme analysis revealed that prawns fed 13% lipid diets had significantly lower hepatopancreatic trypsin and lipase activities compared to those fed 10% and 7% lipid diets. As the hepatopancreas is the primary digestive and absorptive organ in crustaceans, reduced digestive enzyme activity suggests diminished digestive capacity, possibly representing a self-regulatory response following excessive lipid accumulation. Our results also showed that prawns fed high-lipid diets (13% lipid) had the highest body lipid content (Table 3) and greatest hepatic lipid accumulation (Figure 1).

Crustacean larvae have limited capacity for endogenous phospholipid synthesis [22], making dietary phospholipid supplementation essential for growth and survival of postlarvae and juveniles [10-13]. Studies on *L. vannamei* [10,12] and black tiger shrimp (*Penaeus monodon*) [23] have demonstrated improved juvenile performance with increasing dietary phospholipid levels. Sui et al. [24] reported that appropriate lecithin supplementation accelerated molting and growth while improving survival in Chinese mitten crab (*Eriocheir sinensis*) larvae. Hou et al. [25] found that optimal soybean phospholipid levels (41.96 g/kg) enhanced growth performance and feed utilization in juvenile swimming crab (*Portunus trituberculatus*). Furthermore, soybean phospholipid, rich in unsaturated fatty acids, plays a crucial role in organ development and growth in young animals [26-28]. Our findings that high-phospholipid diets promoted better growth and feed efficiency in *M. nipponense* align with these reports.

Previous research has shown that dietary phospholipid supplementation improves lipid utilization efficiency [29]. Our results demonstrated significant interactions between dietary lipid (as lipid mixture inclusion) and phospholipid (as soybean phospholipid proportion) levels on growth performance (Table 2), hepatopancreatic lipase activity (Table 3), and hepatopancreatic T-AOC (Table 4), with a trend toward significant interaction for survival rate (Table 2). Specifically, higher phospholipid levels improved growth performance, increased survival, enhanced lipase activity, and reduced body (Table 3) and hepatic (Figure 1) lipid content in high-lipid groups. This may be attributed to the amphiphilic nature of phospholipid molecules, which contain both hydrophobic fatty acid chains and hydrophilic groups, enabling them to function as surfactants and emulsifiers. Phospholipids can further disperse lipids entering the intestine, increasing the contact surface area between fats and lipophilic substances with the intestinal mucosa, thereby enhancing lipid digestion and absorption. Additionally, phospholipids are essential components for lipoprotein assembly and secretion, playing a specific role in chylomicron and lipoprotein synthesis [30], and can regulate lipid metabolism through effects on lipoprotein synthesis. These findings suggest that dietary lipid and phospholipid should be maintained in an appropriate ratio for *M. nipponense*, though the optimal proportion requires further investigation.

Under normal conditions, the production and scavenging of free radicals exist in

equilibrium, with SOD and T-AOC serving as important indicators of reactive oxygen species scavenging capacity. Phospholipids possess antioxidant properties [31], and Xu et al. [32] reported that soybean phospholipid enhanced hepatic antioxidant capacity in mice, with high-phospholipid groups significantly outperforming low-phospholipid groups. In contrast, our study found no significant effect of dietary phospholipid level on hepatopancreatic SOD activity or T-AOC in *M. nipponense* (Table 3), possibly because the emulsifying capacity of phospholipids stabilizes once the critical micelle concentration is exceeded, beyond which emulsification effects no longer increase [33].

Conclusion

In summary, dietary lipid and phospholipid should be maintained in an appropriate ratio for oriental river prawn. Based on the results of this study, a dietary lipid level of 10% with 4% soybean phospholipid supplementation is optimal for *M. nipponense*.

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