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Effect of Lysolecithin Replacement of Soybean Lecithin Oil on the Survival, Growth Performance and Immunity of *Macrobrachium nipponense* Juveniles

Chang Guoliang[1,2]*, Pan Zhengjun[1,2], Zhu Chuankun[1,2], Zhao Haitao[1,2], Yan Zhang[3], Summaya Rajput[4] and Laghari M. Younis[4]*

[1]*Jiangsu Collaborative Innovation Center of Regional Modern Agriculture and Environmental Protection, Huaiyin Normal University, Jiangsu, China*
[2]*Laboratory for Breeding of Special Aquatic Organisms, Huai'an, 223300, China*
[3]*Key Laboratory of Aquatic Genomics, Ministry of Agriculture, and Beijing Key, Laboratory of Fishery Biotechnology, Chinese Academy of Fishery Sciences, 100141, Beijing*
[4]*Department of Freshwater Biology and Fisheries, University of Sindh, Jamshoro, Sindh-Pakistan*

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Authors' Contribution
YZ designed the project and guided to conduct the experiment. PZ, ZC and ZH conducted the experiments. SR assisted in data recording and compiling. CG and LMY assisted in statistical data analysis, compiling and article writing.

Key words
***Macrobrachium nipponense*, Survival, Growth, Immunity**

ABSTRACT

Macrobrachium nipponense is called the oriental river prawn or blue prawn and is an important aquaculture species in China, Japan and Southeast Asian countries. But up to now, no special commercial formulated feed has been developed for *M. nipponense* that meets its nutritional requirements. Generally, soybean lecithin oil (SO) is used as a major source of phospholipids and an emulsifier in aquatic animal feed, which is very costly. In recent years, as a good emulsifier, lysolecithin attracted increasing interest in fish species. In the present study, lysolecithin as a substitute for SO was used to observe the growth and immune performance of *M. nipponense*. Four types of diets containing similar basal compositions but differing in lysolecithin ratios were used. lysolecithin 0.1% was added to all diets except the control diet (0.0% lysolecithin). The contents of SO in the four diets(Diet# 1, Diet# 2, Diet# 3 and Diet# 4) were 2%, 1%, 0.5% and 0.0%, respectively. Furthermore, antioxidant enzyme activity (T-SOD, AKP, ACP and POD) in the hepatopancreas, serum and muscle were determined. The maximum final weight gain rate (WGR) and standard growth rate (SGR) observed with Diet# 4, showed average increases of 52.51±7.91 and 0.70±0.086 g, respectively. This result suggests that the SGR and WGR of prawns fed a diet containing 0% SO were higher than those of the other experimental groups. Furthermore, the survival rate also increased by decreasing the SO level in the feed contents. The highest relative hepatosomatic index (HSI) was also observed in Diet# 2. Significant changes were found in T-SOD, AKP and ACP enzyme activity of *M. nipponense* different organizations. The lowest level of serum T-SOD was in prawns fed with Diet #4, while the highest level of ACP and AKP was all in prawns fed with Diet# 3 ($P < 0.05$). This study suggested a diet containing 0.1% lysolecithin, replacing the appropriate amount of SO, can improved growth performance and immunity of *M. nipponense*. Therefore, lysolecithin has a good application potential to replace a certain amount of SO in the diet of *M. nipponense*.

INTRODUCTION

Macrobrachium nipponense also called the oriental river prawn or the blue prawn, is an important aquaculture species in China, Japan and Southeast Asian countries (Yang *et al*., 2004). Since few years, the trend of oriental river prawn intensive culture is developing rapidly in China, especially in the middle and lower reaches of the Yangtze River. The major interest of its intensive culture is the characteristics of short maturation cycles, high profitability, and delicious taste and flavor (Zhou *et*

* Corresponding author: changgl888@163.com, laghariyounis@yahoo.com
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al., 2020). The production of *M. nipponense* only in China was recorded to be approximately 228,765 tons in 2020 (Bureau of Fisheries Management, Chinese Ministry of Agriculture, 2021). Unfortunately, no special commercial formulated feed has been developed for *M. nipponense* that meets its nutritional requirements (Zhou *et al*., 2020). It is encouraging to note that in recent years, researchers has shown some nutritional factors that affects the growth performance and immunity of *M. nipponense* juveniles, including protein (Zhang *et al*., 2017; Zhou *et al*., 2020; Lv *et al*., 2021), lipid (Gu e t al., 2017; Luo *et al*., 2017; Ding *et al*., 2018; Cui *et al*., 2019; Jiang *et al*., 2019; Li *et al*., 2020; Jin *et al*., 2022), carbohydrate (Kong *et al*., 2019; Ding *et al*., 2022), vitamins (Ettefaghdoost and Haghighi, 2021; Sun *et al*., 2022; Xiong *et al*., 2022) and minerals (Kong *et al*., 2014, 2017; Wang *et al*., 2021).

Lipids play an important role in optimal growth and health of prawns (Muralisankar *et al*., 2014; Si *et al*., 2014; Cui *et al*., 2019; Li *et al*., 2020). Among lipid types, phospholipids (PLs) are important components for maintaining the structure and function of cellular membranes, emulsifying lipids in the gut and improving intestinal absorption of long-chain fatty acids (Tocher *et al*., 2008). Studies have proven that adding a certain level of phospholipids to the diet can promote growth and improve immunity in prawn (Briggs *et al*., 1988; Cui *et al*., 2019).

Generally, the aquafeed industry uses soybean lecithin oil (SO) as a major source of phospholipids and an emulsifier, ranging from 1% to 3%, in commercial prawn feed. However, SO increases the price of feed. Lysolecithin, also called lysophosphatidylcholine (LPC), is obtained by hydrolyzing soybean lecithin with phospholipase A_2. Compared with ordinary soybean lecithin, lysolechitin improves the ability to bind protein and starch, enhances the oil in water emulsification performance, has stronger adaptability to temperature and has a small amount of addition (about 1/10 of ordinary soybean lecithin). In recent years, as a good emulsifier, lysolecithin attracted increasing interest in fish species such as crucian crap (*Carassais auratus* gibelio) (Li *et al*., 2010a), hybrid tilapia (*Oreochromis aureus*♂×*Oreochromis niloticus*♀) (Li *et al*., 2010b), channel catfish (*Ictalurus punctatus*) (Liu *et al*., 2020), and rainbow trout (*Oncorhynchus mykiss*) (Taghavizadeh *et al*., 2020; Adhami *et al*., 2021), with most studies revealing positive effects of lysolecithin in the diets on fish growth performances. Hence, in order to reduce the supplemental level of SO in the diet of *M. nipponense*, this study was designed to investigate effect of lysolecithin as far the replacement of soybean lecithin oil, on the growth performance and immunity.

MATERIALS AND METHODS

Experimental diets

Four experimental diets were tested in the present study. The diets were formulated according to the nutrition standards of *M. nipponense*. Each diet contained a similar basal composition but differed in the lysolecithin ratio. Lysolecithin 0.1% was added to all diets except the control diet (Diet# 1), which had 0.0%. Hence, Diets# 1, 2, 3 and 4 comprised 2.0%, 1.0%, 0.5% and 0.0% of soybean lecithin oil (SO), respectively. The reduced SO in the diet was replaced by rapeseed meal of corresponding quality (Table I). The experimental diets were formulated to meet the nutritional requirement of *M. nipponense* juveniles at this stage of growth as recommended by Si *et al*. (2014). The diets were made into sinking pellets using a pellet machine. The pellets were stored in sealed plastic bags at room temperature before using in the feeding trial.

Table I. Formulations and analyses of the crude protein, total lipid, crude fiber and ash contents (g kg^{-1} weight) of the experimental diets used in the juvenile *M. nipponense* feeding experiments.

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal	130.0	130.0	130.0	130.0
Rapeseed meal	200.0	209.0	214.0	219.0
Strong flour	180.0	180.0	180.0	180.0
Fermented soybean meal	160.0	160.0	160.0	160.0
Soybean meal	150.0	150.0	150.0	150.0
Rice bran	30.0	30.0	30.0	30.0
Shrimp powder	30.0	30.0	30.0	30.0
Squid powder	20.0	20.0	20.0	20.0
Shrimp shell powder	20.0	20.0	20.0	20.0
Zeolite powder	20.0	20.0	20.0	20.0
Monocalcium phosphate	20.0	20.0	20.0	20.0
Sodium carboxymethyl cellulose	10.0	10.0	10.0	10.0
Premix	10.0	10.0	10.0	10.0
Soybean lecithin oil (SO)	20.0	10.0	5.0	0.0
Lysolecithin[1]	0.0	1.0	1.0	1.0
Analysed composition				
Moisture	102.1	101.3	103.5	104.1
Crude protein	353.4	356.1	357.6	359.2
Total lipid	67.4	63.2	61.2	59.1
Ash	109.6	108.1	108.5	109.0

Premix: 1 kg of diet contained vitamin A, 540,000 IU; vitamin D_3, 90,000 IU; vitamin B_6, 1200 mg; vitamin B_{12}, 2.4 mg; vitamin C, 17,500 mg; vitamin E, 4800 mg; nicotinamide, 58,000 mg; vitamin K_3, 240 mg; calcium pantothenate, 1504 mg; vitaminB_1, 184 mg; vitamin B_2, 720 mg; fllic acid, 60 mg; biotin, em 6 mg; inositol, 6000 mg; Mg, 15000 mg; Mn, 6000 mg; Zn, 8000 mg; Cu, 400 mg; Fe, 15000 mg; Se, 40.23 mg; I, 75.7 mg. [1]Containing 20% lysolecithin.

Prawns, experimental conditions and feeding

Pond-reared *M. nipponense* juveniles were obtained from a local farm in Jiangsu Province, China. These juveniles were acclimatized in a 2.7m^3 tank at the Laboratory for Breeding of Special Aquatic Organisms, Huai'an, for two weeks before starting the experimental trial. A total of 720 healthy juvenile *M. nipponense* were selected randomly with an average weight of 0.8±0.20 g and randomly stocked in twelve experimental plastic tanks, each with 60 individuals, with a 280L (2.3m^2) fresh water capacity. Place some plastic water plants in each tank for these juveniles to hide in. The twelve tanks with prawns were divided into 4 groups and fed 4 types of diets. Three replicates were conducted for each type of diet that was prepared for blue prawns during the experiment. Each tank was supplied with two airstones and maintained at a similar air flow rate of 10 L/min. a daily water exchange of 20% was carried out in the morning for each tank, during which leftover food and any dead animals were removed. The water temperature were ambient (17 to 26ºC). The prawns were fed twice a day (07:00 and 18:00) at 3%~6% of the body weight per day for a period of 60 days.

Survival, growth performance and HSI

After sixty days of feeding, all survived individuals were starved for 24h, as to obtain an accurate final body weight. The prawns were dissected and hepatopancreas were collected from each tank. The parameters were measured using the following equations:

Survival (%) = (final prawn number/initial prawn number) × 100

HSI (%) = (Hepatopancreas weight /body weight) × 100

WGR (%) = ((final body weight - initial body weight)/ initial body weight) ×100

SGR (%d^{-1}) = ((ln final body weight -ln initial body weight)/days) × 100

Immune parameters

Hemolymph samples were collected from the pericardial sinus of prawn with sterile syringes. The hemolymph samples from 8-10 prawns were combined into one sample and placed into a sterile 0.5ml centrifuge tube. Then the centrifuge tube containing hemolymph samples were placed in a 4ºC environment overnight. On the second day, the hemolymph samples were centrifuged at 3500 rpm for 5 min and the serum was collected and diluted 3 times with 0.86% (w/v) sodium chloride solution to measure the immune parameters.

After hemolymph collection, the hepatopancreas and muscles of prawn were quickly dissected on ice (hepatopancreas were weighed to calculate HSI) and these samples were homogenated in 9 volumes (w:v) of pre-cooled 0.86% (w/v) sodium chloride solution with a small tissue dispersion machine, and then centrifuged at 3500 rpm for 10 min (Song *et al*., 2019). The supernatant was collected to a sterile centrifuge tube and the immune activity was quickly determined.

Antioxidant enzyme activity (T-SOD, AKP, ACP and POD) in the serum, hepatopancreas and muscle was determined using commercial kits (Nanjing Jiancheng, Nanjing, China). The supernatant is diluted into different concentrations according to the determination methods of various indicators, and the protein concentration of them was analyzed by Bradford assay (Bradford protein assay kit, Nanjing Jiancheng, Nanjing, China). All indicators were performed according to the manufacturer's instructions.

Statistical analysis

All results are presented as the mean±standard deviation (SD). One-way ANOVA was used to determine the differences between different diet groups, and $P < 0.05$ was considered a significant difference. Prior to statistical analysis, homogeneity in variance of data was tested with Levene's test. All statistical analyses were performed with the SPSS package (version 16.0).

RESULTS

Survival, growth performance and HSI

The highest final WGR and SGR were observed with Diet 4, with average increases of 52.51±7.91 and 0.702±0.086, respectively (Table II). While the lowest final WGR and SGR were observed with Diet 2. But for HSI, the highest and lowest of Diet groups were reversed. The highest and lowest survival rate was observed with Diet 3 and Diet 2, respectively. However, there were no significant differences among the four groups for all these indicators ($P > 0.05$).

Immunological analysis

The antioxidant enzyme activity of various enzymes, namely, total superoxide dismutase (T-SOD), peroxidase (POD), alkaline phosphatase (AKP) and acid phosphatase (ACP), was analyzed in the hepatopancreas, muscle and serum of *M. nipponense*.

Total superoxide dismutase (T-SOD)

The level of serum T-SOD in prawns fed with Diet 1, Diet 2 and Diet 3 was significantly higher than that of Diet 4 (596.1±28.1U/ml) ($P < 0.05$), but there was not a significant difference in Diet 2 and Diet 3 compared to Diet 1 (Fig. 1A). The highest and lowest hepatopancreas T-SOD were

Table II. Effects of the four experimental diets on weight gain, survival and the HSI of juvenile *M. nipponense*.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4
Initial body weight (g)	0.79±0.16	0.83±0.15	0.77±0.007	0.78±0.14
Final body weight (g)	1.13±0.44	1.12±0.16	1.12±0.06	1.18±0.16
WGR (%)	40.13±27.59	36.38±5.42	45.36±6.96	52.51±7.91
SGR (% d^{-1})	0.55±0.33	0.52±0.07	0.62±0.08	0.70±0.09
Survival (%)	77.5±16.9	75.8±4.8	80.6±11.2	80.1±13.2
HSI (%)	5.44±0.23	5.51±0.45	5.23±0.31	5.15±0.36

WGR, weight gain ratio; SGR, standard growth ratio; HIS, hepatosomatic index. For details of diets, see Table I.

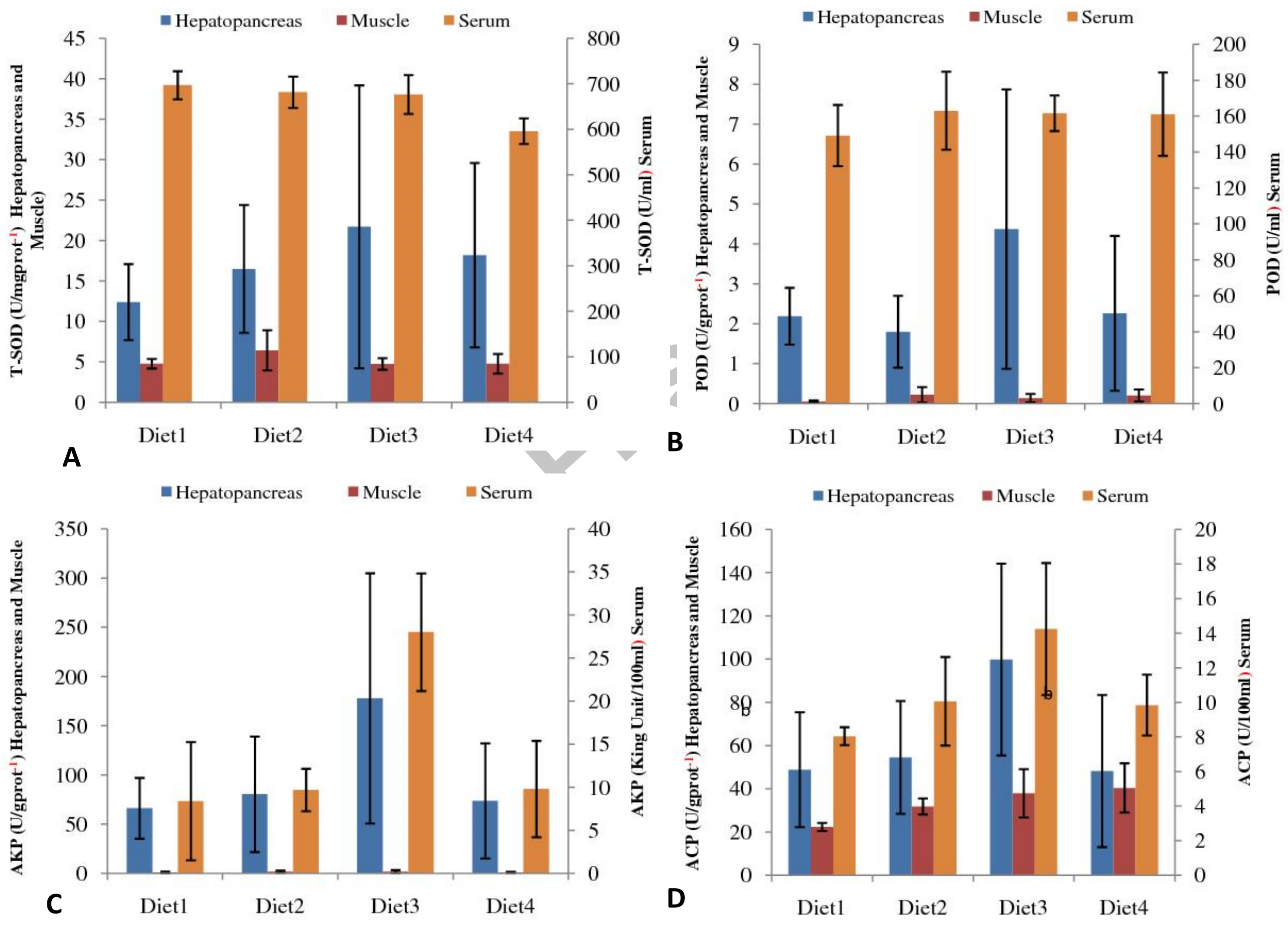

Fig. 1. Total superoxide dismutase (A), Peroxidase (B), Alkaline phosphatase (C) and acid phosphatase (D) levels in the hepatopancereas, muscle and serum of *Macrobrachium nipponense* juveniles.

observed in prawns fed with Diet 3 (21.7±17.5 U/mgprot^{-1}) and Diet 1 (12.4±4.7 U/mgprot^{-1}), respectively. While the highest muscle T-SOD was observed in prawns fed with Diet 2 (6.43±2.48 U/mgprot^{-1}). The enzyme activities of the other three groups were about 4.80 U/ mgprot^{-1}. However, there was not a significant difference in hepatopancreas and muslce T-SOD in prawns fed with four groups diets.

Peroxidase (POD)

The level of serum and muscle POD in prawns fed with Diet 1 (149.2±17.0U/ml and 0.052±0.025 U/gprot^{-1}, respectively) was lower than that of other three diet groups, while The level of hepatopancreas POD in prawns fed with Diet 3 (4.37±3.50 U/gprot^{-1}) was higher than that of other three diet groups. However, no significant difference (P >

0.05) was observed in POD antioxidant activities among the four experimental groups (Fig. 1B).

Alkaline phosphatase (AKP)

The level of hepatopancreas and serum AKP in prawns fed with Diet 3(177.9±127.1U/gprot^{-1} and 28.02±6.83 King Unit/100ml, respectively) was higher than that of Diet 1, Diet 2 and Diet 3, but significant difference was only observed in serum ($P < 0.05$). The levels of muscle AKP were all lower than 2.0 U/gprot^{-1}, and there was no significant difference in four diet groups ($P > 0.05$) (Fig. 1C).

Acid phosphatase (ACP)

Significant difference ($P < 0.05$) was observed in the level of three organization ACP. The level of hepatopancreas ACP in prawns fed with Diet 3 (99.8±44.4U/gprot) was significant higher than that of Diet 1(48.8±26.6U/gprot) and Diet 4(48.2±35.2U/gpro) ($P < 0.05$). The level of muslce ACP in prawns fed with Diet 3 (37.9±11.2 U/gpro^{-1}) and Diet 4(37.9±11.2 U/gprot^{-1}) was significant higher than that of Diet 1 (22.3±1.9U/gprot) ($P < 0.05$). The level of serum ACP in prawns fed with Diet 3 (14.24±3.82 U/100ml) was significant higher than that of Diet 1 (8.04±0.52 U/100ml) ($P < 0.05$) (Fig. 1D).

DISCUSSION

In the present study, a lysolecithin-rich diet (0.1% lysolecithin) was shown to be a viable alternative to SO in the diets of juvenile *M. nipponense*. The decrease in SO percent down to 0% led to increase in the WGR and SGR of *M. nipponense* prawns. The results confirmed that decreasing the SO amount in the prawn diet and replacing with lysolecithin increased the WGR, SGR and survival rate of juvenile *M. nipponense* prawns. Studies have shown that adding lysolecithin supplements in diets at about 0.125%~0.25% (hybrid tilapia), 0.1% (crucian carp) and 0.1%~0.2% (rainbow trout) can significantly increase the WGR and SGR (Li *et al*., 2010a, b; Liu *et al*., 2020). The optimum levels of lysolecithin for these freshwater species vary mainly due to the different nutritional requirements of different aquatic animals. On the other hand, the source of lysolecithin used varied with the active substances in it. The juvenile prawn survival rates in this study were satisfactory and comparable to other similar diet studies (Kim *et al*., 2013; Sisouvong *et al*., 2013; Kangpanich and Senanan, 2015). Decreasing the oil percentage in the diet (Kangpanich and Senanan, 2015; Kangpanich *et al*., 2017) in the current study did not compromise juvenile growth and improved the weight gain and SGR and the mortality rate. Generally, the non-SO diets yielded better growth performance for prawn juveniles than those fed the SO diet. The current results concurred with several studies in which SO was partially or entirely replaced in prawn feed (Kangpanich and Senanan, 2015; Kangpanich *et al*., 2017). However, for improved growth performance, juvenile *M. nipponense* prawns appeared to respond better to diets containing a combination of 0% SO and 1% lysolecithin. The best growth performance (highest WGR, SGR and survival rate) of *M. nipponense* occurred for prawns fed Diet 4. The reason was that lysolecithin could effectively improve feed utilization and fat metabolism (Li *et al*., 2010a; Liu *et al*., 2020). In addition, the growth performance of juvenile prawns fed Deit 2 and Deit 3 (containing SO and lysolecithin in the diet) did not show the best, and the reasons for this need to be confirmed by further experiments.

The activities of the antioxidant enzymes T-SOD, POD, AKP and ACP were observed in serum, hepatopancreas and muscle. SOD is one of the key enzymes involved in cellular defense against reactive oxygen species in living organisms, and it is an important indicator of antioxidant capacity (Sudipta *et al*., 2014). There was not a significant difference in hepatopancreas and muscle T-SOD in prawns fed with four groups diets. However, the T-SOD level was significantly decreased in the serum samples of individuals fed the lowest content of SO (Diet 4). This was not consistent with the existing research results, diets supplemented with 0.05%~0.1% lysolecithin could significantly increase the content of SOD in *C. uratus* plasma (Li *et al*., 2010a).

Significant difference was observed in the level of three organization ACP and serum AKP, and the highest level of ACP and AKP was all in prawns fed with Diet 3. ACP is one of the hydrolytic enzymes involved in lipid metabolism (Athenstaedt and Daum, 1999). ACP plays an important role in a variety of metabolic processes of aquatic organisms, and it is also a vital lysosomal enzyme that has a role in nonspecific immune defense against ambient stressors (Liang *et al*., 2014). It is directly involved in the metabolism of phosphorus in the organism, and is also related to the metabolism of DNA, RNA proteins and lipids. In addition, it plays an important role in calcium uptake, calcium phosphate deposition, bone formation, chitin secretion and formation (Chen *et al*., 1996). Numerous factors, such as environmental conditions, host physiology and pathogen biology, impact the blood parameters of fish.

After SO in the diet was replaced by lysolecithin at different levels, significant changes were found in T-SOD, AKP and ACP enzyme activity of *M. nipponense* different organizations. According to the results of enzyme activities, it can be speculated that the diet 3 with 0.5% SO and 0.1% lysolecithin has good antioxidant capacity.

This study suggested a diet containing 0.1% lysolecithin, replacing the appropriate amount of SO, can improved growth performance and immunity of *Macrobrachium nipponense*. Therefore, we believe that lysolecithin substitute for SO has a good application potential in the diet of *M. nipponense*.

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IRB approval and ethical statement

The authors declare that the experiments were approved and conducted following the recommendation of the Care and Use of Animals for Scientific Purposes established by the Academic Ethics Committee of Huaiyin Normal University, China.

Statement of conflict of interest

The authors have declared no conflict of interest.

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