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Evaluating dose response lysolecithin supplementation on growth, antioxidant response, and immunity in giant river prawn *Macrobrachium rosenbergii*

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ABSTRACT

This study evaluated the effects of dietary lysolecithin (LL) supplementation on growth performance, body composition, biochemical responses, antioxidant capacity, digestive enzyme activity, histological study, and disease resistance in *Macrobrachium rosenbergii*. Six isonitrogenous and isocaloric diets were prepared for the dose-response trial by supplementing LL to the basal diet at six graded levels (0 g/kg, 2 g/kg, 4 g/kg, 6 g/kg, 8 g/kg, and 10 g/kg of dry weight) while propotionally reducing fish oil content. The prawns were fed for 56 days, after which various physiological and biochemical parameters were assessed. The results showed that dietary LL significantly increased final body weight, weight gain, and specific growth rate, with the optimal inclusion level estimated at 4.8 g/kg based on a second-order polynomial regression model. Additionally, LL supplementation increased superoxide dismutase activity and glutathione levels while reducing malondialdehyde levels. LL also improved digestive enzyme activities, reduced lipid accumulation in the hepatopancreas, lowered hemolymph triglyceride levels, and enhanced intestinal amylase and trypsin activities. Furthermore, prawns fed with diets containing 4–10 g/kg LL exhibited significantly reduced cumulative mortality following a challenge with *Aeromonas hydrophila*. To conclude, these findings demonstrated that dietary LL regulates metabolism, improves growth performance, antioxidant capacity, digestive function, hepatopancreatic health, and disease resistance in *Macrobrachium rosenbergii*, with an optimal recommended inclusion level estimated at 4.8 g/kg based on quadratic regression analysis.

1. Introduction

The giant river prawn (*Macrobrachium rosenbergii*) is a key species in freshwater aquaculture worldwide due to its rapid growth characteristics and favorable meat quality, widely raised throughout Southeast Asia and China (New and Nair, 2012; Qiu et al., 2023). However, with the trend toward intensified aquaculture farming systems, several physiological challenges have become increasingly evident, including reduced feed conversion efficiency, lipid metabolic disorders, and impaired immune function (Boyd et al., 2020; Naylor et al., 2021). These issues collectively hinder the developmental progression and physiological stability of *M. rosenbergii* (Sun et al., 2022, 2020; Yang et al., 2024). It has been revealed through research that dysregulated lipid metabolism not only increase in lipid droplet accumulation in the hepatopancreas and heightened oxidative stress but also impairs digestive enzyme function and weakens immune defense (Cui et al., 2023; Liu et al., 2022; Pu et al., 2024). Thus, the creation of effective feed additives for optimized lipid metabolism and improved aquaculture performance has become one of the foremost research objectives in contemporary aquatic nutrition.

In crustaceans, dietary lecithin acts not only as an emulsifier, but also as an important biological multifunctional agent that regulates processes within an organism (Chen et al., 2023; Ukwela et al., 2024). Phosphatidylcholine, as the major constituent of phospholipids in biomembranes, is responsible for preserving the systems of biomembranes within organelles, especially keeping the cellular membranes intact

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during severe conditions or when under stress (Van Der Veen et al., 2017). Moreover, lecithin promotes the formation of lipoprotein-like particles that enhance lipid digestion and absorption efficiency. Recent studies further indicate that lecithin can up-regulate hepatopancreatic lipid transport mechanisms, facilitating the mobilization of cholesterol and triglycerides for vital physiological processes (Yang and Chen, 2022). Traditionally, research has predominantly focused on complete soybean-derived and egg yolk lecithins; recently, lysolecithin has gained attention due to its superior emulsifying properties and enhanced bioavailability (Alhajj et al., 2020; Jala et al., 2016; Tan et al., 2020). Lysolecithin has the potential as a functional ingredient in aquafeed, improving lipid digestion and absorption and regulating lipoprotein metabolism (Dong et al., 2024; Jafari et al., 2024; Weng et al., 2022).

Thus, establishing the optimal dietary inclusion level of lecithin through a dose-response approach may facilitate the formulation of economically feasible diets to enhance growth performance and overall health in crustaceans. To date, no studies have investigated the dose-response effects of dietary lecithin in *M. rosenbergii*. Present study utilizes *M. rosenbergii* as the model species, evaluating six isonitrogenous and isocaloric diets supplemented with varying lysolecithin levels (ranging from 0 to 10 g/kg). The study aims to assess the effects of dietary lecithin on the growth performance, fatty acid composition, hemolymph biochemical indexes, antioxidant capacity of the hepatopancreas, and activity of digestive enzymes in the intestine. Meanwhile, the observation of their microscopic structure and *Aeromonas hydrophila* challenge tests are used jointly to investigate the mechanism of promoting the health of *M. rosenbergii* by lecithin.

2. Materials and methods

2.1. Animal ethical approval

All animal experiments were performed in accordance with international standards of animal care and laboratory animal use and were approved and reviewed by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia (UPM/IACUC/AUP-R059/2024).

2.2. Experimental diets

Lysolecithin (LL) was sponsored by Linyi Zhengnengliang Biological Co.,Ltd. (Shandong, China). This study formulated six isonitrogenous (43 % protein) and isocaloric (8 % fat) diets with graded levels of LL supplementation (0, 2, 4, 6, 8, and 10 g/kg) to evaluate their effects on *M. rosenbergi*i. The selected lysolecithin levels and feeding period were designed based on previous studies that demonstrated physiological and growth benefits in aquatic animals (Qiu et al., 2025; Xu et al., 2022; Yan et al., 2025). The ingredient composition and proximate analysis of the experimental diets are summarized in Table 1, whereas the fatty acid composition is detailed in Table 2. All dried ingredients were finely ground and passed through a 200 μm sieve, weighed in proportion, and thoroughly mixed. Fish oil and LL were sequentially added to the mixture and stirred for 15 min. Subsequently, an appropriate amount of water was added, followed by an additional 10 min of mixing. Isocaloric conditions among dietary treatments were maintained by incrementally reducing fish oil levels with increasing lysolecithin inclusion, given its role as a principal energy source. Carboxymethyl cellulose was incorporated at 2 % as a binder during pellet formation. The resulting blends were extruded using a single-screw extruder (KE19, Brabender, Germany) and formed into pellets through a 1.2 mm die. The pellets were dried at 50 °C until the moisture content was below 10 % and then stored at -20 °C for future use.

Table 1
Ingredient composition and proximate analysis of the experimental diets (% dry matter basis).

Ingredients (%)	0	2	4	6	8	10
Fish meal	25.00	25.00	25.00	25.00	25.00	25.00
Poultry by-product	6.00	6.00	6.00	6.00	6.00	6.00
Shrimp meal	5.00	5.00	5.00	5.00	5.00	5.00
Brewers dried yeast	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00
Starch	21.00	21.00	21.00	21.00	21.00	21.00
Wheat gluten	6.00	5.00	5.00	5.00	5.00	5.00
Cholesterol	0.50	0.50	0.50	0.50	0.50	0.50
Fish oil	4.00	3.80	3.60	3.40	3.20	3.00
$Ca(H_2PO_4)_2$	2.00	2.00	2.00	2.00	2.00	2.00
Choline chloride (50 %)	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[a]	1.00	1.00	1.00	1.00	1.00	1.00
Carboxymethyl cellulose	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[b]	1.00	1.00	1.00	1.00	1.00	1.00
Lysolecithin	0.00	0.20	0.40	0.60	0.80	1.00
Analyzed nutrients composition (% dry matter basis)						
Crude Protein	43.80	43.35	42.35	43.77	43.69	42.59
Crude lipid	8.05	7.78	7.96	7.93	7.54	7.83
Moisture	8.29	8.10	7.85	7.96	8.10	9.21
Ash	16.06	16.03	16.02	16.48	15.85	15.78

Note: [a,b] The vitamin and mineral premix composition was adapted from our previous study (Qiu, Xu, and Li et al., 2023).

Table 2
Fatty acid composition of the experimental diets (% of total fatty acids).

Fatty acid	0	2	4	6	8	10
C14:0	3.06	3.349	3.375	3.229	3.225	3.246
C16:0	24.87	23.485	23.951	23.913	23.752	23.91
C18:0	8.06	7.296	7.363	7.333	7.516	7.433
C16:1	4.56	4.858	4.902	4.772	4.662	4.744
C18:1	25.97	22.654	22.965	22.153	22.361	22.037
C18:2n-6	13.59	12.725	13.169	13.664	14.268	14.766
C20:4n-6	1.72	1.941	1.856	1.862	1.879	1.754
C18:3n-3	1.54	1.668	1.7	1.696	1.803	1.852
C20:5n-3	5.21	4.584	4.094	3.815	4.21	3.895
C22:6n-3	9.14	9.925	9.633	8.914	9.259	9.233
∑SFA	35.99	34.13	34.689	34.475	34.493	34.59
∑MUFA	30.53	27.512	27.867	26.925	27.023	26.781
∑n - 6 PUFA	15.31	14.666	15.025	15.526	16.147	16.52
∑n -3 PUFA	15.89	16.177	15.427	14.425	15.272	14.980
∑n-6/∑n-3 PUFA	0.96	0.907	0.974	1.076	1.057	1.103

Note: Not all analyzed fatty acids are included in this table; only the major ones are presented. Fatty acids present in minor or trace amounts, or those not detected, are omitted. Values represent the average of two measurements.
[a]SFA: C14:0, C16:0, C18:0
[b]MUFA: C16:1, C18:1
[c]∑n - 6 PUFA: C18:2n-6, C20:4n-6
[d]∑n - 3 PUFA: C18:3n-3, C20:5n-3, C22:6n-3

2.3. Experiment animals and management

The *M. rosenbergii* (PL-5) were obtained from the International Institute of Aquaculture and Aquatic Sciences (I-AQUAS) at Universiti Putra Malaysia. Before the experiment, the prawns were acclimated for 14 days in a 1500 L rearing tank and fed a mixed diet of brine shrimp (*Artemia*) and basal feed. Subsequently, 1800 individuals (~0.04 g in weight) were randomly assigned to 18 tanks (100 prawns per tank), with six dietary treatments, each treatment having three replicate tanks. The prawns were fed twice daily (09:00 and 17:00), with the feed amounting to 10 % of their average body weight, which was gradually reduce to 6 % at the end of the 56- day feeding trial. During this period, leftover feed, feces, and molts were removed daily using the siphoning method, and water quality was maintained with the following parameters: 30 ± 0.4 °C, dissolved oxygen above 5.0 mg/L, pH maintained at 7.8 ± 0.2, total ammonia nitrogen and nitrite nitrogen below 0.2 mg/L and 0.05 mg/L, respectively.

2.4. Sample collection

At the end of the feeding trial, the prawns were counted and weighed after fasting for 24 h to assess their growth condition. Hemolymph was withdrawn from the ventral sinus, located at the base of the first abdominal segment of the prawn with a 1 mL syringe and a 26 gauge needle, and mixed with Alsever solution (R1016, Solarbio, China) at a 1:1 ratio as an anticoagulant. For each replicate tank, hemolymph was collected from six prawns and pooled. After centrifugation at 2000 ×g for 10 min at 4 °C, the supernatant was collected. The same prawns were then dissected to collect intestinal tissues for biochemical analysis. All samples were stored at -80 °C until analysis.

2.5. Growth performance parameters

The weight gain, feed conversion ratio, specific growth rate and survival rate were calculated as follows:

Weight gain (WG, %) =100 × (Final weight - Initial weight) / initial weight ;

Feed conversion ratio (FCR) = feed intake (g)/WG (g) ;

Specific growth rate (SGR, % day^{-1}) = [Ln final weight (g)-Ln initial weight (g)]/experimental period (days)] × 100 ;

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

2.6. Proximate composition and fatty acid analysis

The approximate composition of the feed and prawn samples was analyzed. Moisture, crude protein, and ash contents were determined according to AOAC (2006) standard methods, while crude lipid was extracted using the chloroform–methanol method of Folch et al. (1957). Moisture content was determined by drying at 105 °C until a constant weight was achieved. Crude protein (N × 6.25) was quantified using a Dumas nitrogen analyzer (Dumatherm, Gerhardt, Germany), and ash content was determined by incinerating at 550 °C in a muffle furnace for 5 h. The lipid extracts were subsequently esterified following the AOAC (2006) protocol. During the preparation of fatty acid methyl esters (FAME), the lipid extract is first treated with 0.5 M methanol NaOH under heating, and then esterified with methanol and BF_3. Subsequently, n-heptane was added to recover the methyl esters, and the mixture was washed with saturated NaCl solution. After ultrasonic shaking, the upper n-heptane phase was carefully collected and stored in a 10 mL glass vial for analysis. Finally, the fatty acid composition was determined using gas chromatography (Nexis GC-2030, Shimadzu, Japan).

2.7. Biochemical analysis

Commercial kits from the Nanjing Bioengineering Research Institute (Nanjing, China) were used to measure various biochemical indicators. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured in the hemolymph. In the hepatopancreas, malondialdehyde (MDA), superoxide dismutase (SOD), glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), and reduced glutathione (GSH) levels were quantified. The activities of α-amylase (α-AL), trypsin, and lipase were determined in the intestinal samples. All measurements were strictly conducted according to the manufacturer's instructions.

2.8. Challenge test

Following the feeding trial, 20 prawns were randomly selected from each tank for a bacterial challenge with *Aeromonas hydrophila*. The bacterium, originally isolated from diseased giant freshwater prawn (*M. rosenbergii*) as reported by Chong et al. (2020), was cultured in tryptic soy broth (TSB) at 37 °C for 24 h and subsequently diluted to a concentration of 1×10^5 CFU/mL. This concentration corresponds to the previously determined 7-day median lethal dose (LD_{50}) for *M. rosenbergii* based on preliminary trials, and the virulence of the isolate was reconfirmed through LD_{50} testing. This LD_{50} dose was selected to ensure a standardised pathogenic pressure suitable for assessing host immune response and survival, consistent with previous methodologies (Okasha et al., 2025; Qiu et al., 2025; Sherif et al., 2025). In the challenged group, each prawn received an intramuscular injection of 30 μL of the bacterial suspension into the second or third abdominal segment. Control prawns were injected with an equal volume of sterile phosphate-buffered saline (PBS). Mortality was monitored and recorded daily for 7 consecutive days.

2.9. Statistical analysis

Data from the dose–response trial were first tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. When both assumptions were satisfied, one-way analysis of variance (ANOVA) was performed using JMP Pro, Version 16 (SAS Institute Inc., Cary, NC, USA) to determine significant differences among dietary treatments, and post hoc comparisons were conducted using Tukey's HSD test. Statistical significance was accepted at $p < 0.05$.

For the dose–response evaluation of dietary lysolecithin levels, orthogonal polynomial contrasts were performed using the GLM procedure in SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA) to assess linear, quadratic, and cubic trends. When significant effects were detected ($p < 0.05$), regression analysis was conducted to determine the best-fitting model and estimate the optimal lysolecithin level.

3. Results

3.1. Growth performance

The effects of increasing dietary levels of LL supplementation on the growth performance and survival of *Macrobrachium rosenbergii* are shown in Table 3. The dietary treatment with 4 g/kg lysolecithin (LL) significantly increased the FBW, WG, and SGR of prawns compared with the basal dietary treatment ($p < 0.05$). FCR tended to decrease at lower levels of lysolecithin (LL) supplementation and then increased again at higher levels, but these changes were not statistically significant ($p > 0.05$). Similarly, no significant differences in SR were observed among the dietary treatments ($p > 0.05$). Based on the quadratic polynomial regression model of SGR, the optimal dietary LL supplementation level for *Macrobrachium rosenbergii* was estimated at 4.8 g/kg (Fig. 1).

3.2. Proximate composition and fatty acid profile

The effect of adding different levels of LL to prawn feed on its body composition is shown in Table 4. Compared to the basal dietary treatment, the crude fat content in the 4 g/kg LL dietary treatment was significantly lower ($P < 0.05$), and it showed a decreasing trend with increasing levels of LL, reaching the lowest point in the 6 g/kg LL dietary treatments and above ($P < 0.05$). There were no significant differences in moisture, crude protein, and ash content among the groups ($P > 0.05$). The results of the fatty acid composition analysis are shown in Table 5. Compared to the basal dietary treatment, the content of C20:4n-6 in the 10 g/kg LL dietary treatment significantly increased ($P < 0.05$), while the content of C18:3n-3 in the 10 g/kg LL dietary

Table 3
Effects of dietary lysolecithin levels on the growth performance and survival of *Macrobrachium rosenbergii*.

Parameters	Dietary Lysolecithin levels (g/kg)						Pr > F	Pooled SE
	0	2	4	6	8	10		
FBW(g)	0.96^{b}	1.05^{ab}	1.14^{a}	1.06^{ab}	0.99^{b}	0.96^{b}	0.0044	0.03
WG (%)	2302.52^{b}	2515.51^{ab}	2746.32^{a}	2557.32^{ab}	2374.94^{b}	2288.34^{b}	0.0043	70.31
FCR	1.90	1.91	1.53	1.74	1.78	1.99	0.1586	0.12
SGR(%)	5.68^{b}	5.82^{ab}	5.98^{a}	5.86^{ab}	5.73^{b}	5.67^{b}	0.0049	0.05
SR(%)	0.80	0.74	0.82	0.78	0.82	0.76	0.6921	4.18

Note: Initial body weight (IBW), final body weight (FBW), weight gain (WG), survival rate (SR), feed conversion ratio (FCR), specific growth rate (SGR). Values represent the means of three replicate groups (n = 3). Different letters within the same row indicate significant differences between groups ($P < 0.05$).

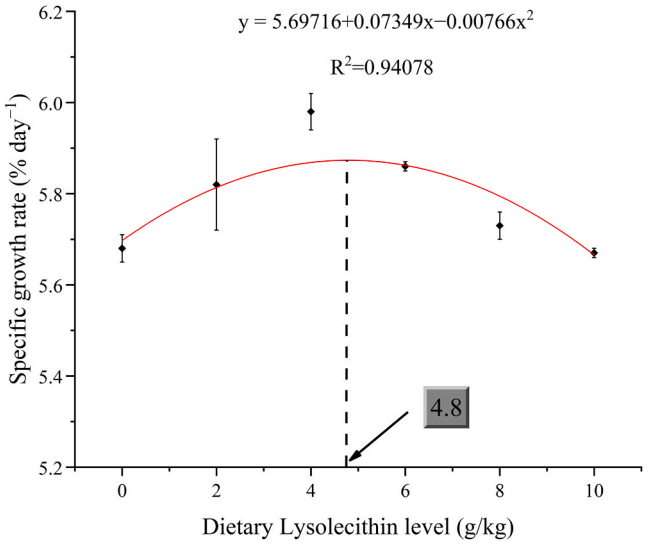

Fig. 1. Response of specific growth rate (SGR) of *Macrobrachium rosenbergii* to dietary lysolecithin (LL) supplementation levels. Each point represents the mean ± standard error (n = 3). The relationship was fitted using a quadratic polynomial regression model ($p < 0.05$), and the optimal dietary LL level 4.8 g/kg.

treatment significantly decreased ($P < 0.05$). The contents of the other fatty acids showed no significant difference compared to the basal dietary treatment ($P > 0.05$).

3.3. Hemolymph immunity

The effect of adding different levels of LL to prawn feed on hemolymph biochemical indicators is shown in Table 6. Compared to the basal dietary treatment, dietary supplementation with 4 g/kg and 10 g/kg LL dietary treatments significantly decreased serum TG levels ($P < 0.05$). In addition, the addition of LL did not show a significant difference in HDL-C and LDL-C levels ($P > 0.05$). There were also no significant differences in TC levels between the groups ($P > 0.05$).

3.4. Hepatopancreas antioxidant capacity

The effects of adding different levels of LL to prawn feed on the activity of hepatopancreas antioxidant enzymes and lipid peroxidation levels are shown in Fig. 2. Compared to the basal dietary treatment, the addition of 4 g/kg LL dietary treatment in the feed significantly increased GOT and GPT activities ($P < 0.05$), while the GPT activity in the 8 g/kg LL dietary treatment also significantly increased ($P < 0.05$). The SOD activity in the 4 and 10 g/kg LL dietary treatments was significantly higher than that in the basal dietary treatment ($P < 0.05$). In addition, the MDA levels in the 2, 4, 6, and 8 g/kg LL dietary treatments were significantly lower than those in the basal dietary treatment ($P < 0.05$). The CAT levels in the 2, 4, 6, and 10 g/kg LL dietary treatments were significantly higher than those in the basal dietary treatment ($P < 0.05$), Meanwhile, the GSH activity in the 4, 6, and 10 g/kg LL dietary treatments was significantly higher than that in the basal dietary treatment ($P < 0.05$).

3.5. Intestinal digestibility

The effect of adding different levels of LL to prawn feed on intestinal digestive enzyme activity is shown in Fig. 3. Compared to the basal dietary treatment, the amylase activity in the 2, 4, 6, and 10 g/kg LL dietary treatments was significantly higher ($P < 0.05$). The activity of trypsin in the 4, 6, and 8 g/kg LL dietary treatments was significantly higher than that of the basal dietary treatment ($P < 0.05$). In contrast, there were no significant differences in lipase activity among the experimental groups ($P > 0.05$).

3.6. Cumulative mortality

The effect of adding different levels of LL to prawn feed on the cumulative mortality rate of *M. rosenbergii* infected with *Aeromonas hydrophila* is shown in Fig. 4. Compared to the basal dietary treatment, the cumulative mortality rates in the 4, 6, 8, and 10 g/kg LL dietary treatments were significantly lower ($P < 0.05$). Among them, the mortality rates in the 8 and 10 g/kg LL dietary treatments were the lowest and gradually stabilized after 72 h post-infection. In contrast, the cumulative mortality rate in the 0 g/kg LL dietary treatment was the highest and rose rapidly within 48 h post-infection.

Table 4
Effects of dietary lysolecithin levels on the body composition of *Macrobrachium rosenbergii*.

Parameters	Dietary Lysolecithin levels (g/kg)						Pr > F	Pooled SE
	0	2	4	6	8	10		
Moisture	75.08	74.44	74.78	76.47	74.95	74.85	0.1961	0.53
Crude protein	16.886	17.70	17.22	16.37	17.58	17.35	0.1226	0.33
Crude lipid	2.35^{a}	2.35^{a}	1.94^{ab}	1.44^{c}	1.60^{bc}	1.40^{c}	< 0.0001	0.1
Ash	4.88	4.39	4.97	4.41	4.61	4.49	0.5601	0.27

Values represent the means of three replicate groups (n = 3). Different letters within the same row indicate significant differences between groups ($P < 0.05$)

Table 5
Effects of dietary lysolecithin levels on the fatty acid composition of *Macrobrachium rosenbergii*.

Fatty acid	Dietary Lysolecithin levels (g/kg)						Pr > F	Pooled SE
	0	2	4	6	8	10		
C14:0	2.78	2.47	1.96	1.99	1.92	2.61	0.0263	0.19
C16:00	24.93	25.31	25.20	23.67	24.18	24.29	0.1736	0.47
C18:0	10.53	10.59	10.96	10.42	10.80	9.64	0.7567	0.64
C16:1	2.78	2.50	2.53	2.50	2.32	2.68	0.4880	0.17
C18:1	26.65^{ab}	26.90^{ab}	27.84^{a}	24.81^{ab}	25.85^{ab}	22.78^{b}	0.0460	1.01
C18:2n-6	8.96	9.39	9.86	9.38	9.89	9.74	0.5646	0.40
C20:4n-6	3.22^{b}	3.41^{ab}	3.30^{ab}	3.99^{ab}	3.96^{ab}	4.04^{a}	0.0096	0.17
C18:3n-3	0.69^{a}	0.64^{a}	0.62^{a}	0.63^{a}	0.61^{a}	0.23^{b}	0.0148	0.08
C20:5n-3	7.48^{ab}	7.57^{ab}	6.98^{b}	8.58^{ab}	8.35^{ab}	10.30^{a}	0.0347	0.63
C22:6n-3	6.36	6.41	5.70	7.45	6.77	6.98	0.1149	0.40
∑SFA	38.23	38.37	38.13	36.08	36.90	36.54	0.2189	0.77
∑MUFA	29.43^{ab}	29.40^{ab}	30.36^{a}	27.30^{ab}	28.17^{ab}	25.45^{b}	0.0459	1.00
∑n - 6 PUFA	12.18	12.80	13.16	13.37	13.85	13.78	0.0636	037
∑n - 3 PUFA	14.53	14.62	13.30	16.18	15.72	17.51	0.0795	0.91
∑n-6/∑n-3 PUFA	0.84	0.88	0.99	0.84	0.89	0.79	0.2603	0.05

Values represent the means of three replicate groups (n = 3). Different letters within the same row indicate significant differences between groups ($P < 0.05$).

Table 6
Effects of dietary lysolecithin levels on Hemolymph biochemical parameters in *Macrobrachium rosenbergii*.

Parameters	Dietary Lysolecithin levels (g/kg)						Pr > F	Pooled SE
	0	2	4	6	8	10		
TC (mmol/L)	0.19	0.17	0.19	0.23	0.22	0.19	0.1250	0.0161
TG (mmol/L)	0.99^{a}	0.61^{ab}	0.50^{b}	0.73^{ab}	0.70^{ab}	0.48^{b}	0.0363	0.1013
HDL-C (mmol/L)	0.42^{ab}	0.41^{b}	0.46^{ab}	0.45^{ab}	0.50^{a}	0.42^{ab}	0.0255	0.0175
LDL-C (mmol/L)	0.18^{ab}	0.17^{b}	0.19^{ab}	0.21^{ab}	0.24^{a}	0.23^{a}	0.0043	0.0137

Note: Total cholesterol (TC), Triglycerides (TG), High-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C). Values represent the means of three replicate groups (n = 6). Different letters within the same row indicate significant differences between groups ($P < 0.05$).

4. Discussion

Growth performance is an important metric when determining aquaculture feed quality, usually measured using several parameters such as rate of WG, SGR, and FCR (Li et al., 2024; Qiu et al., 2023). The research revealed that inclusion of 2–4 g/kg of LL in the diet significantly improved the growth performance of *M. rosenbergii* indicated by increased WG and SGR. Moreover, based on the second-degree polynomial regression model, the optimal growth response breaking point was revealed at approximately 4.8 g/kg LL supplementation; beyond this level, growth benefits became nonsignificant and exhibited a declining trend. This may be due to how excessive lecithin interferes with fat balance mechanisms, causing elevated fat accumulation (Zheng et al., 2024). Lin et al. (2017) also observed improved growth results upon inclusion of moderate amounts of phospholipid content in the diet in juvenile hybrid snakehead (*Channa argus*×*Channa maculata*), although over-supply may reduce conversion efficiency. The underlying mechanism may involve the interaction between bile salts and lecithin that facilitates fat absorption (Tan et al., 2022; Zhou et al., 2025).

The lipid contents in feed ingredients have a major influence on the body composition and fatty acid quality of aquatic animals, hence impacting both growth and economic value (Liu et al., 2022; Peng et al., 2017). This study observed that the dietary inclusion of moderate levels of LL significantly reduced whole-body crude fat content in *M. rosenbergii*. Moreover, excessive dietary supplementation beyond the recommended levels, despite further reductions in whole-body lipid deposition, may induce metabolic dysregulation, thereby impairing lipid utilization efficiency and leading to diminished growth performance. Incremental LL supplementation resulted in coordinated changes in the fatty acid composition of prawn muscle across both the n-6 and n-3 pathways (Table 5). At the highest level (10 g/kg), C20:4n-6 increased significantly ($p = 0.0096$), while its precursor C18:2n-6 remained unchanged ($p = 0.5646$). In the n-3 pathway, C18:3n-3 decreased ($p = 0.0148$), whereas C20:5n-3 increased ($p = 0.0347$), and C22:6n-3 did not vary significantly ($p = 0.1149$). These combined responses suggest that excessive LL supplementation may modulate the activity of desaturase and elongase enzymes, thereby altering the conversion balance between precursor and product fatty acids. This finding is in concordance with Song et al. (2019), where they postulated that excessive doses of supplemental phospholipid may interfere with the blue swimmer crab fatty acid metabolism. Similarly, (Li et al., 2014a; 2014b) noted that supplementing with excessive amounts of phospholipid into the diet of juvenile swimming crab (*Portunus trituberculatus*) may lead to interference with the fatty acid metabolism, decrease lipid consumption efficacy, and potentially exert negative effects on their growth. Overall, optimized supplementation of LL enhances functional nutrient deposition by promoting the bioavailability and accumulation of beneficial fatty acids (Cai et al., 2017).

Hemolymph is also instrumental in lipid metabolism and is an essential criterion for determining systemic metabolic condition in aquatic species (Ciaramella et al., 2014). Changes in biochemical indices in hemolymph also reinforced LL's lipid-regulating action. Triglyceride (TG) levels significantly decreased under the 4 and 10 g/kg LL dietary treatments, implying improved clearance of lipids as well as reduced circulating fat accumulation. This may be due to LL's capacity to promote emulsification and transport of lipids, thus allowing more efficient utilization and deposition in target organs such as muscle or hepatopancreas. The same decreases in serum TG levels have also occurred in juvenile turbot (*Scophthalmus maximus L.*) and largemouth bass (*Micropterus salmoides*) under LL supplementation (Li et al., 2019; Zheng et al., 2024), which again supports its systemic regulation of lipids. Meanwhile, levels of TC, HDL-C, as well as LDL-C, had no notable differences, which suggests that LL mainly regulates triglyceride metabolism without disturbing overall cholesterol transport processes. The results prove that LL plays a part in circulatory balance in lipids, in addition to its action in hepatopancreatic lipid deposition and antioxidant activity.

Oxidative stress represents a common biological challenge in aquatic animals, where reactive oxygen particles in excess may initiate fat

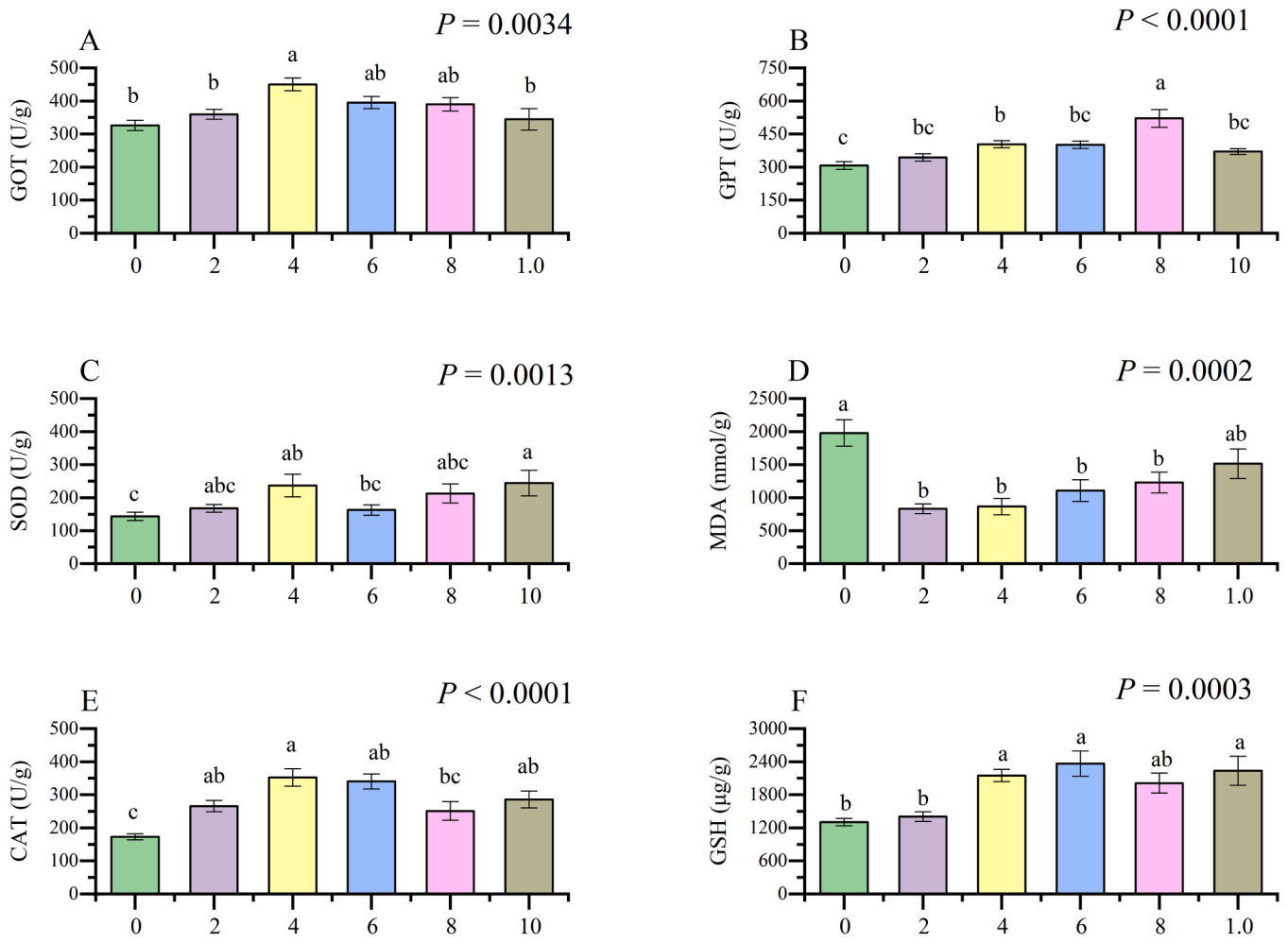

Fig. 2. Effects of dietary lysolecithin levels on hepatopancreas antioxidant and biochemical parameters in *Macrobrachium rosenbergii*. (A) Glutamic oxaloacetic transaminase (GOT), (B) Glutamic pyruvic transaminase (GPT), (C) Superoxide dismutase (SOD), (D) Malondialdehyde (MDA), (E) Catalase (CAT), and (F) Reduced Glutathione (GSH). Error bars represented the mean ± standard error (n = 6). Bars with different superscript letters indicate statistically significant differences ($P < 0.05$).

oxidation reactions that damage cellular organelles and membranes (Birnie Gauvin et al., 2017; Giulio et al., 1989). The body typically depends on antioxidant enzymes such as SOD, CAT, and GSH to remove free radicals and reduce oxidative damage (Dawood et al., 2021; Hoseinifar et al., 2020). The findings of the present study indicate that SOD, CAT, and GSH had significantly elevated activities in the 4 g/kg LL dietary treatment, while the level of MDA significantly decreased, indicating that such a concentration can significantly enhance the antioxidant ability of the body and alleviate oxidative damage. The findings in line with previous study by Yang et al., (2023) on the blunt snout bream species of *Megalobrama amblycephala* fingerlings, which revealed that proper supplementation of phospholipids not only enhances SOD activity but also maximizes the antioxidant activity of CAT and GSH, thus alleviating lipid peroxidation damage. It is important to note that the present study demonstrated a decrease in CAT and GSH activities in the 10 g/kg LL dietary treatment compared to the 4 g/kg LL dietary treatment, accompanied by an increase in MDA levels. This suggests that excessive LL supplementation may impose metabolic stress on the antioxidant system, suppressing the normal activities of CAT and GSH, and thus weakening the overall antioxidant defense capacity. The findings are consistent with findings reported by Lin et al. (2024), who demonstrated that supplementing yellow catfish diets with appropriate levels of phospholipids could alleviate oxidative stress induced by high-fat diets. However, excessive supplementation may increase the production of reactive oxygen species (ROS), consequently exacerbating oxidative damage. Furthermore, studies by Melo et al. (2024) and Antonopoulou et al. (2014) also established that excessive consumption of phospholipids could interfere with the redox balance of the body, leading to reduced catalase (CAT) activity and increased oxidative stress, thereby weakening the overall effectiveness of the antioxidant defense mechanism. Additionally, measurement of GOT and GPT, key markers of pancreatic and hepatic function, provides crucial evidence of organ responses to oxidative stress through alterations in their enzyme activities (Dong et al., 2013; Qiu et al., 2023). In the present study, activities of GOT and GPT significantly increased under the 4 g/kg LL dietary treatment, while GPT activity also rose notably under the 8 g/kg LL dietary treatment. Cai et al. (2017) reported that excessive phospholipid supplementation could increase hepatic lipid deposition and oxidative stress, thereby initiating a harmful cycle that exacerbates the very condition the supplementation aims to correct.

Digestive enzyme activity is also a critical parameter to assess the efficacy of digestion of feeds and the absorption of nutrients by aquatic animals (Prabu et al., 2017; Qiu et al., 2023). This study demonstrated that dietary LL supplementation modulated the activities of digestive enzymes, indicating its potential role in enhancing digestive efficiency and nutrient utilization in *M. rosenbergii*. Dietary LL supplementation significantly enhances amylase and trypsin activities, with optimal effects observed at 4–6 g/kg LL indicating improved carbohydrate and protein digestion, respectively. This improvement is likely due to the amphiphilic nature of LL, which enhances nutrient emulsification and

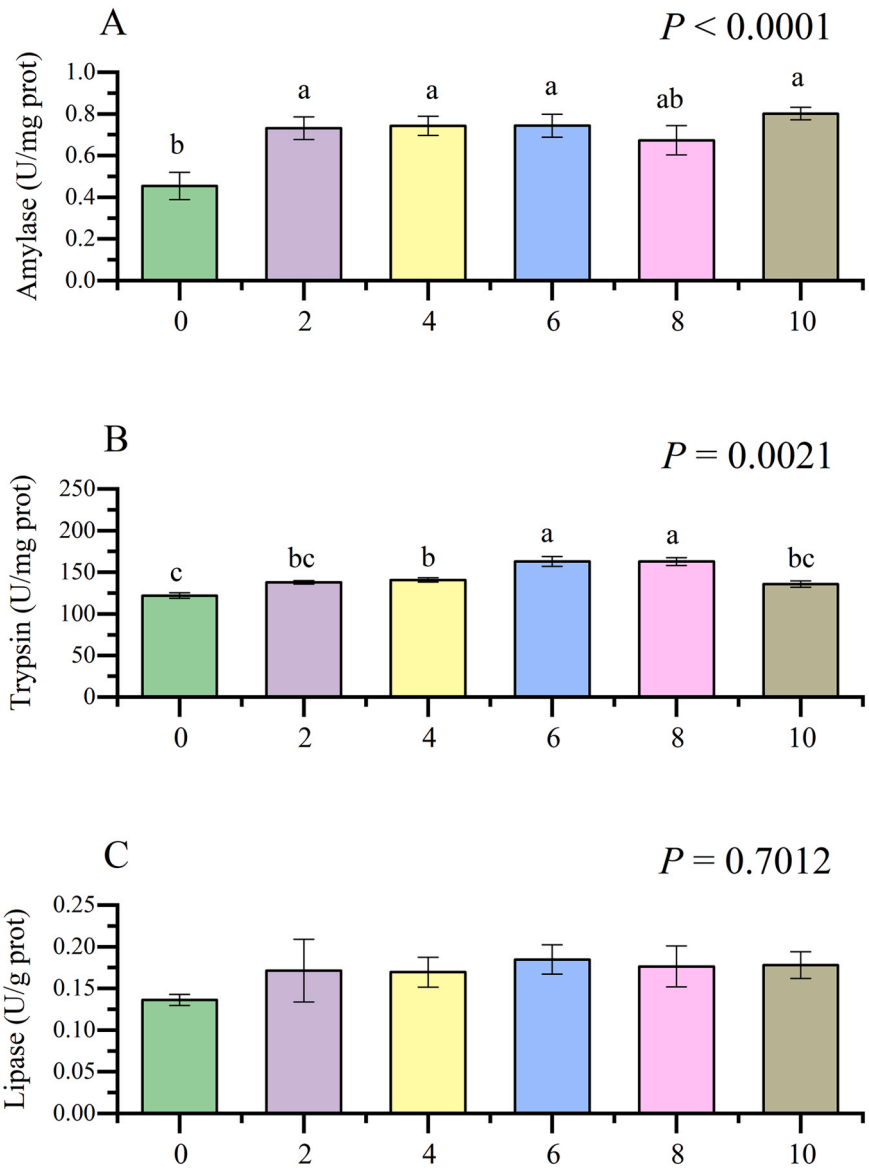

Fig. 3. Effects of dietary lysolecithin levels on intestinal digestive enzyme activities in *Macrobrachium rosenbergii*. (A) Amylase, (B) Trypsin, and (C) Lipase activities. Error bars represented the mean ± standard error (n = 6). Bars with different superscript letters indicate statistically significant differences ($P < 0.05$).

facilitates enzyme-substrate interactions within the intestinal lumen. Furthermore, elevated trypsin activity suggests enhanced proteolytic capacity, potentially leading to increased amino acid absorption and improved growth performance. Similarly, previous studies have reported that increased intestinal secretion of enzymes and uptake of nutrients in fish, as well as crustaceans, due to supplementation with phospholipid (Chen et al., 2024; Wang et al., 2022). No change in lipase activity was significantly affected in all the dietary treatments, which reflects that digestion of lipids might have reached saturation or was possibly being regulated by other processes such as bile acid interaction (Li et al., 2024). Overall, these outcomes reflect that LL is contributing to digestive proficiency, thereby lending stronger evidence to its applicability as a functional feed supplement.

Crustaceans do not have an immune system of their own and have predominantly innate defense mechanisms, such as humoral and cellular immunity (Vázquez et al., 2009). Research has demonstrated that proper supplementation with phospholipid significantly improves the activity of immune-dependent enzymes in aquatic animals. For instance, in juvenile grass carp, proper phospholipid supplementation has been demonstrated to promote non-specific immune responses, enhance complement component 3 (C3) content, and induce acid phosphatase activity, thus effectively enhancing the organism's immune function in total (Chen et al., 2015; Feng et al., 2016). Lecithin containing a high amount of bioactive compounds like phosphatidylcholine and phosphatidylserine has been deemed to be able to modulate immune cell function, promote phagocytic, and enhance disease resistance (Vázquez et al., 2009; Wee et al., 2023). In this study, dietary LL supplementation significantly reduced cumulative mortality of *M. rosenbergii* following an *Aeromonas hydrophila* challenge compared to the basal dietary treatment, with the greatest protective effects observed at 8 and 10 g/kg LL dietary treatments. These results indicate that LL effectively enhances immune function and disease resistance, possibly through improved cell membrane fluidity and enhanced immune cell signaling. Additionally, appropriate LL supplementation appears to support digestive enzyme activities, optimize tissue fatty acid composition, and strengthen antioxidant defenses, collectively promoting better growth performance and overall health. However, despite excellent immune protection at the highest dose (10 g/kg), further research is needed to assess potential risks of long-term excessive supplementation, such as lipid metabolism disturbances. Future studies should focus on

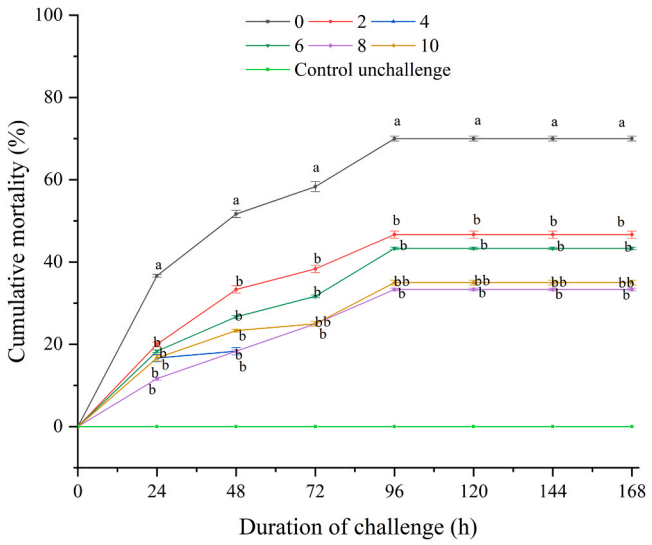

Fig. 4. Cumulative mortality of *Macrobrachium rosenbergii* fed diets supplemented with different levels of LL after *Aeromonas hydrophila* challenge. The unchallenged basal dietary treatment is also shown. Error bars indicated the mean ± standard error (n = 3). Lines with different superscript letters indicate statistically significant differences ($P < 0.05$).

determining the optimal dosage of LL and understanding its long-term effects, providing scientific evidence for the practical use of phospholipids in crustacean diets.

5. Conclusion

This study demonstrates that dietary LL supplementation has the capacity to enhance the growth performance, antioxidant activity, lipid metabolism, and disease resistance of *M. rosenbergii* and validates its application as a functional aquaculture feed additive. A quadratic analysis of specific-growth rate recommended an optimal dietary inclusion level of 4.8 g/kg for *M. rosenbergii*.

CRediT authorship contribution statement

de Cruz Clement R: Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Chou Min Chong:** Writing – review & editing, Supervision. **Qiyou Xu:** Writing – review & editing, Supervision. **Zongsheng Qiu:** Writing – original draft, Methodology, Investigation, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Alhajj, M., Montero, N., Yarce, C., Salamanca, C., 2020. Lecithins from vegetable, land, and marine animal sources and their potential applications for cosmetic, food, and pharmaceutical sectors. Cosmetics. https://doi.org/10.3390/cosmetics7040087.

Antonopoulou, E., Kousidou, E., Tserga, E., Feidantsis, K., Chatzifotis, S., 2014. Dietary lipid levels in meagre (*Argyrosomus regius*): effects on biochemical and molecular indicators of liver. Aquaculture 428429, 265–271. https://doi.org/10.1016/J.AQUACULTURE.2014.03.024.

AOAC, 2006. Official Methods of Analysis, 18th editioned., 11. Association of Official Analytical Chemists.

Birnie Gauvin, K., Costantini, D., Cooke, S., Willmore, W., 2017. A comparative and evolutionary approach to oxidative stress in fish: a review. Fish Fish. 18, 928–942. https://doi.org/10.1111/FAF.12215.

Boyd, C., D Abramo, L., Glencross, B., Huyben, D., Juarez, L., Lockwood, G., McNevin, A., Tacon, A., Teletchea, F., Tomasso, J., Tucker, C., Valenti, W., 2020. Achieving sustainable aquaculture: historical and current perspectives and future needs and challenges. J. World Aquac. Soc. https://doi.org/10.1111/jwas.12714.

Cai, Z., Mai, K., Ai, Q., 2017. Regulation of hepatic lipid deposition by phospholipid in large yellow croaker. Br. J. Nutr. 118, 999–1009. https://doi.org/10.1017/S000711451700294X.

Chen, C., Jiang, Z., Liang, Z., Wu, S., Yang, H., Xu, C., Li, E., 2023. Response of growth performance, antioxidant capacity, and gut microbiota to dietary cholesterol and lecithin in the juvenile redclaw crayfish, cherax quadricarinatus. Aquaculture. https://doi.org/10.1016/j.aquaculture.2023.740401.

Chen, Y., Jiang, W.D., Liu, Y., Jiang, J., Wu, P., Zhao, J., Kuang, S., Tang, L., Tang, W., Zhang, Y.A., Zhou, X.Q., Feng, L., 2015. Exogenous phospholipids supplementation improves growth and modulates immune response and physical barrier referring to NF-κB, TOR, MLCK and Nrf2 signaling factors in the intestine of juvenile grass carp (*Ctenopharyngodon idella*). Fish. Shellfish Immunol. 47 (1), 46–62. https://doi.org/10.1016/j.fsi.2015.08.024.

Chen, J., Sui, C., Hu, Y., Qin, H., Zhang, D., Wei, J., Cao, B., Li, Q., 2024. Effects of dietary phospholipids on growth performance, antioxidant capacity, and lipid metabolism of juvenile Chinese sturgeon (*Acipenser sinensis*), a critically endangered sturgeon in the Yangtze river. Aquac. Rep. https://doi.org/10.1016/j.aqrep.2024.102366.

Chong, C., Yaakob, A., Maha, A., 2020. Passive transfer of immunity from tilapia antisera enhanced in-vitro phagocytosis of *Aeromonas hydrophila* in naïve *Macrobrachium rosenbergii* haemocytes. J. Environ. Biol. 41, 1147–1152. https://doi.org/10.22438/jeb/41/5(si)/ms_05.

Ciaramella, M., Battison, A., Horney, B., 2014. Measurement of tissue lipid reserves in the American lobster (*Homarus americanus*): hemolymph metabolites as potential biomarkers of nutritional status. J. Crustace Biol. 34, 629–638. https://doi.org/10.1163/1937240X-00002262.

Cui, J., Zhang, Y., Liu, L., Zhang, Q., Xu, S., Guo, M., 2023. Polystyrene microplastics induced inflammation with activating the TLR2 signal by excessive accumulation of ROS in hepatopancreas of carp (*Cyprinus carpio*). Ecotoxicol. Environ. Saf. 251, 114539. https://doi.org/10.1016/j.ecoenv.2023.114539.

Dawood, M., Basuini, M., Yılmaz, S., Abdel Latif, H., Kari, Z., Razab, M.K.A.A., Ahmed, H., Alagawany, M., Gewaily, M., 2021. Selenium nanoparticles as a natural antioxidant and metabolic regulator in aquaculture: a review. Antioxidants 10. https://doi.org/10.3390/antiox10091364.

Dong, M., Feng, L., Kuang, S., Liu, Y., Jiang, J., Hu, K., Jiang, W.D., Li, S.H., Tang, L., Zhou, X.Q., 2013. Growth, body composition, intestinal enzyme activities and microflora of juvenile jian carp (*Cyprinus carpio var. jian*) fed graded levels of dietary phosphorus. Aquac. Nutr. 19, 1–14. https://doi.org/10.1111/J.1365-2095.2011.00926.X.

Dong, Z., Ke, T., Li, N., Zhang, B., Wang, B., Kang, J., Zhang, Y., Qian, T., Liu, L., Cai, W., Storebakken, T., Shi, B., Zhang, Y., 2024. Dietary lysolecithin enhances the value of high-lipid diet supplemented with fish stearin oil in post-juvenile largemouth bass *Micropterus salmoides*. Aquac. Rep. https://doi.org/10.1016/j.aqrep.2024.102365.

Feng, L., Chen, Y., Jiang, W.D., Liu, Y., Jiang, J., Wu, P., Zhao, J., Kuang, S., Tang, L., Tang, W., Zhang, Y.A., Zhou, X.Q., 2016. Modulation of immune response, physical barrier and related signaling factors in the gills of juvenile grass carp (*Ctenopharyngodon idella*) fed supplemented diet with phospholipids. Fish. Shellfish Immunol. 48, 79–93. https://doi.org/10.1016/j.fsi.2015.11.020.

Folch, J., Lees, M., Stanley, G.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226 (1), 497–509.

Giulio, R., Washburn, P., Wenning, R., Winston, G., Jewell, C., 1989. Biochemical responses in aquatic animals: a review of determinants of oxidative stress. Environ. Toxicol. Chem. 8, 1103–1123. https://doi.org/10.1002/ETC.5620081203.

Hoseinifar, S., Yousefi, S., Van Doan, H., Ashouri, G., Gioacchini, G., Maradonna, F., Carnevali, O., 2020. Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. Rev. Fish. Sci. Aquac. 29, 198–217. https://doi.org/10.1080/23308249.2020.1795616.

Jafari, F., Agh, N., Noori, F., Gisbert, E., Mozanzadeh, M., 2024. Supplementing lysolecithin in corn-oil based diet enhanced growth and improved body biochemical composition in juvenile stellate sturgeon (*Acipenser stellatus*). Anim. Feed Sci. Technol. https://doi.org/10.1016/j.anifeedsci.2024.115945.

Jala, R., Chen, B., Li, H., Zhang, Y., Cheong, L., Yang, T., Xu, X., 2016. Enzymatic preparation and characterization of soybean lecithin-based emulsifiers. Grasas Y Aceites 67, 168. https://doi.org/10.3989/GYA.0571161.

Li, Y., Gao, J., Huang, S., 2014. Effects of different dietary phospholipid levels on growth performance, fatty acid composition, PPAR gene expressions and antioxidant responses of blunt snout bream *Megalobrama amblycephala* fingerlings. Fish. Physiol. Biochem. 41, 423–436. https://doi.org/10.1007/s10695-014-9994-8.

Li, X., Jiteng, W., Han, T., Shuixin, H., Jiang, Y., Wang, C., 2014. Effect of dietary phospholipids levels and sources on growth performance, fatty acid composition of the juvenile swimming crab, portunus trituberculatus. Aquaculture 430, 166–172. https://doi.org/10.1016/J.AQUACULTURE.2014.03.037.

Li, B., Li, Z., Sun, Y., Wang, S., Huang, B., Wang, J., 2019. Effects of dietary lysolecithin (LPC) on growth, apparent digestibility of nutrient and lipid metabolism in juvenile turbot *Scophthalmus maximus l.* Aquac. Fish. https://doi.org/10.1016/J.AAF.2018.11.003.

Li, L., Liu, T., Li, J., Yang, Y., Liu, H., Zhang, P., 2024. Effectiveness of bile acids as a feed supplement to improve growth performance, feed utilization, lipid metabolism, digestive enzymes, and hepatic antioxidant status in aquaculture animals: a meta-analysis. Aquac. Rep. https://doi.org/10.1016/j.aqrep.2024.102121.

Lin, X., Cao, Y., Wang, X., Meng, X., Huang, H., Zhou, H., Deng, J., Tan, B., 2024. Suitable dietary phospholipids improve lipid deposition, but excessive dietary phospholipids disorder lipid metabolism in juvenile leopard coral grouper (*Plectropomus leopardus*). Aquac. Rep. https://doi.org/10.1016/j.aqrep.2024.102265.

Lin, S.M., Li, F., Yuangsoi, B., Doolgindachbaporn, S., 2017. Effect of dietary phospholipid levels on growth, lipid metabolism, and antioxidative status of juvenile hybrid snakehead (*Channa argus*×*channa maculata*). Fish. Physiol. Biochem. 44, 401–410. https://doi.org/10.1007/s10695-017-0443-3.

Liu, M., Sun, C., Zheng, X., Zhou, Q., Liu, B., Zhou, Y., Xu, P., 2022. Comparative proteomic analysis revealed the mechanism of tea tree oil targeting lipid metabolism and antioxidant system to protect hepatopancreatic health in *Macrobrachium rosenbergii*. Front. Immunol. 13. https://doi.org/10.3389/fimmu.2022.906435.

Liu, Y., Yan, Y., Han, Z., Zheng, Y., Wang, X., Zhang, M., Li, H., Xu, J., Chen, X.N., Ding, Z., Cheng, H.L., 2022. Comparative effects of dietary soybean oil and fish oil on the growth performance, fatty acid composition and lipid metabolic signaling of grass carp, ctenopharyngodon idella. Aquac. Rep. https://doi.org/10.1016/j.aqrep.2021.101002.

Melo, N., De Souza, S.P., Konig, I., De Jesus Paula, D.A., Ferreira, I.S., Luz, R.K., Murgas, L., 2024. Sensitivity of different organs and tissues as biomarkers of oxidative stress in juvenile tambaqui (*Colossoma macropomum*) submitted to fasting. Comp. Biochem. Physiol. Part a Mol. Integr. Physiol., 111595 https://doi.org/10.1016/j.cbpa.2024.111595.

Naylor, R., Hardy, R., Buschmann, A., Bush, S., Cao, L., Klinger, D., Little, D., Lubchenco, J., Shumway, S., Troell, M., 2021. A 20-year retrospective review of global aquaculture. Nature 591, 551–563. https://doi.org/10.1038/s41586-021-03308-6.

New, M., Nair, M., 2012. Global scale of freshwater prawn farming. Aquac. Res. 43, 960–969. https://doi.org/10.1111/J.1365-2109.2011.03008.X.

Okasha, L.A., Tawfeek, W.S., Elbaz, S., Sherif, A.H., 2025. Lactobacillus plantarum ameliorates stress caused by pseudomonas aeruginosa infection in Nile tilapia. Res. Vet. Sci. 192, 105721. https://doi.org/10.1016/j.rvsc.2025.105721.

Peng, M., Xu, W., Tan, P., Du, J., Mai, K., Zhou, H., Zhang, Y., Nian, R., Macq, B., Ai, Q., 2017. Effect of dietary fatty acid composition on growth, fatty acids composition and hepatic lipid metabolism in juvenile turbot (*Scophthalmus maximus l.*) fed diets with required n3 LC-PUFAs. Aquaculture 479, 591–600. https://doi.org/10.1016/J.AQUACULTURE.2017.06.032.

Prabu, E., Felix, S., Felix, N., Ahilan, B., Ruby, P., 2017. An overview on significance of fish nutrition in aquaculture industry. Int. J. Fish. Aquat. Stud. 5, 349–355.

Pu, C., Liu, Y., Zhu, J., Jianshuang, Cui, M., Mehdi, O.M., Wang, B., Wang, A., Zhang, C., 2024. Mechanisms insights into bisphenol S-induced oxidative stress, lipid metabolism disruption, and autophagy dysfunction in freshwater crayfish. J. Hazard. Mater. 479, 135704. https://doi.org/10.1016/j.jhazmat.2024.135704.

Qiu, Z., Xu, Q., Chong, C.M., de Cruz, C.R., 2025. Lecithin-enriched diet improves growth performance, lipid metabolism and disease resistance in giant river prawn (*Macrobrachium rosenbergii*). Aquaculture, 743016.

Qiu, Z., Xu, Q., Li, S., Zheng, D., Zhang, R., Zhao, J., Wang, T., 2023. Effects of probiotics on the water quality, growth performance, immunity, digestion, and intestinal flora of giant freshwater prawn (*Macrobrachium rosenbergii*) in the biofloc culture system. Water. https://doi.org/10.3390/w15061211.

Qiu, Z., Xu, Q., Xie, D., Zhao, J., Yamamoto, F., Xu, H., Zhao, J., 2023. Effects of the replacement of dietary fish meal with poultry By-Product meal on growth and intestinal health of Chinese Soft-Shelled turtle (*Pelodiscus sinensis*). Anim. Open Access J. MDPI 13. https://doi.org/10.3390/ani13050865.

Qiu, Z., Zhao, J., Xie, D., De Cruz, C., Zhao, J., Xu, H., Xu, Q., 2023. Effects of replacing fish meal with enzymatic cottonseed protein on the growth performance, immunity, antioxidation, and intestinal health of Chinese Soft-Shelled turtle (*Pelodiscus sinensis*). Aquac. Nutr. 2023. https://doi.org/10.1155/2023/6628805.

Sherif, A.H., Fadel, A., Kasem, E.A., Mahfouz, M.E., Eldessouki, E.A., Fahmy, H.A., 2025. The nanocomposite of chitosan-vitamin c modulates the expression of immune and antioxidant-related genes in Nile tilapia stressed with lead (Pb). Aquac. Int. 33 (2), 136. https://doi.org/10.1007/s10499-024-01806-8.

Song, D., Shi, B., Liyun, D., Jin, M., Sun, P., Jiao, L., Zhou, Q., 2019. Regulation of dietary phospholipids on growth performance, antioxidant activities, phospholipid metabolism and vitellogenesis in prereproductive phase of female swimming crabs, *Portunus trituberculatus*. Aquaculture. https://doi.org/10.1016/J.AQUACULTURE.2019.734230.

Sun, S., Korheina, D., Fu, H., Ge, X., 2020. Chronic exposure to dietary antibiotics affects intestinal health and antibiotic resistance gene abundance in oriental river prawn (*Macrobrachium nipponense*), and provokes human health risk. Sci. Total Environ. 720, 137478. https://doi.org/10.1016/j.scitotenv.2020.137478.

Sun, C., Shan, F., Liu, M., Liu, B., Zhou, Q., Zheng, X., Xu, X., 2022. High-Fat-Diet-Induced oxidative stress in giant freshwater prawn (*Macrobrachium rosenbergii*) via NF-κB/NO signal pathway and the amelioration of vitamin e. Antioxidants 11. https://doi.org/10.3390/antiox11020228.

Tan, Y., Zhang, Z., Mundo, J.M., Mcclements, D., 2020. Factors impacting lipid digestion and nutraceutical bioaccessibility assessed by standardized gastrointestinal model (INFOGEST): emulsifier type. Food Res. Int. 137, 109739. https://doi.org/10.1016/J.FOODRES.2020.109739.

Tan, P., Zhang, P., Zhang, L., Zhu, W., Wang, L., Chen, R., Zhu, Q., Xu, D., 2022. Effects of soybean lecithin on growth performance, intestine morphology, and liver tissue metabolism in rock bream (*Oplegnathus fasciatus*) larvae, 9. https://doi.org/10.3389/fmars.2022.942259.

Ukwela, E.J., Muhammad, S.R.S., Mazelan, S., Mohamad, S.J., Wen, C.C., Ponnumony, V., Al-Khayat, J., Rosas, V., Liew, H.J., 2024. Benefits of phospholipids in aquafeed development: a review. Planet. Sustain. https://doi.org/10.46754/ps.2024.01.002.

Van Der Veen, J., Kennelly, J., Wan, S., Vance, J., Vance, D., Jacobs, R., 2017. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. Biochim. Et. Biophys. Acta Biomembr. 1859 (9 Pt B), 1558–1572. https://doi.org/10.1016/j.bbamem.2017.04.006.

Vázquez, L., Alpuche, J., Maldonado, G., Agundis, C., Pereyra-Morales, A., Zenteno, E., 2009. Review: immunity mechanisms in crustaceans. Innate Immun. 15, 179–188. https://doi.org/10.1177/1753425909102876.

Wang, S., Han, Z., Turchini, G.M., Wang, X., Fang, Z., Chen, N., Xie, R., Zhang, H., Li, S., 2022. Effects of dietary phospholipids on growth performance, digestive enzymes activity and intestinal health of largemouth bass (*Micropterus salmoides*) larvae. Front. Immunol. 12, 827946.

Wee, W., Téllez-Isaías, G., Kari, Z.A., Cheadoloh, R., Kabir, M.A., Mat, K., Sukri, S.A.M., Rahman, M.M., Rusli, N., Wei, L., 2023. The roles of soybean lecithin in aquafeed: a crucial need and update. Front. Vet. Sci. 10. https://doi.org/10.3389/fvets.2023.1188659.

Weng, M., Zhang, W., Zhang, Z., Tang, Y., Lai, W., Dan, Z., Liu, Y., Zheng, J., Gao, S., Mai, K., Ai, Q., 2022. Effects of dietary lysolecithin on growth performance, serum biochemical indexes, antioxidant capacity, lipid metabolism and inflammation-related genes expression of juvenile large yellow croaker (*Larimichthys crocea*). Fish. Shellfish Immunol. https://doi.org/10.1016/j.fsi.2022.07.020.

Xu, H., Luo, X., Bi, Q., Wang, Z., Meng, X., Liu, J., Duan, M., Wei, Y., Liang, M., 2022. Effects of dietary lysophosphatidylcholine on growth performance and lipid metabolism of juvenile turbot. Aquac. Nutr. 2022 (1), 3515101. https://doi.org/10.1155/2022/3515101.

Yan, H., Wang, Y., Liang, H., Duan, Y., Wang, J., Zhou, C., Huang, Z., 2025. Effects of lysophospholipids on the antioxidant capacity, digestive performance, and intestinal microbiota of litopenaeus vannamei. Biology 14 (1), 90.

Yang, F., Chen, G., 2022. The nutritional functions of dietary sphingomyelin and its applications in food. Front. Nutr. 9, 1002574.

Yang, C., Liu, B., Pan, L., Xia, D., Sun, C., Zheng, X., Chen, P., Hu, H., Zhou, Q., 2024. Impact of soybean bioactive peptides on growth, lipid metabolism, antioxidant ability, molecular responses, and gut microbiota of oriental river prawn (*Macrobrachium nipponense*) fed with a Low-Fishmeal diet. Biology 14. https://doi.org/10.3390/biology14010011.

Yang, W., Wu, J., Song, R., Li, Z., Jia, X., Qian, P., Zhang, H., Zhang, P., Xue, X., Li, S., Xie, Y., Ye, J., Dong, G., Wu, C., 2023. Effects of dietary soybean lecithin on growth performances, body composition, serum biochemical parameters, digestive and metabolic abilities in largemouth bass *Micropterus salmoides*. Aquac. Rep. https://doi.org/10.1016/j.aqrep.2023.101528.

Zheng, H., Wang, B., Li, Q., Zhao, T., Xu, P., Song, Y.F., Luo, Z., 2024. Dietary lecithin attenuates adverse effects of high fat diet on growth performance, lipid metabolism, endoplasmic reticulum stress and antioxidant capacity in the intestine of largemouth bass (*Micropterus salmoides*). Aquaculture. https://doi.org/10.1016/j.aquaculture.2024.741688.

Zhou, Z., Zhang, P., Tan, P., Chen, R., Hu, W., Wang, L., Zhang, Y., Xu, D., 2025. Artemia nauplii enriched with soybean lecithin enhances growth performance, intestine morphology, and desiccation stress resistance in yellow drum (*Nibea albiflora*) larvae. Metabolites 15. https://doi.org/10.3390/metabo15010063.