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EFFECTS OF DIETARY LYSOPHOSPHOLIPIDS ON GROWTH PERFORMANCE, DIGESTIVE ENZYME ACTIVITY, AND IMMUNE RESPONSES IN PACIFIC WHITE SHRIMP (*Litopenaeus vannamei)*

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Abstract: This experiment was assigned in a completely randomised design with four treatments and four replications. A 28-day feeding trial in Pacific white shrimp with initial weight of 1.55 ± 0.02 gram/individual was conducted by feeding, with and without lysophospholipids namely LYPOTECH EC, (a) Positive Control (PC) diet (2% fish oil), (b) Negative Control (NC) diet (1% fish oil), (c) PC with 0.06% Lypotech EC (T1), and (d) NC with 0.06% Lypotech EC (T2). The results of all growth performances showed no significant differences in all experiments ($P > 0.05$). However, total production, average daily gain, specific growth rate, and protein efficiency ratio in T1 showed higher numerical values than NC, whereas FCR was lower than NC ($P < 0.1$). Phenoloxidase, lysozyme, and glutathione activities showed no significant differences between all experiments ($P > 0.05$). Superoxide dismutase in shrimp fed T1 was significantly higher than the others ($P < 0.05$). Lipase activity in shrimp-fed NC was significantly lower compared to other groups, whereas protease and amylase activities were significantly higher in shrimp-fed T2 ($P < 0.05$). In conclusion, dietary lysophospholipids 0.06% (Lypotech EC) can improve shrimp growth performance and nutrient utilisation of the combination dietary lipid of fish oil and soybean oil, including partial substituting fish oil under the proportion of 1:16.

Keywords: Lysophospholipids, substituting fish oil, soybean oil, pacific white shrimp, growth performance.

Introduction

Marine fish oils are an important lipid source in aquatic feeds, which are abundant in Long-Chain Polyunsaturated Fatty Acids (LC-PUFA) and omega 3 such as Eicosanoid Pentanoic Acids (EPA) and Docosahexaenoic Acid (DHA) due to satisfy essential fatty acids requirements and being as a high-density energy supply in dietary of all fish and crustaceans such as Pacific white shrimp (Oliva-Teles *et al.*, 2022). However, global fish oil and fishmeal production has significantly declined and fluctuated according to lower quotas from fisheries policy and governance, poor climate dominated by *El Niño-Southern Oscillation*, and high fuel prices. In contrast, the demand for fisheries processed into fishmeal and fish oil has continued to increase by a fast-growing aquaculture industry as well as pet-food and pharmaceutical industries (FAO, 2023). The fluctuations in fish oil and fishmeal production, including high price variation, result in looking for alternative products or raw materials that can compensate and supply essential amino acids and Polyunsaturated Fatty Acids (PUFA) associated with some ingredients that can be applied to enhance and optimise nutrients requirement for aquafeeds.

Global Vegetable Oils (VO) production has increased with less restriction to supply which has been reaching 222.8 million tonnes in 2023 with four major terrestrial plants such as palm, soybean, rapeseed, and sunflower, respectively (USDA, 2024). Nowadays, these vegetable oils are currently substituted and considered good alternative lipid sources that are cost-effective and sustainable. However, the major comparative characteristic between fish and vegetable oils is fatty acid composition, in which VO generally lacks essential fatty acids such as LC-PUFA, DHA, and EPA. In addition, vegetable oils consist of fatty acids with chain lengths lower than C_{18} (linoleic acid). In contrast, fish oils and marine products are abundant in LC-PUFA, which can supply the availability of energy up to 70% to 80%, cholesterol, and phospholipids in aquatic nutrition (Turchini *et al*., 2009). The effects of substitution of fish oils or partial replacement, including reduction of the total amount of dietary lipids, need to minimise deleterious effects and consider several strategies for improving lipid utilisation.

Emulsifiers are widely used in animal feed industries, especially marine shrimp such as white leg shrimp or Pacific white shrimp (González-Félix *et al*., 2003; NRC, 2011; Li *et al*., 2023; Liou *et al*., 2023). Soy lecithin (phospholipids), bile salts, Glycerol Monolaurate (GML), and lysophospholipids, including lysolecithin are generally known as commercial natural emulsifiers and have a high market potential in livestock and aquatic feed (Siyal *et al*., 2017; Ullah *et al*., 2023).

However, physical properties such as Hydrophilic-Lipophilic Balance (HLB) and Critical Micelle Concentration (CMC), which have described important functions in terms of the balance of the size and strength of the hydrophilic and lipophilic moieties of emulsifier molecules are characteristic and availability of emulsifiers as the efficiency, stability, and effectiveness, respectively. Lysophospholipids provide a greater emulsifying capacity related to increased interfacial area (higher HLB value) and enhanced micelles formation (lower CMC value). The effect of dietary lysophospholipids can facilitate more efficient lipase activity than phospholipids in animal feeds (Zubay, 1984; Gutiérrez-Méndez *et al*., 2022).

Bile salts or bile acids are synthesised from cholesterol, which this endogenous molecule performs as a natural emulsifier, hormone, and signalling molecule in vertebrates. The benefits of bile salts include improving growth, promoting antioxidant capacity and non-specific immune response, facilitating lipid digestion, and providing more beneficial effects on the function and structure of intestinal microbiota in shrimp. Nevertheless, side effects of bile salt were found with 0.5-gram kilogram^{-1} and the consideration of bile salt supplementation should concern feed cost in Pacific white shrimp (Su *et al*., 2021). In terms of using GML, few studies have been conducted in aquatic feed, and more investigation of the inclusion rate has been required to determine the optimal recommendation in Pacific white shrimp.

However, the study of 700 mg kilogram^{-1} GML supplementation in the shrimp diet showed improved growth, increased digestive enzyme activity such as lipase and protease, and enhanced immune responses (antioxidant and antimicrobial activity). In contrast, high doses of dietary GML induced high-fat accumulation in the hepatopancreas, which might be a negative effect on shrimp (Wang *et al*., 2020). The consideration of dietary emulsifiers in shrimp feed should realise optimal supplementation in terms of efficiency, safety margin, cost-effectiveness, and overall health benefits, which can refer to previous studies of livestock and finfish experiments.

Lysophospholipids are phospholipids derivatives that are almost derived from soy lecithin in the commercial feed industry and produced by hydrolysing one molecule of hydrophobic fatty acids with phospholipase A1 or A2 digestion (Anshi, 2007). As the result of enzymatic digestion, lysophospholipids have better function of emulsification properties than lecithin and bile salts such as CMC is 0.02 to 0.2 mmol/L, which is lower than CMC of lechin (0.3 to 2 mmol/L) (Khan *et al*., 2018). Bile salts (2 to 13 mmol/L) (Pavlović *et al*., 2018) and emulsification capacity is five times higher than lecithin or phospholipids (Anshi, 2007). CMC is a key parameter of emulsifying properties, including surface tension and Hydrophilic-Lipophilic-Balance (HLB) as highly efficient emulsifiers (AKM *et al*., 2017).

Lysophospholipids play an essential role as natural emulsifiers, which are characterised by lower CMC and higher numbers of HLB, show significantly improved lipid digestion, nutrient absorption, and transportation into small intestine in commercial livestock such as broiler chickens (Zampiga *et al*., 2016; Haetinger *et al*., 2021), weaning pigs (Zhao *et al*., 2015), and lactating sows (Zhao *et al*., 2017). In aquatic feed, the studies of lysophospholipids demonstrated the improvement in growth performance, nutrient utilisation, protein efficient ratio, and antioxidant properties in channel catfish (Liu *et al*., 2020) and turbot (Xu *et al*., 2022). The high proportion of 13% total lysophospholipids showed significantly higher lipid digestion compared to the other emulsifiers such as Arabic gum, soy lecithin, and synthetic emulsifiers such as Tween 20, Tween 80, and Polozamer188 in spiny lobster diet (Rodríguez-Viera *et al*., 2022). In terms of dietary oil reduction, the effects of lysophospholipids supplementation could reduce the inclusion of dietary lipids as palm oil and showed an improvement in fatty acid digestibility in tiger shrimp (Khan *et al*., 2018a).

Due to the key function of lysophospholipids, dietary lysophospholipids with a reduction in fish oil and combined with soybean oil, particularly in Pacific white shrimp. Note that it has not been previously studied and demonstrated beneficial effects that could be resilient to aquatic industry feed among the problems of fluctuation in fish oil production. This study aims to evaluate dietary lysophospholipids' efficacy on growth parameters, feed utilisation, digestive enzyme activity, and immune responses in diets of partially replaced fish oil with vegetable oil substitution in Pacific white shrimp.

Materials and Methods

Experimental Diets Preparation

Four isonitrogenous diets (crude protein 40.63%) were formulated and feed composition was shown based on nutrient requirement (NRC, 2011) in Table 1. The Positive Control (PC) diet contained 2% fish oil and 1.5%

Table 1: Experimental diet composition of Pacific white shrimp based on NRC, 2011

Ingredients (%)	PC	T1	NC	T2
Plant-based protein	41.00	41.00	41.00	41.00
Animal-by products	24.00	24.00	24.00	24.00
Wheat flour	24.00	24.00	24.00	24.00
Broken rice	2.33	2.27	3.33	3.27
Tuna fish oil	2.00	2.00	1.00	1.00
Soybean oil	1.50	1.50	1.50	1.50
Soy lecithin	1.00	1.00	1.00	1.00
Mix vitamins and minerals	3.65	3.65	3.65	3.65
Mix amino acids	0.22	0.22	0.22	0.22
Pellet binder	0.20	0.20	0.20	0.20
Antioxidant	0.10	0.10	0.10	0.10
Lypotech EC	0.00	0.06	0.00	0.06
Total	**100.00**	**100.00**	**100.00**	**100.00**

Remark: Micronutrient premix-preservation is supplied by DSM Nutritional Products (Thailand) Ltd. and has the following components: Vitamin and mineral mix (supplements per kg of the mixed feed): vitamin A, 3,500 IU; vitamin D3, 1,500 IU; vitamin E, 75 mg; vitamin B1, 12.5 mg; vitamin B2, 10 mg; vitamin B6, 12.5 mg; vitamin B12, 10 µg; vitamin K3, 15 mg; ascorbic acid, 100 mg; Niacin, 50 mg; Pantothenic acid, 40 mg; folic acid, 5 mg; biotin, 500 µg; Fe, 15 mg; Mn, 15 mg; Zn, 50 mg; I, 0.5 mg; Cu, 12.5 mg; Se, 0.175 mg; Co, 0.1 mg.

soybean oil, whereas the Negative Control (NC) diet contained 1% fish oil and 1.5% soybean oil. Lypotech EC (containing lysophospholipids 40 gram/kilogram, Bioscience Animal Health Public Co. Ltd., Samut Sakhon, Thailand) was supplemented at 0.06% into PC and NC formulation as T1 and T2, respectively. All coarse ingredients were finely ground, passed through a 250-µm mesh screen, and mixed for 10 minutes in the mixer machine. Liquid materials such as fish oil, soybean oil, lecithin, and water were added to a mixer to form a dough and then transferred to a mincer machine to make the pellet through a 1.5-mm die.

Consequently, the pellets were steamed at 100°C for 15 minutes and dried in a hot air oven at 80°C for six hours to reduce moisture content. Finally, pellets were cooled down at room temperature with ventilation for 24 hours, sealed, and kept until used. The chemical analysis of the experimental feed of PC and NC diets is presented in Table 2. The chemical composition of both control groups was analysed using the AOAC (2000) method. The cholesterol concentration of the experimental feed was determined by gas chromatography-single quadrupole mass spectrometer (GCMS-QP2020 NX, Shimadzu, Japan) coupled to the PAL autosampler system (CTC Analytics AG, Switzerland) after lipid extraction according to the method of Folch *et al*. (1957) and derivatised by adopting the method proposed by Kunz and Matysik (2019). Cholesterol was normalised to the closest elution solution using the myristic acid-d27-TMS, trimethysilylation, as the deuterated internal standard.

Experimental Shrimp and Feeding Trials

Juvenile Pacific white shrimp were obtained from a Samut Songkhram province, Thailand commercial farm. Shrimp were acclimatised in continuously aerated 1,000 L fibreglass tanks and fed a commercial diet for 14 days. After acclimatisation, 400 healthy shrimp were randomly transferred into the aquarium (0.53 x 1.22 x 0.37 m) for four treatments with four replicates at a density of 25 shrimp per aquarium and 150 shrimp/m^3 after being fasted 24 hours. The average initial weight was 1.55 ± 0.03 grams per shrimp. All shrimp were fed four times daily at 08.00, 12.00, 16.00, and 20.00. The initial feeding rate was calculated at 12% of shrimp body weight and then was adjusted every two weeks to adjust satiation feeding. During the experimental period, water quality was monitored and controlled as follows: pH 7.5-8.5, temperature ranged from 28°C to 30°C, salinity adjusted at 12 ppt, dissolved oxygen was more than 5 mg/L, and nitrate concentration was lower than 0.5 mg/L. The water in the aquarium was changed at 60% every two days. The trial lasted four weeks.

Table 2: Chemical composition analysis of the experimental feed

Chemical Analysis Parameter	Positive Control (PC)	Negative Control (NC)
Proximate analysis		
Moisture (%)	10.94 ± 4.13	10.81 ± 3.78
Crude protein (%)	40.38 ± 0.49	40.34 ± 0.48
Gross energy (kcal/kg)	4,256.3 ± 16.77	4,206.8 ± 9.57
Crude lipid (%)	6.57 ± 0.63	5.56 ± 0.66
Ash (%)	9.49 ± 0.52	9.00 ± 0.10
Cholesterol analysis		
Cholesterol concentration (ppm)	1,049.03 ± 83.40^b	934.60 ± 48.83^a

Remark: Data with different superscripts as $^{a, b, c}$ values in the same parameter are significantly different (P < 0.05).

Growth Performance and Nutrient Utilisation

At the end of four weeks, shrimp were fasted for 24 hours before sampling collection. All shrimp from each replicated were stunned using the thermal shock of ice slurry and then counted and weighed all shrimp to evaluate growth and feed utilisation parameters as below:

- Average Daily Gain (ADG, gram/day) = (total final body weight, g – total initial body weight, g)/feeding days.
- Specific Growth Rate (SGR, % day) = 100 x (Ln total final body weight – Ln total initial body weight, g)/feeding days.
- Survival rate (%) = (final shrimp population × 100)/initial shrimp population.
- Feed Conversion Ratio (FCR) = dry weight feed consumed, g/(total final body weight, g – total initial body weight, g).
- Protein Efficiency Ratio (PER) = (total final body weight, g – total initial body weight, g)/protein intake (dry weight, g).

Hematological and Immune Responses Parameter

After the feeding trial, 12 shrimp per each treatment were randomly collected for the hemolymph (three shrimp per replicate) to analyse total hemocyte count, total protein, and immune responses parameters such as phenoloxidase enzyme activity (PO), lysozyme, Superoxide Dismutase (SOD), and total Glutathione (GSH). Hemolymph was withdrawn from the ventral sinus using 1.0 mL syringes with anticoagulant (sodium citrate) as a ratio of 1:1 and pooled in an Eppendorf. After centrifugation at 4,000 rpm/min for 10 minutes, blood samples were determined hemolymph protein using the Lowry method (Lowry *et al*., 1951), total hemocyte count and phenoloxidase enzyme activity (PO) according to the method by Encarnacion *et al*. (2012). Lysozyme, SOD, and total GSH were analysed using the assay kits of SIGMA ALDRICH (19160-1KT-F, M3770, and CS0260-1KT), respectively.

Determination of Digestive Enzyme Activity

12 shrimp were collected from each treatment at the end of this experiment to study digestive enzyme activity. Hepatopancreas were weighed and homogenised in 50 mM Tris-HCl (pH 7.5 by 1:3 (w/v). The homogenate was centrifuged at 3,000 rpm for 15 minutes at 4°C. Consequently, the supernatant was collected and centrifuged at 12,000 rpm for 15 minutes at 4°C and kept at -20°C for total protein content and enzymatic analysis. Total protein content was determined by the Lowry method and used bovine serum albumin as a standard protein (Lowry *et al*., 1951). Lipase activity was determined according to Markweg *et al*. (1995). One unit of lipase activity (U mg^{-1} protein) was defined as the generation of 1 millimole (mM) of p-nitrophenol per minute per milligram (mg) of protein. Protease activity was analysed following the modified casein method (Pan *et al*., 2005). One unit of protease activity (U mg^{-1} protein) was defined as 1 mM of tyrosine released through the hydrolysis of casein in one minute. Amylase activity was measured in accordance with the Bernfeld method to determine the increase of reducing sugar from the hydrolysis of α -D (1,4) glycosidic bond in polysaccharides and stained with 3,5-dinitrosalicylic acid (DNS) (Bernfeld, 1955). One unit of amylase activity (U mg^{-1} protein) was defined as the production of 1 mM of glucose per minute per milligram (mg) of protein.

Statistical Analysis

All Data were determined in normality and homogeneity of variance using SPSS Statistics 29.0 software (IBM, USA). All results in different groups were analysed using a one-way ANOVA analysis and Duncan multiple comparison tests. Significance differences among the experimental groups were regarded as $P < 0.05$ and alphabetical notation was used to mark the differences. The results are expressed in mean ± standard deviation (mean ± SD).

Results and Discussion

Growth Performance and Nutrient Utilisation

The results of growth performance are shown in Figure 1. All growth performances in this experiment were not significant differences ($P > 0.05$). However, the results exhibited a trend of improvement in some growth performances such as total production (p-value = 0.068), average daily gain (p-value = 0.065), specific growth rate (p-value = 0.099), and protein efficiency ratio (p-value = 0.075) in shrimp fed T1 diet of 0.06% Lypotech EC compared to a NC group. In contrast, the feed conversion ratio trended lower than the other groups (p-value = 0.072). In addition, all growth performances between PC and T2 showed no significant differences ($P > 0.05$). The lowest growth performance and the highest FCR were shown in shrimp-fed NC. Survival Rate (SR) was not significantly different. However, shrimp fed with 0.06% Lypotech EC in T1 revealed higher numerical numbers, 85.33% compared to 84.00% (PC), 82.67% (T2), and 82.67% (NC), respectively.

Lipid is an essential nutrient that is a source of energy and contributes essential fatty acids to supply more function in the growth and survival of animals (NRC, 2011). Digestion of lipids in the digestive tract needs an emulsifier such as bile salts in vertebrates, which are secreted from the gall bladder after being synthesised from the liver. Bile salts create micelles formation and induce lipase enzymes to get easier digestion, then absorb essential nutrients through epithelial cells in the small intestine (Macierzanka *et al*., 2019). In addition, bile salts have functioned as formation micelles to increase lipid digestibility. They play a role as natural emulsifiers, which have the ability to mix lipids to be micelles and incorporate phospholipids and cholesterol. The increasing solubility of cholesterol micelles can enhance digestion and absorption of lipids and fat-soluble vitamins in shrimp (Chiang, 2013). However, studies of the expression of bile acid synthesis genes have been discovered in recent years and have been limited and demonstrated, indicating that shrimp can be synthesised bile acids de novo (Kumar *et al*., 2020; 2021). Thus, emulsification in the digestive tract could potentially reduce efficiency and require dietary supplementation to optimise lipid metabolism and utilisation. For instance, supplemented emulsifiers in the shrimp diet such as lysophospholipids can facilitate micelles formation by promoting lipase activity. This improves lipid digestion, including transportation and absorption via hepatopancreatic cells.

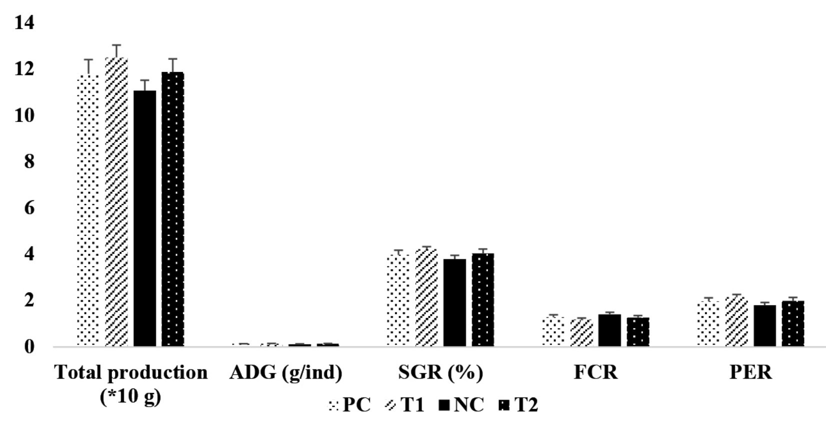

Figure 1: Growth performance and nutrient utilisation of Pacific white shrimp (*L. vannamei*) fed experimental diets with 0.06% lysophospholipids supplementation in T1 and T2 for four weeks. Remark: Data with different superscripts as [a, b, c] values in the same parameter are significantly different ($P < 0.05$). Data without superscript alphabets are not significantly different ($P > 0.05$)

Due to the benefits of lysophospholipids, the current trend to reduce fat and energy levels in feed formulation contributes to the study of aquatic feed formulation. Lu *et al*. (2022) reported that the growth performance of largemouth bass was not significantly affected by low crude protein and crude lipids when supplemented with lysophospholipids compared to the control diet. The supplementation results showed no negative effect but could improve hepatic lipid metabolism, increase intestinal digestive enzyme activity, and enhance protein deposition, including regulated structure and diversity of the intestinal flora (Lu *et al*., 2022).

Similarly, the study with a reduction of 1% dietary fish oil supplemented with lysophospholipids could enhance nutrient digestion and absorption, including the liver glycolipid metabolising enzyme activity in largemouth bass (Che *et al*., 2023). Previous studies demonstrated that the optimal level of lysophospholipids in turbot diet also improved lipid digestion and showed significant upregulation of lipolytic gene expression (Xu *et al*., 2022). In rainbow trout, dietary lysophospholipids enhance fat powder digestion and decrease the effects of fish oil replacement when used as the alternative lipid in the fish diet such as fat powder (Adhami *et al*., 2021).

However, there are quite a few reports about the effects of lysophospholipids supplementation in terms of optimisation with partial or complete reduction of fish oils or marine sources. For example, the effect of dietary lysophospholipids with palm oil showed higher growth performance, lipid digestibility, and fatty acid composition of muscle and non-muscle portions. For example, Arachidonic Acid (ARA), EPA, and DHA are compared to the other treatments with soy lecithin or phospholipid supplementation in tiger shrimp (Khan *et al*., 2018a). This is similar to supplemented lysophospholipids with partial substitution of marine protein and oil sources, which showed better performance of growth and final product quality in tiger shrimp (Khan *et al*., 2018b). Initially, this finding suggests that the effects of dietary lysophospholipids tend to improve growth performance and nutrient utilisation via lipid digestibility and metabolism as energy values, including supporting fish oil reduction in shrimp diets. However, this experiment needed an attention check to reduce Type II error, which is generally used in hypothesis testing and describes the error that occurs when one fails to reject a null hypothesis that is false and called a false negative (Ganeshpurkar *et al*., 2018). This statistical term often occurs from sample sizes that are too small. Further studies should consider the influential factors to improve power in statistical tests such as a larger population of experimental animals, an extended duration period, and different age groups of Pacific white shrimp to approve the effects of lysophospholipid supplementation.

Hematological and Immune Responses Parameter

Total Hemocyte Count (THC) and immune response parameters such as PO, lysozyme, SOD activity, and GSH are shown in Figure 2. Hematological parameters such as THC and almost immune responses excluding SOD were no significant differences between treatments ($P > 0.05$). In contrast, SOD in T1 with 0.06% Lypotech EC showed significant highest value ($P < 0.05$).

Physiological and pathological status are generally observed by hematological parameters as well as normal health of stress indicators in the animals (Dani, 2021). In aquaculture practices, haematological indices are obviously significant and quick tools to monitor the effect of stress conditions on fish health (Fazio *et al*., 2012; Nabi *et al*., 2022). However, the immune system in crustaceans such as shrimp depends on non-specific immune responses to resist infectious pathogens. THC and PO are crucial non-specific immunological parameters in crustaceans to detect health status and stress markers of infectious disease and environmental impact (Rodrıguez & Le Moullac, 2000). This study showed no significant effects of 0.06% Lypotech EC supplementation on THC and

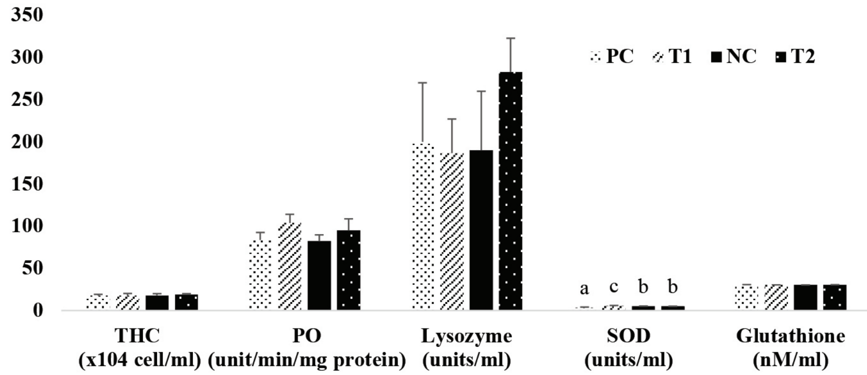

Figure 2: Hematological and immune responses of Pacific white shrimp (*L. vannamei*) fed experimental diets with 0.06% lysophospholipids supplementation in T1 and T2 for four weeks. Remark: Data with different superscripts as [a, b, c] values in the same parameter are significantly different ($P < 0.05$). Data without superscript alphabets are not significantly different ($P > 0.05$)

PO activity compared between the control and treatment groups, which could indicate no adverse effects on shrimp. Nonetheless, PO activity was observed in an increase in numerical value in shrimp fed with 0.06% Lypotech EC™ in T1, which compared to NC and T2 ($P < 0.1$). Lysozyme is one of non-specific innate immunity and is well known as the earliest antibacterial protein against bacterial infection in invertebrates such as shrimp.

The lytic activity of lysozyme in several infectious pathogens has been studied well, especially in Vibrio spp. such as V. parahaemolyticus, V. alginolyticus, and V. cholerae, which are significant pathogens in Pacific white shrimp (Burge *et al*., 2007; Tassanakajon *et al*., 2011). Nevertheless, all treatment groups had no significant differences ($P > 0.05$). Previous studies of dietary lysophospholipids effects on lysozyme in crustaceans have been limited, whereas lysozyme activity showed an increase in rainbow trout diet supplemented with 2 g kg^{-1} lysophospholipids (Taghavizadeh *et al*., 2020). The benefit of lysophospholipid supplementation may positively affect growth due to enhanced immunity. The effects of dietary lysophospholipids on PO and lysozyme activity non-specific immune responses in Pacific white shrimp have few published studies and are recommended to evaluate differential lysophospholipids and concentrations on growth and immune responses in further studies.

Antioxidant capacity is generally observed as an indicator to analyse oxidative stress and antioxidant defence systems in terrestrial (S. Li *et al*., 2015) and aquatic animals (Di Giulio *et al*., 1989; Birnie-Gauvin *et al*., 2017). SOD plays an important role as an enzyme-metabolising superoxide radical in shrimp (Campa-Córdova *et al*., 2002). Free radicals or ROS such as superoxide anion, singlet oxygen, hydroxyl radical, and hydrogen peroxide have occurred by phagocytosis and oxidative stress under normal conditions and infectious status. Therefore, SOD converts this reactive oxygen to hydrogen peroxide in cellular metabolism.

Glutathione Peroxidase (GPx) protects the cells by removing hydrogen peroxide, which is derived from GSH, a substrate that stimulates this antioxidant enzyme (Y. Li *et al*., 2016). The result of this study showed that SOD levels in shrimp fed PC with 0.06% Lypotech EC (T1) were significantly higher than the others ($P < 0.05$). In addition, the reports of dietary lysophospholipids on the antioxidant capacity of blunt snout bream (Y. Li *et al*., 2015), channel catfish (Liu *et al*., 2020), crucian carp (H.-X. Li *et al*., 2010), and juvenile large yellow croaker (Weng *et al*., 2022) had significantly increased SOD. GPX and catalase activities can enhance liver homeostasis and reduce oxidative stress by these antioxidant enzymes. Hence, this study shows the result of 0.06% Lypotech EC dietary supplementation on the SOD activity of Pacific white shrimp increased, improving immune responses in terms of antioxidant responses to protect against cellular oxidative damage.

Determination of Digestive Enzyme Activity

Digestive enzyme activity in terms of lipase, protease, and total amylase activity are shown in Figure 3. Protease and total amylase activities in shrimp fed on a Lypotech EC-supplemented diet in T2 were significantly higher compared to other groups. The protease, amylase, and lipase activity in NC had significantly lowest ($P < 0.05$). The effect of lysophospholipids has shown better benefits for lipid metabolism, including promoting fatty acid catabolism via emulsification of lipids. Moreover, it could enhance nutrient digestion by increasing the contact area between feed particles during lipid digestion and digestive enzymes, which relatively promotes the digestive enzyme activity in the midgut (Khan *et al*., 2018a; Guoliang *et al*., 2023). In previous studies, the effects of lysophospholipids supplementation have significantly increased lipase, protease, and amylase activities in carp (Guerden *et al*., 2008), channel catfish (Liu *et al*., 2020), largemouth bass (Che *et al*., 2023), and large yellow croaker (Weng *et al*., 2022). The results of this study in shrimp fed 0.06% Lypotech EC show significantly higher lipase, protease, and total amylase in T2 compared to a negative diet without lysophospholipids supplementation (NC) ($P < 0.05$) due to their functions in terms of improving lipids emulsification properties and nutrient utilisation.

The reduction of fish oil in a NC diet without lysophospholipid supplementation (NC) showed a decrease in digestive enzyme activity than others. In contrast, dietary lysophospholipids could significantly compensate for these digestive enzymes' activity. Fish oils are abundant in essential fatty acids, phospholipids, and cholesterol and are more digestible than plant-based oils. Finally, this study presents the efficiency with higher emulsifying properties of lysophospholipids compared to phospholipids. It had decreased in the NC, which could increase digestive enzyme activity by enhancing the surface area and creating micro micelles to allow for more efficient lipase, protease, and amylase activity. The effects of this optimal dietary supplementation level of lysophospholipids in an energy-reduction diet in this experiment might be an initial study of Pacific white shrimp. Therefore, further studies need to investigate the duration of the feeding period and differentiate optimal amounts of

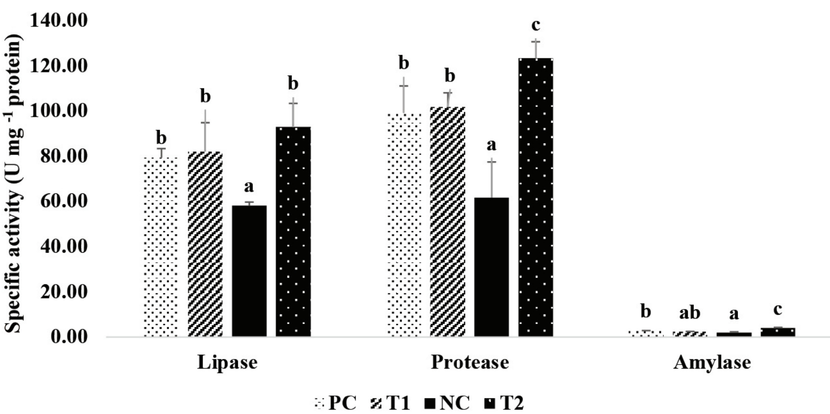

Figure 3: Digestive enzyme activities of Pacific white shrimp (*L. vannamei*) fed experimental diets with 0.06% lysophospholipids supplementation in T1 and T2 for four weeks. Remark: Data with different superscripts as a, b, c values in the same parameter are significantly different ($P < 0.05$). Data without superscript alphabets are not significantly different ($P > 0.05$)

lysophospholipids supplementation on other benefits such as apparent digestibility coefficient and nutrient accumulation. This is especially true for fatty acids composition, amino acids profile in the whole body, the cephalothorax, and muscle of Pacific white shrimp in terms of partial or complete fish oil substitution.

The findings of this study suggest that supplemented 0.06% lysophospholipid fed in Pacific white shrimp shows a tendency to promote growth performance. This improves digestive enzyme activity and immune responses, especially in shrimp with lower dietary energy levels. The results of using Lypotech EC can substitute fish oil with no negative effect under the proportion of Lypotech EC: Dietary fish oil reduction as 1:16.

Conclusions

In summary, the dietary addition of 0.06% lysophospholipids, Lypotech EC could effectively enhance benefits on growth performance and feed utilisation and promote immune responses, particularly antioxidative stress enzyme, by emulsification effects and improving lipid metabolism. In contrast, supplementing lysophospholipids in a low-energy diet, especially in partial fish oil replacement with soybean oil could induce digestive enzyme activity in Pacific white shrimp.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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