

Lyso-phospholipid supplementation to replace lecithin and improve growth performance in Pacific white shrimp

A low inclusion of a strong emulsifier can replace a higher inclusion of a weak emulsifier and maintain lipid digestion and absorption.

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In shrimp and other crustaceans, weight gain responses to different levels of dietary lipid indicate that highest gains are generally achieved at dietary levels of 5-6% inclusion (NRC, 2011). Higher levels (>10%) often retard growth because of the inefficiency of shrimp to use lipids. Shrimp lack the bile juice system, which results in a less efficient emulsification process and a subsequent decreased formation and absorption of lipid micelles, leading to a reduced hepatopancreas function.

Digestibility enhancers based on natural emulsifying agents selected for their compatibility with the digestive system of shrimp can complement the process of emulsification, digestion, and absorption. These facilitate the break-down of lipids into small droplets that can be dispersed throughout the water, thus allowing for more efficient action by lipases. Emulsifiers are also associated with the products of lipid digestion and contribute to the formation of small micelles that are more easily transported and absorbed by the hepatopancreatic cells. As a result, the ability of shrimp to efficiently use lipids as essential nutrients and sources of energy for growth is improved.

Lecithin is the most common form of phospholipids used in animal nutrition and as such has traditionally been used as emulsifier in shrimp feeds. The two lipophilic fatty acid tails contained in the phospholipid molecule make lecithin an efficient emulsifying agent for water-in-oil emulsions (i.e. limited amount of water is added to a lipid rich environment) but a weak emulsifier for the oil-in-water conditions (i.e. limited amount of lipids is added to a water rich environment) such as those of the digestive tract of shrimp (Figure 1). Lyso-phospholipids

are obtained by controlled enzymatic hydrolysis of phospholipids whereby one of the fatty acid tails is removed. Since lyso-phospholipids have only one fatty acid residue per molecule, they are more hydrophilic than phospholipids and therefore have increased potential to serve as an oil-in-water emulsifier in the digestive tract of shrimp. The present study evaluated the efficacy of lyso-phospholipids to replace lecithin and promote growth performance in Pacific white shrimp.

Experimental diets

The formulation and proximate composition of the experimental diets are described in Table 1. A total of three experimental diets containing 15% fish meal and 1.7% fish oil were formulated. The positive control (Pos CTRL) contained 1.5% lecithin, while lecithin was reduced from 1.5% to 0.75% in the negative control (Neg CTRL). A lyso-phospholipid-based emulsifier (Aqualyso STD[®] Adisseo, France) at 0.1% was supplemented in the negative control diet (Neg CTRL + Aqualyso STD) aiming to compensate for the reduced inclusion rate of lecithin. Sinking shrimp pellets were produced using a mincer with a 2mm diameter die, dried in an oven at 60°C and stored at -20°C until used.

Experimental set up

Experimental feeds were randomly assigned to 12 tanks (70L, four replicates per treatment) in a close recirculation system. The system consisted of a common filter, biofilter, protein skimmer and UV light to maintain the water quality. The water temperature of the rearing system was controlled at 28±1°C. Shrimp *Litopenaeus vannamei* were fed at 6% of their wet weight four times per day at 07:00, 12:00, 17:00 and 22:00 h. Shrimp were weighed once every 2 weeks and half of rearing water was exchanged after weighing. Shrimp were fed the experimental diets for an 8-week period. At the end of the feeding trial, shrimp were weighed individually and growth performance including weight gain (WG), specific growth rate (SGR) and survival rate were calculated.

Data were assessed for normality and variance homogeneity using the Kolmogorov-Smirnov test and Bartlett's test,

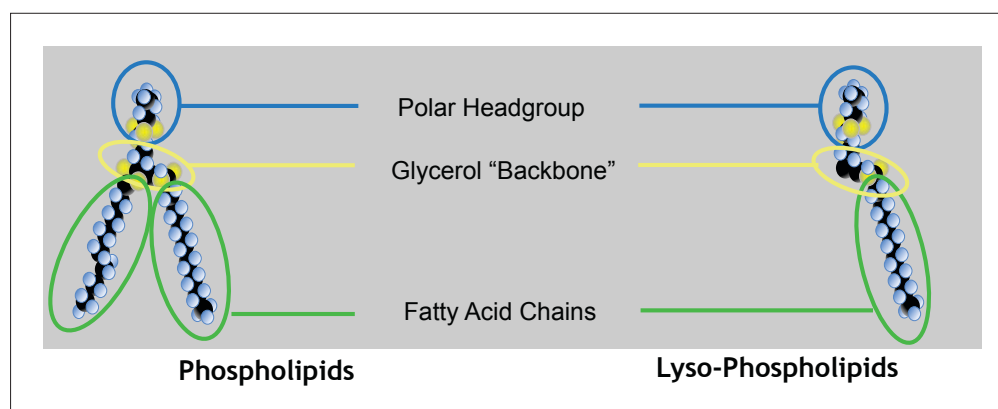


Figure 1. Structure of phospholipids and lyso-phospholipids. Lyso-phospholipids are obtained by enzymatic hydrolysis of phospholipids. The removal of one fatty acid increases the hydrophilicity and therefore lyso-phospholipids serve as better oil-in-water emulsifiers than phospholipids.

	Pos CTRL	Neg CTRL	Neg CTRL + Aqualso STD®
Ingredients (%)			
Fish meal	15	15	15
Corn protein concentrate	5	5	5
Soybean meal	30	30	30
Rice bran	10	10	10
Wheat	20	20	20
Squid liver meal	4	4	4
Shrimp head meal	4	4	4
Fish oil	1.7	1.7	1.7
Lecithin - Soy (70%)	1.5	0.75	0.75
Trace mineral premix	1	1	1
Vitamin premix	2	2	2
Gluten (wheat)	2	2	2
Alpha-starch	3.3	3.3	3.3
CMC	0.5	1.25	1.15
Emulsifier	0	0	0.1
Proximate composition (%)			
Moisture	9.65	9.92	9.79
Ash	7.22	7.14	7.16
Crude protein	36.86	37.29	37.64
Ether extract	7.99	6.34	6.23

Table 1. Formulation of the experimental diets.

respectively. The results were analysed by a one-way analysis of variance (ANOVA). When the ANOVA identified differences among the groups, multiple comparisons were made among the means using the Duncan's multiple range test. Statistical significance was determined as $p < 0.05$

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Results

Shrimp fed the negative control diet (0.75% lecithin) showed numerically lower weight gain and specific growth rate (SGR) than shrimp fed the positive control diet with 1.5% lecithin (Figure 2). This can be attributed to insufficient emulsifying capacity and consequently to poorer lipid digestion and utilisation. However, lyso-

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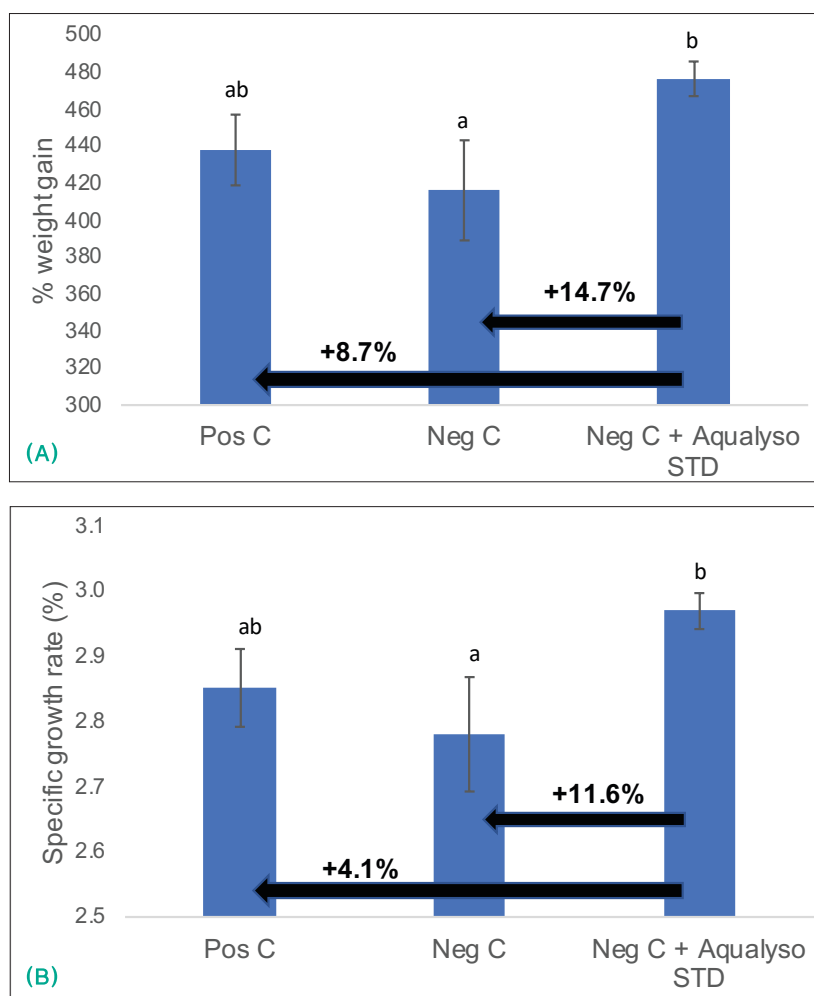


Figure 2. Weight gain (A) and specific growth rate (B) of *Litopenaeus vannamei* shrimp fed different diets for 8 weeks. Each bar was presented as mean with standard deviation from four replicates (n=4) of all dietary treatments. Percent improvement achieved with treatment diet (Neg CTRL+ Aqualyso STD®) is given relative to Neg CTRL and Pos CTRL. Different superscripts indicate significant ($p < 0.05$) difference among the dietary treatments.

Pos CTRL: positive control; Neg CTRL: negative control. WG = (final body weight – initial body weight)/initial body weight \times 100. SGR = (ln final body weight – ln initial body weight)/feeding days.

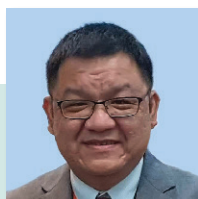
In summary, the present study provides evidence on the efficacy of lyso-phospholipid as a strong emulsifying agent and a replacement for lecithin in shrimp feeds. Supplementation of 0.1% Aqualyso STD can spare 0.75% of lecithin and enhance growth performance of Pacific white shrimp.

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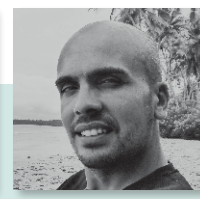
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phospholipid supplementation (Aqualyso STD at 0.1%) in diets with low lecithin levels (0.75%) significantly enhanced % weight gain and SGR by 14.7% and 11.6%, respectively, in relation to the negative control group. Numerical improvements in the same performance parameters were also detected in relation to the positive control. These results suggested that the replacement of a large inclusion of a weak emulsifier by the small inclusion of a strong emulsifier was an effective strategy to at least maintain lipid digestion and absorption as well as growth performance.

Hosseini et al. (2018) reported that the performance benefits of lyso-phospholipid supplementation may be attributed to their effect on cell membrane enterocytes. Lyso-phospholipids can positively alter membrane permeability and fluidity and consequently promote a higher flux rate of nutrients across the cell membrane (Chen et al., 2019). Several studies have reported positive growth effects of lyso-phospholipid supplementation in fish, including turbot and channel catfish, as well as an increment of lipase and alkaline phosphatase activities (Liu et al., 2019; Li et al., 2019). These observations could be explained by the more efficient oil-in-water emulsification achieved by lyso-phospholipids in the digestive tract and therefore the more active surface for enzymatic action.



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