Lysophospholipid-based digestive enhancer to boost growth performance and digestion mechanisms of shrimp under different dietary cholesterol levels

By Yu-Hung Lin and Waldo G. Nuez-Ortín

For many years the aquaculture industry has sought to improve sustainability through reduction in the use of fishmeal in aquafeeds. Soybean meal is a widely used alternative to fish meal in shrimp feed because of its well-balanced nutritional composition and easy availability. However, the use of soybean meal in shrimp feed should be carefully considered due to its deficiency of some nutrients, especially cholesterol. Cholesterol is a critical dietary requirement for shrimp which lacks the ability to *de novo* synthesise this nutrient (NRC, 2011). Cholesterol requirements for white shrimp are reported to be 0.11-0.14% (NRC, 2011), while cholesterol levels in standard shrimp feed formulations currently range between 0.05% and 0.1%. Therefore, increasing soybean meal inclusion levels decrease cholesterol concentrations in the shrimp hepatopancreas and haemolymph (Lin et al., 2017). Such hypocholesterolemia is attributed to both deficiency of cholesterol in soybean meal and poor cholesterol utilisation by the shrimp.

Shrimp lack a bile juice system, as a result of which the lipid digestion process is not as efficient as in fish. Digestibility enhancers based on natural emulsifying agents have been proven to be capable of complementing the process of emulsification and optimising the digestion and utilisation of important lipidic nutrients such as cholesterol.

Aqualyso® (Adisseo) is a lysophospholipid-based additive produced by the controlled hydrolysis of phospholipids in soybean lecithin with phospholipase A2. Lecithin facilitates the digestion and absorption of lipids. Given the superior emulsifying properties of Aqualyso in relation to lecithin, a common application nowadays in shrimp feeds is lecithin replacement to reduce formulation cost and maintain growth performance. A growth trial was conducted to investigate the benefits of lysophospholipid-based Aqualyso in current shrimp feed formulations containing high and low concentrations of cholesterol. Dietary levels of fish meal and soybean meal were adjusted to provide high (0.12%) and low (0.05%) cholesterol concentrations in the experimental feeds.

Experimental setup

Four isoproteic and isolipidic experimental feeds were formulated and are presented in Table 1. Two control feeds containing 1% lecithin were designed, with either high or low concentrations of cholesterol: high cholesterol diet (HIGH CHOL, 0.12%) and low cholesterol diet (LOW CHOL, 0.05%). Aqualyso (AQL) was supplemented at 0.1% into both formulations: HIGH CHOL+0.1% AQL and LOW CHOL+0.1% AQL. 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 use.

The four experimental feeds were randomly assigned to 12 tanks (300L, 3 replicates per treatment) in a close recirculation system. Twenty shrimp (2.39±0.02g) were stocked in each tank. The system consisted of biological filters, protein skimmer and UV light to maintain water quality. The water temperature of the rearing system was controlled at 28 ± 1°C. The shrimp were fed to 6% of their wet weight 4 times per day at 07:00, 12:00, 17:00 and 22:00h. Shrimp were weighed once every 2 weeks and half of the rearing water was exchanged at the same time. Shrimp were fed the experimental diets over 8 weeks.

	HIGH CHOL	HIGH CHOL + 0.1% AQL	LOW CHOL	LOW CHOL + 0.1% AQL
Ingredients (%)				
Fish meal	20	20	7	7
Soybean meal	25	25	43.5	43.5
Fish oil	3	3	1	1
Soybean oil (refined)	0	0	3	3
Soy lecithin	1	1	1	1
Aqualyso®	0	0.1	0	0.1
Alpha-cellulose	6.5	6.0	3.0	2.5
Others*	29.9	29.9	29.9	29.9
Composition (%)				
Moisture	9.32	11.04	9.96	11.01
Ash	7.62	6.40	7.57	6.72
Crude protein	37.15	36.68	37.15	36.85
Crude fat	7.64	7.90	7.74	8.12
Cholesterol	0.118	0.052	0.122	0.053

*includes 15% fermented soybean meal, 8% corn starch, 10% alpha-starch, 7% squid liver meal, 1.5% choline chloride, 1% vitamin premix and 2% mineral premix.

Table 1. Formulation and composition of the experimental diets.

At the end of the feeding trial, the shrimp were bulk weighed to calculate growth performance. Following this, hepatopancreas were randomly collected from two shrimp in each tank. Gene expression of digestive enzymes, including chymotrypsin, trypsin, amylase and lipase, were measured. Primer design and relative quantification followed the description by Castro-Ruiz et al. (2021) and Livak and Schmittgen (2001).

Data was assessed for normality and variance homogeneity using the Kolmogorov-Smirnov test and Bartlett's test, 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 by setting the aggregate type I error to $p<0.05$.

Supplementation supports growth performance

Supplementation of the lysophospholipid-based digestive enhancer supported feed intake and growth performance of shrimp fed high and low cholesterol feeds (Figure 1). Feed intake significantly improved by 13 and 21% with

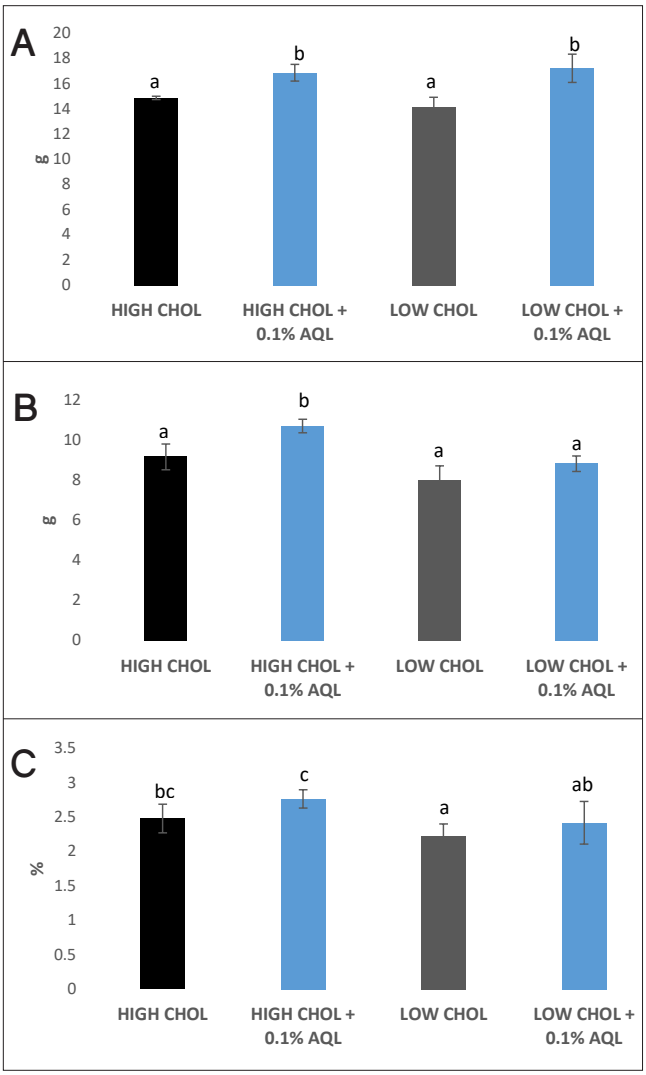

Figure 1. Effects of dietary lysophospholipids (Aqualyso®, AQL) on feed intake (A), weight gain (B) and specific growth rate (C) of white shrimp fed diets with high cholesterol (HIGH CHOL) or low cholesterol (LOW CHOL). Bars with different letters indicate significant differences ($p<0.05$).

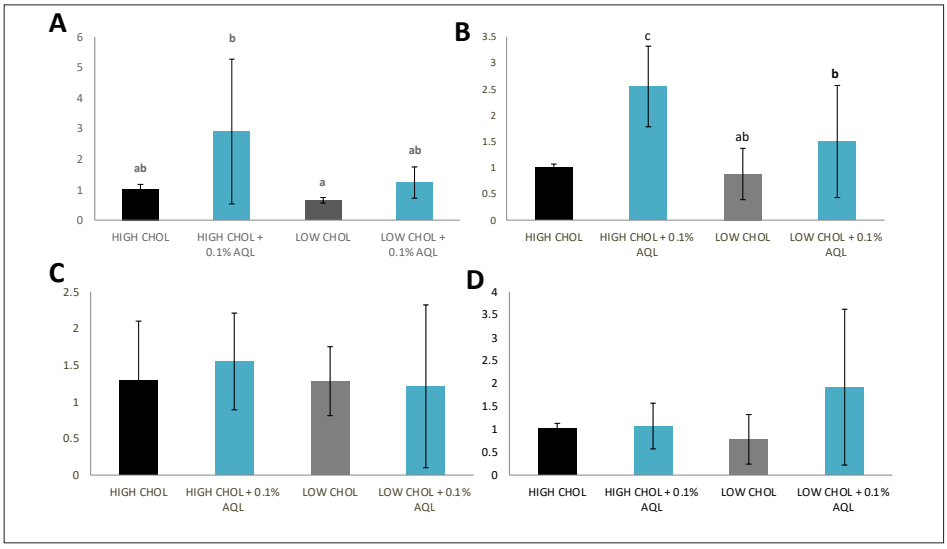

Figure 2. Effects of dietary lysophospholipids (Aqualyso®, AQL) on relative gene expression of chymotrypsin (A), trypsin (B), amylase (C) and lipase (D) of white shrimp fed diets with high cholesterol (HIGH CHOL) or low cholesterol (LOW CHOL). Bars with different letters indicate that they are significantly different ($p<0.05$) from one another.

supplementation in the high and low cholesterol feeds, respectively. The best growth effects were found in the high cholesterol feed supplemented with Aqualyso, showing significant improvements of 16% and 11% in weight gain and specific growth rate (SGR), respectively. A similar pattern was observed in the low cholesterol feed; with supplementation the numerical improvements were 10% and 8% in weight gain and SGR, respectively. More interestingly, supplementation in the low cholesterol feed matched the performance of the high cholesterol feed, proving the efficacy of this lysophospholipid-based digestive enhancer to reduce formulation cost while maintaining the performance of a better quality feed. Additive supplementation did not significantly affect feed conversion efficiencies of the diets containing high and low cholesterol levels. Neither cholesterol levels nor additive supplementation affected survival, which averaged 76% across all treatments.

We have previously demonstrated that 0.1% lysophospholipid-based digestive enhancer can successfully replace 0.75-1% lecithin of a control feed containing 2% lecithin (Lin et al. 2021). In the present study, an average amount of 1% soy lecithin was added to all diets to satisfy the minimal phospholipid requirement for shrimp (NRC, 2011). Under such formulation strategy, the similar growth performance between the high cholesterol feed and the Aqualyso-supplemented low cholesterol feed can be attributed to the additive effect to improve digestive emulsification and promote a more efficient absorption and utilisation of cholesterol in 1% lecithin feeds.

Previous studies in fish, such as with turbot and channel catfish, have also reported a positive effect of lysophospholipid supplementation that is believed to be linked to a better absorption and utilisation of essential lipidic nutrients (Li et al., 2019; Liu et al., 2019). In salmon, more recent unpublished data show that Aqualyso accelerates the absorption and transport of nutrients in the intestine as well as the processing of nutrients in the liver.

Influencing digestive enzymatic activity

The present study also showed that Aqualyso can potentially influence digestive enzymatic activity. Gene expression analysis of proteases, such as chymotrypsin and trypsin, were upregulated in both high and low cholesterol feeds (Figure 2). However, only the supplementation in the high cholesterol feed proved to be significant. A similar but not significant upregulation pattern was observed for amylase and lipase (Figure 2). Specifically, the lysophospholipid supplementation numerically induced a 1.5-fold change in the gene expression of lipase in the low cholesterol feed, suggesting an optimisation of lipase activity due to enhanced emulsification. The regulatory pattern in the expression of proteases, amylase and lipase were in line with the increased intake and better performance observed in the Aqualyso supplemented groups, indicating that improved performance can be attributed to increased feed intake supported by the digestive mechanisms keeping up with the increasing digestive demands.

In summary, we demonstrated in this present study that 0.1% lysophospholipid-based Aqualyso® improves growth performance and digestive enzyme gene expression in shrimp fed different cholesterol levels. Given the need for further reducing fishmeal inclusion in shrimp feeds, along with the increasing cost of lecithin, Aqualyso supplementation under lecithin replacement strategies seems an effective strategy to promote sustainability and optimise the feed cost and performance of shrimp feeds.

The Aqua Nutrition Platform by Adisseo continues to combine species-specific research on Aqualyso application strategies and formulation experience while providing services related to feed formulation and processing.

Professor Yu-Hung Lin is with the Department of Aquaculture, National Pingtung University of Science and Technology, Taiwan.
Email: yuhunglin@mail.npust.edu.tw

Waldo G. Nuez-Ortín, PhD is Lead Scientist Aquaculture, Adisseo, Belgium.
Email: waldo.nuezortin@adisseo.com