

## Cost efficient replacement of cholesterol, n-3 HUFA and phospholipids in feed for white shrimp (*Penaeus vannamei*)



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Bile salts are natural emulsifiers capable of enhancing the digestive capacity for lipids in the digestive system of shrimp by improving the lipid emulsification and micelle formation, resulting in a faster absorption of lipids in the hepatopancreas. Furthermore, bile salts constitute an alternative source for the steroid ring that shrimp cannot synthesize, which is at the basis of their requirement for dietary cholesterol. Bile salts have a species-specific composition and molecular structure. The commercial availability of bile salts is mostly restricted to pharmaceutical grade applications of purified cholic acid from bovine origin, extracted from healthy

animals from BSE free countries, due to its elevated cost, not applicable in animal feeds. They need to comply with EU regulations on maximum threshold levels on undesirable substances (e.g. heavy metals, PCB's, dioxine, pesticide residues, antibiotic residues).

Although the use of bile salts in shrimp nutrition has been empirically documented, it is not known to what extent the efficacy of bile salts is affected by its origin, composition and/or molecular structure.

Shrimp are incapable to biosynthesize n-3 highly unsaturated fatty acids (HUFA) and cholesterol, and have a limited

capacity to produce phospholipids de novo. Therefore, these essential lipids need to be supplied through the feed for optimizing growth and health of farmed shrimp. The increased cost and/or limited availability of essential fat sources for shrimp feed, particularly fish oil, cholesterol and/or lecithin, have become a challenge for shrimp nutritionists during recent years. Nutritionists are looking for alternatives to replace these expensive raw materials without jeopardizing the performance of the feed, and meeting the nutrient requirements of the shrimp

The current study aims at determining the potential of different sources of bile

salts as partial replacement of cholesterol, n-3 HUFA and phospholipids in practical grow out diets for the white shrimp (*Penaeus vannamei*).

## Treatments

Five diets were processed using a pilot scale pelletizer (pre-conditioning during 2 min @ 90°C mash temperature; die 2 mm x 40 mm; post-conditioning @ 90 °C, 20 min). A positive control diet (CON HI) and a negative control diet (CON LO) only differed in terms of levels of essential lipids by reducing the level of fish oil (-0.5%), reducing the level of lecithin (-0.5%) and eliminating the cholesterol supplement in CON LO (Table 1). Three treatments were designed as follows: LO/LIPO : CON LO diet supplemented with a mixture of bile salts from bovine origin (LIPOGEST, Nutriad); LO/CHA : CON LO diet supplemented with purified cholic acid from bovine origin; LO/PG: CON LO diet supplemented with a mixture of bile salts from pig origin.

## Conditions of the feeding trial

The feeding trial was conducted at the Brackish Aquaculture Development Center test facility in Jepara, Indonesia for 56 days. The experimental setup consisted of cylindrical, 1-mt, flat-bottom fibre glass tanks.

Seawater was prepared by pumping through a 1-µm GAF filter bag, and recirculated over a 1.6-mt coral biofilter. Salinity was adjusted to 25 ppt by the addition of underground freshwater, and disinfection with 3 mg/l hypochlorite powder for 24 hours. Shrimp (*P. vannamei*) of 1 g were stocked at 20 shrimp/tank and acclimated for one week prior to the start of the trial. Experimental feeds were tested in

Table 1: Feed formulation and analysis for the experimental diets.

Ingredients (%)	CON HI	CON LO
Peruvian Fishmeal (65.7% Protein, 16.6% Ash)	13.00%	13.00%
Tuna Fishmeal (60.7% Protein, 21.6% Ash)	5.00%	5.00%
Soybean Meal (48% Protein)	34.38%	34.11%
Rape Seed Meal (34% Protein)	7.00%	7.00%
Corn Gluten (65% Protein)	2.00%	2.00%
Wheat bran	5.00%	5.00%
Rice Bran	7.00%	7.00%
Whole wheat	19.09%	20.39%
Squid meal (80% Protein)	3.00%	3.00%
Shrimp Premix*	2.00%	2.00%
Lecithine (Liquid, 60% PL)	1.50%	1.00%
Fish oil	1.00%	0.50%
Cholesterol (92% purity)	0.025%	-
Total	100	100
*Premix providing vitamins, minerals, trace elements and binder.		
Analysis as % product		
Crude protein	37.35	37.31
Crude fat after hydrolysis	6.35	5.61
Crude ash	7.56	7.66
Crude fibre	3.2	3.3
HUFA	0.89	0.75
Cholesterol	0.080	0.064
Moisture	11.46	10.62

triplicate and uneaten feed was recuperated to estimate the correct feed intake. Water temperature (27.1±0.9°C), salinity, and other quality parameters remained within acceptable limits during the tests.

## Results & Discussion

Survival was excellent and averaged 90%. Overall shrimp growth, up to 1.8 g/week towards the end of the trial, was excellent under the conditions of clear water culture in small tanks. Growth and feed utilization were significantly

affected by the reduction of the essential lipids in the CON LO treatment compared to the CON HI treatment, growth -14%, food conversion ratio (FCR) +16% and protein efficiency ratio (PER) -14%. Supplementation of the different types of bile salts to the CON LO diet resulted in an improved growth and significantly improved feed utilization, which were no longer significantly different from the results in the positive control. Also PER was significantly improved by adding bile salts, indicating that the energy in the diet is used in a more efficient way, making available more protein for tissue growth (Table 1, Figure 1,2&3). Although

Figure 1: Growth expressed as gram/week for *P. vannamei* fed the experimental diets for 56 days.

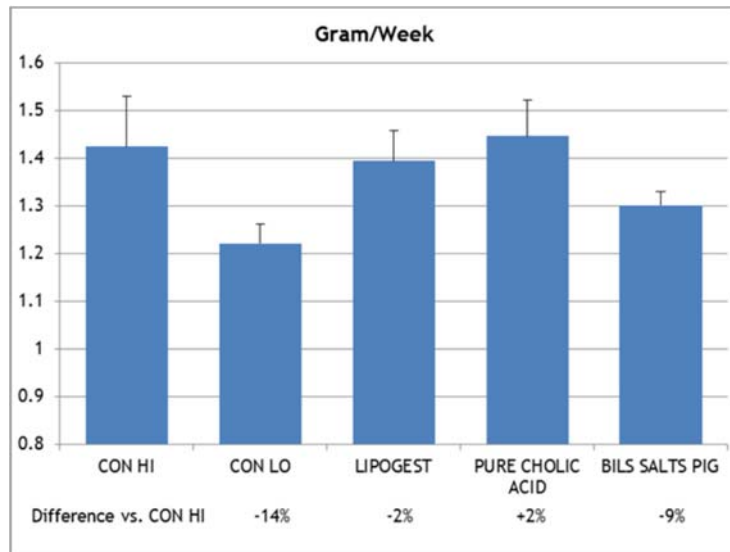


Figure 2: Feed Conversion ratio for *P. vannamei* fed the experimental diets for 56 days

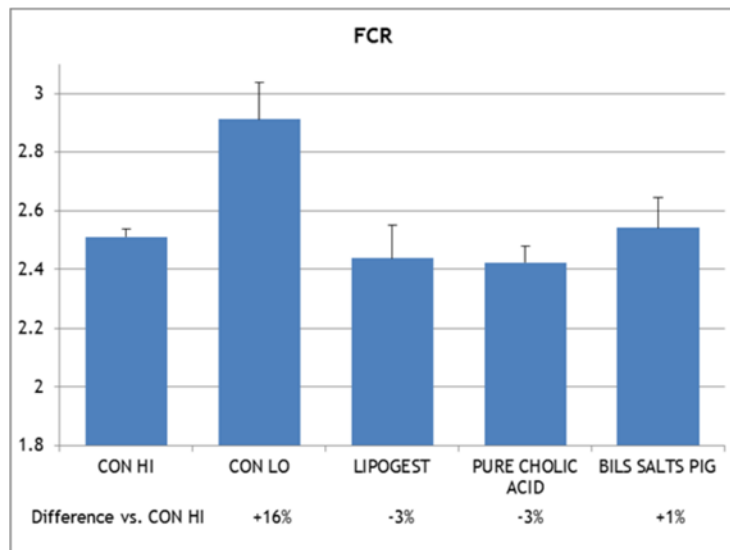
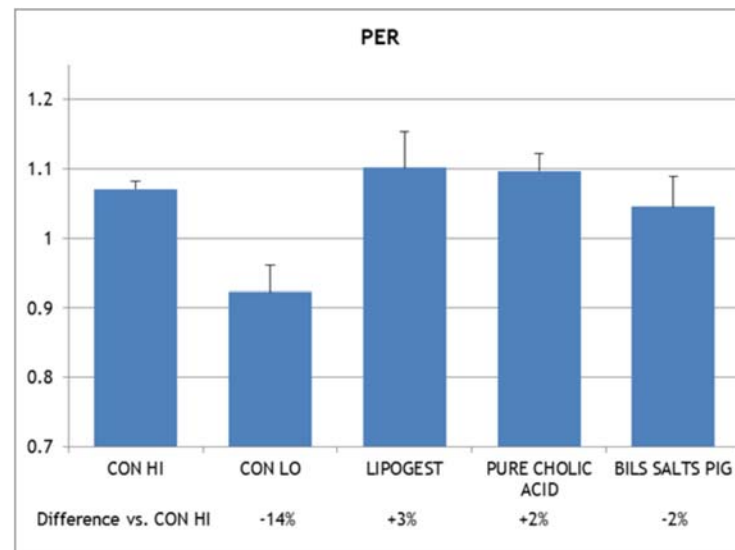


Figure 3: Protein Efficiency Ratio for *P. vannamei* fed the experimental diets for 56 days



the different bile salt supplementations did not result in statistically significant differences, the addition of purified cholic acid and the bile salts from bovine origin showed numerically better performance in terms of growth (7-11%), FCR (4-5%) and PER (6%) compared to supplementing bile salts from pig origin.

## Conclusion

Reducing the level of essential lipids including cholesterol, phospholipids and n-3 HUFA significantly affected growth, feed conversion and protein efficiency in white shrimp.

Adding bile salts to the diet lower in essential lipids restored the performance of the shrimp to the same level as the control diet with elevated levels of essential lipids. This study indicated that the source and composition of the bile salt supplement may affect the performance in shrimp nutrition.

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## More information



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