Current Status of Lipid Nutrition of Pacific White Shrimp, *Litopenaeus vannamei.*

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ABSTRACT

In the Americas *Litopenaeus vannamei* is the leading farm-raised shrimp species. To promote the expansion of this industry it is necessary to develop nutritionally complete diets, therefore knowledge of the species' nutritional requirements is essential, and so far, incomplete. Early research on lipid nutrition was performed on closed thelycum species like *Marsupenaeus japonicus*, *Fenneropenaeus chinensis*, *F. indicus* and *Penaeus monodon*, while *L. vannamei* in contrast, is an open thelycum species. Because there are differences in feeding habits among penaeids, even among developmental stages, generalizations as to their nutritional requirements are most likely inaccurate. Some studies have demonstrated that juvenile *L. vannamei* is able to satisfy its essential fatty acid requirements with highly unsaturated fatty acids from either n-3 or n-6 families, which showed higher nutritive value than polyunsaturated fatty acids. A significant interaction between dietary PL and cholesterol on shrimp growth also has been observed. However, research efforts are still needed to gain a better understanding of the metabolism of lipids in shrimp, the interactions among themselves and with other nutrients, and to define quantitative nutritional requirements for essential lipids throughout the life cycle of this species.

Key words: Shrimp nutrition; *Litopenaeus vannamei*; lipids; fatty acids; phospholipids

INTRODUCTION

Penaeid shrimp require dietary lipids to satisfy a variety of metabolic functions. Dietary lipids are a highly digestible and concentrated source of energy which supply 9 kcal/g, about double of that supplied by either carbohydrate or protein (Mead *et al*., 1986). Additionally, dietary lipids are a source of essential fatty acids (EFA), phospholipids, sterols and carotenoids, required for growth, survival and the normal metabolic function of all organisms.

González-Félix, M. L., Perez-Velazquez, M., 2002. Current status of lipid nutrition of Pacific white shrimp, *Litopenaeus vannamei*. In: Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M. G., Simoes, N. (Eds.). Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola. 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

It has been demonstrated that shrimp have a limited ability to synthesize *de novo* the n-6 and n-3 families of fatty acids (FA), including the polyunsaturated linoleic (18:2n-6, LOA) and linolenic (18:3n-3, LNA) acids. They also have a limited ability to elongate and desaturate these polyunsaturated fatty acids (PUFA) to highly unsaturated fatty acids (HUFA) such as arachidonic (20:4n-6, AA), eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids (Kanazawa, Teshima & Ono, 1979a; Kanazawa *et al.*, 1979b; Kayama *et al.*, 1980). Consequently, these FA are considered EFA. However, greater activity of LNA over LOA as an EFA has been reported for *Marsupenaeus japonicus* (Bate) (*Kanazawa et al.*, 1979c) and *Fenneropenaeus chinensis* (Osbeck) (Xu *et al.*, 1994), but for *Penaeus monodon* (Fabricius) (Glencross & Smith, 1999) and *Fenneropenaeus indicus* (Milne Edwards) (Read, 1981) LOA promoted a greater growth response. Interestingly, some observations indicate that shrimp might require some combination of n-3 and n-6 FA in their diet. Xu *et al.* (1994) observed greater growth in *F. chinensis* fed a diet with LOA and LNA each at 0.5% of diet, while Chandge & Paulraj (1998) made similar observations in *F. indicus*. Glencross & Smith (1999) observed that a 2:3 ratio of LOA to LNA was optimum for *P. monodon*. Nevertheless, HUFA have demonstrated the greatest nutritive value for these closed thelycum species; for instance, Kanazawa, Teshima & Endo (1979d) observed that LOA and LNA were not as successful as EPA or DHA in promoting weight gain of *M. japonicus*. For *F. chinensis*, Xu *et al.*, (1993) observed that n-3 or n-6 HUFA had greater value than PUFA of the same family following this trend: DHA>AA>LNA>LOA. Merican & Shim (1997) observed that growth and survival of juvenile *P. monodon* was enhanced with a lower dietary supplementation of DHA relative to LNA.

The importance of phospholipids (PL) in penaeid shrimp nutrition, including *Litopenaeus vannamei* (Boone), has been demonstrated by many researchers (Coutteau, Camara & Sorgeloos, 1996; Coutteau, Kontara & Sorgeloos, 2000; Lim *et al.*, 1997; Gong *et al.*, 2000a; González-Félix *et al.*, 2002a). PL are major constituents of membranes and are vital to the normal function of every cell and organ. They maintain cell structure and function, and have regulatory activities within the membrane and outside the cell. For instance, they serve as second messengers in cell signaling, an essential process in regulating cell growth, proliferation, differentiation, metabolism, nutrient uptake, ion transport, and even programmed cell death. In addition, there is evidence that PL containing choline, sphingomyelin, and their metabolites are important mediators and modulators of transmembrane signaling (Zeisel, 1993). PL act as emulsifiers and facilitate the digestion and absorption of FA, bile salts and other lipid-soluble matters. They also have a role in the transport of lipids, not only in the transport of absorbed lipids from the gut epithelium into the hemolymph, but also in the transport of lipids between tissues and organs (Coutteau *et al.*, 1997) since they are constituents of lipoproteins.

High density lipoproteins (HDL) and very high density lipoproteins (VHDL) are the main lipoproteins found in some crustacean species (Lee & Puppione, 1978). Phosphatidylcholine (PC) is particularly important because it is an essential component of these lipoproteins (Hertrampf, 1992). PL also act as acyl donors for the lecithin cholesterol acyltransferase (LCAT) to convert cholesterol into cholesterol ester (Mankura, Dalimunthe

& Kayama, 1980; Teshima, 1997). Dietary PL may serve as a source of choline, inositol, EFA or even energy (Coutteau *et al.,* 1997). According to Coutteau *et al.* (2000), it is still unclear whether EFA, preferentially esterified to the *sn*-2 position in PL, are assimilated as a single entity or separately from the PL; thus, EFA associated with dietary PL may have an effect on the composition and function of PL in membranes and/or lipoproteins. Other studies have indicated that dietary PL may improve the efficiency of EFA supplied as neutral lipid due to a more efficient transport and better lipid mobilization from the hepatopancreas to the hemolymph and to other tissues and organs, resulting in enhanced lipid deposition and increased energy availability for growth (Teshima, Kanazawa & Kakuta, 1986a, b; Kontara *et al*., 1998). This may have important implications if PL improve the effectiveness of EFA and thus reduce the quantitative requirements for HUFA. Emphasis has been given to the beneficial effect of PL for early stages or juvenile shrimp because, even though some crustaceans can synthesize PL (Shieh, 1969), their biosynthesis generally cannot meet their metabolic demand (D'Abramo, Bordner & Conklin, 1981; Kanazawa, Teshima & Sakamoto, 1985).

All of the preceding are closed thelycum shrimp species distributed throughout the Indo-West-Pacific. *L. vannamei* in contrast, is an open thelycum species from the Eastern Pacific, and while many aspects of shrimp biology are shared, there are differences in the feeding habits, i.e., from omnivorous to carnivorous, among penaeid shrimp and even among developmental stages, which may be reflected in their nutritional requirements. For example, the protein requirements may vary from 30% of diet for *L. vannamei* (Smith *et al*., 1985) to 57% of diet for *M. japonicus* (Deshimaru & Yone, 1978). Research efforts are still focusing on gaining a better understanding of the metabolism of lipids in crustaceans, as well as on establishing requirements for essential lipids throughout their life cycle. This work reviews recent advances on lipid nutrition of *L. vannamei* and published data concerning their requirements.

ESSENTIAL FATTY ACID REQUIREMENTS IN *Litopenaeus vannamei*

Quantitative requirements for EFA have been rarely reported for penaeid shrimp. Kanazawa, Teshima & Tokiwa (1979e) suggested that a dietary provision of 1% n-3 HUFA could be considered as a minimal value for postlarval penaeids. Shewbart & Mies (1973) showed that optimum growth of *Farfantepenaeus aztecus* (Ives) was achieved by the addition of 1% LNA to the diet. In growth experiments with *P. monodon*, Chen & Tsai (1986) indicated a requirement for HUFA at 0.5-1% of the diet, while Rees *et al*. (1994) observed that postlarvae can grow well on an *Artemia* sp. diet with n-3 HUFA ranging from 12 to 22 mg/g dry weight. Xu *et al.* (1994) suggested that for *F. chinensis* the requirement for LNA may be between 0.7% and 1% of the diet, but once DHA was adequately provided in the diet (around 1%), growth, molt frequency and survival were significantly greater than in animals fed a diet with 1% LNA. They concluded that although n-6 FA like LOA and AA have beneficial effects on growth and survival, n-3 FA, especially DHA are the most potent EFA for this species.

For *L. vannamei*, Lim *et al.* (1997) evaluated the growth response and fatty acid composition of juvenile shrimp fed different dietary lipids. They found that menhaden oil, rich in n-3 HUFA, was the most nutritious for this species, and among plant oils, those rich in LNA had a higher nutritional value than those rich in LOA. They concluded that both n-6 and n-3 FA appear to be essential in the diet, although n-3 HUFA were required for maximum growth, feed efficiency, and survival.

In a 6-week growth trial, González-Félix *et al*. (2002b) evaluated the nutritional value and dietary requirement of LOA and LNA (0.25%, 0.5% or 1% of either fatty acid), including different ratios of LNA/LOA (1, 3 or 9 at a combined inclusion level of 0.5% of diet) for juvenile *L. vannamei*, and compared their nutritional value to that of n-3 HUFA in combination, which showed higher nutritional value by producing significantly ($P < 0.05$) higher final weight and weight gain. Neither LOA nor LNA, alone or in combination, improved growth significantly compared to shrimp fed the basal diet with only palmitic and stearic acids. Dietary requirements for LOA and LNA were not demonstrated under their experimental conditions. Later on, they evaluated the nutritional value of dietary n-3 and n-6 PUFA (e.g., LNA and LOA) and HUFA (e.g., AA, EPA, and DHA), as well as n-3 HUFA in combination, all supplemented at 0.5% of diet (González-Félix *et al*., 2002c). All HUFA showed higher nutritional value than PUFA and produced significantly ($P < 0.05$) higher final weight, weight gain, and total lipid in shrimp muscle (Fig. 1). Fatty acid profiles of shrimp tissues reflected the composition of the dietary lipids. In general, saturated FA were more abundant in the neutral factions, while PUFA and HUFA were more abundant in the polar fractions of tissues. Under their experimental conditions, *L. vannamei* appeared to be able to satisfy its EFA requirements with HUFA from either n-3 (e.g., EPA and DHA) or n-6 (e.g., AA) families, which showed higher nutritive value than PUFA; moreover, the requirement for LOA and LNA was once again, not demonstrated. In spite of reports in which FA of the n-3 family have greater essential values than those of the n-6 family for marine shrimp, *L. vannamei* might equally utilize n-3 and n-6 HUFA as the result of metabolic adaptations related to specific environmental conditions or feeding habits of this species, and possibly phylogenetic differences. In addition, there is evidence that eicosanoids derived from AA might be involved in the molting process of crustaceans and thus influence growth as well (Koskela, Greenwoog & Rothlisberg, 1992). Consequently, this study suggests that the EFA value for this species may be determined by chain length and degree of unsaturation, with long-chain HUFA having greater essential value than shorter-chain FA, regardless of family.

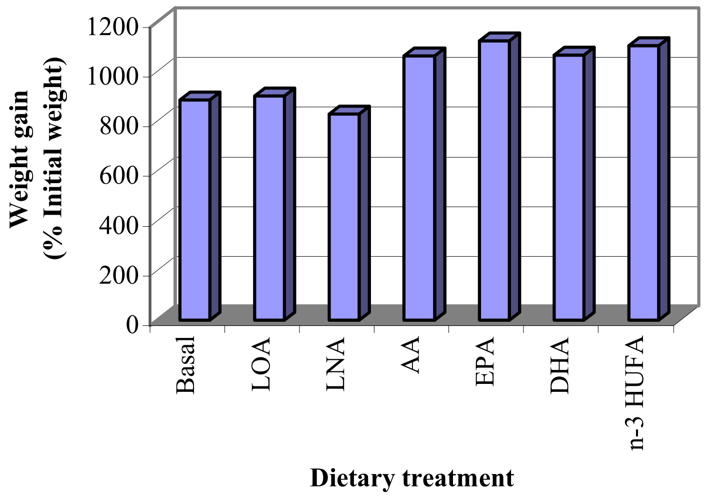

Figure 1. Weight gain of juvenile *L. vannamei* fed different polyunsaturated and highly unsaturated fatty acids.

Effect of dietary lipid level on quantitative EFA and their potential interaction

Studies with fish have indicated that requirements for EFA will vary depending on the supplementation level of dietary lipid (Takeuchi & Watanabe, 1977; Lochmann & Gatlin, 1993). The effect of dietary lipid level on quantitative requirements for EFA by juvenile *L. vannamei* was evaluated in a separate 6-week growth trial by González-Félix *et al*. (2002d). A factorial experiment with three dietary lipid levels (3, 6, and 9%) and three dietary levels (0.5, 1, and 2%) of n-3 HUFA in combination was conducted. An increase in quantitative requirements for EFA with increasing supplementation level of dietary lipid was not demonstrated, and no significant interactions were observed between the dietary factors. *L. vannamei* appeared to be able to satisfy its n-3 HUFA requirements when they were supplied at 0.5% of diet; this dietary level might be even lowered. Depressed growth was observed in shrimp fed diets with n-3 HUFA supplemented at 2%. In addition, this study showed that increasing the dietary lipid level had an effect on lipid composition of shrimp, by increasing lipid deposition in hepatopancreas and muscle tissue, but without a significant effect on growth.

Quantitative lipid requirements for ovarian maturation of L. vannamei broodstock

Wouters *et al*. (2001) determined the quantitative lipid requirements for ovarian maturation of *L. vannamei* broodstock. They tested the effect of total dietary lipid level as well as the total dietary level of HUFA on gonadosomatic index (GSI), hepatosomatic index, survival, body weight, and haemolymphatic vitellogenin concentration. High total dietary lipid levels affected ovarian maturation in a negative way, but the lowest level tested, 8.1%, gave the highest GSI. Increasing total dietary level of HUFA led to increasing levels of HUFA in the ovaries; however, GSI was not affect by the dietary treatments. Total dietary levels of HUFA ranging from 0.6-2.7% thus, had no pronounced effect on ovarian maturation during the length of their study, 21 and 17 days post-ablation. Wouters *et al*. (1999a) had previously reported that *L. vannamei* fed with enriched *Artemia* biomass, and then with *Artemia* fed coconut oil (free of HUFA and cholesterol) instead of the enrichment product, showed a decrease in egg fertilization, repeat performance, and egg production per female, yet the maturation frequency was not affected. In addition, Wouters *et al.* (1999b) had reported that triacylglycerides (TAG) levels increased from 8.3% to 33.8% in *L. vannamei* ovaries, followed by a decrease to 20.6% in spent ovaries and a resultant level of 33.5% in nauplii. They suggested that TAG appear to be selectively incorporated into the eggs and constitute the principal energy source in eggs and nauplii to support embryiogenesis, hatching and naupliar development.

PHOSPHOLIPID REQUIREMENTS IN Litopenaeus vannamei

Quantitative requirements for PL also have been reported. The total PL content recommended by Akiyama, Dominy & Lawrence (1992) is of 2% of diet. Coutteau *et al.* (1996) reported that the growth response of early *L. vannamei* postlarvae was significantly improved by addition of 1.5% soybean PC to the diet. With increasing dietary level of soybean PC, higher proportions of 20:1n-9, 20:5n-3, and total n-6 PUFA were observed in the total FA of shrimp tissue. Cahu *et al*. (1994) observed that dietary PL levels affected PL concentration in *L. vannamei* eggs, and suggested that a broodstock diet should contain more that 2% PL in order to assure that 50% of the total egg lipids is represented by PL, and for maintaining high spawn frequency and fecundity.

Effect of different types of soybean lecithin and their dietary requirement

Gong *et al*. (2001) evaluated different types of soybean lecithin and their dietary requirement for juvenile *L. vannamei* in two 6-week growth trials. In the first trial they tested 1.5, 3, and 5% inclusion levels of type I lecithin (97.6% PL), and 1.5 and 3% levels of type II lecithin (71.4% PL). In the second trial 1, 2, and 4% inclusion levels of type I and II lecithin and 1 and 2% of type III lecithin (48.4% PL) were evaluated. There was no interaction between lecithin type and PL level on shrimp growth or survival. Shrimp growth increased with PL levels up to 3-5% of diet. No significant differences were observed for instantaneous growth rate (IGR) of shrimp fed different types of lecithin at the same inclusion level, and no effect of lecithin type and PL level on shrimp survival was observed. For juvenile *L. vannamei*, they recommended a supplementation level of PL from 3 to 5% of diet.

Effect of dietary PL and different neutral lipids and their potential interaction

González-Félix *et al.* (2002a) evaluated the effect of dietary PL and different neutral lipids, as well as their potential interaction, on growth, survival and fatty acid composition of hepatopancreas and muscle tissue of juvenile *L. vannamei*. The lipid sources tested were coconut, soybean, linseed, peanut, and menhaden oils. They observed no significant differences (at $P < 0.05$) among treatments for survival and no significant interactions between the effects of PL and oil type for any of the responses at the end of this 8-week feeding trial; however, shrimp fed diets containing PL obtained significantly higher final weight and IGR, and lower feed conversion ratios (FCR) than those fed diets containing the same oil and no PL. Shrimp fed diets containing menhaden oil, with and without PL, showed higher final weight and IGR, and lower FCR than the rest of the treatments. These diets had a larger variety of EFA and the highest percentage of AA, EPA and DHA, thus showing a higher nutritional value.

Effect of dietary PL on EFA requirements and their potential interaction

Given that PL might improve the effectiveness of EFA and reduce the quantitative requirements for HUFA, the effect of dietary PL on EFA requirements of juvenile *L. vannamei* and their potential interaction also was evaluated by González-Félix *et al*. (2002e) in a 6-week trial. A 3 x 3 factorial experiment was carried out with increasing levels of soybean lecithin as the dietary PL (0, 1.5, or 3% of diet). Three dietary levels (0, 0.25, or 0.5% of diet) of DHA or n-3 HUFA in combination were tested. No significant interactions between the effects of PL and DHA or n-3 HUFA on growth were detected under their experimental conditions; however, IGR of shrimp was enhanced by addition of either DHA or n-3 HUFA at 0.25% of diet. A higher dietary inclusion level (0.5% of diet) did not further improve growth, and appeared to have a detrimental effect on survival of shrimp. Also, an inclusion level of 3% dietary PL significantly improved IGR of shrimp, and did not show a significant effect on survival. PL supplementation did not consistently increase the proportion of PC or other PL in shrimp muscle. Their results corroborated the beneficial effects of dietary PL on growth of shrimp, as well as their requirement for DHA and n-3 HUFA to achieve maximum growth, although they were similar to those obtained by Kontara, Coutteau & Sorgeloos (1997), where postlarval *M. japonicus* showed increased growth with increasing levels of n-3 HUFA and soybean PC, without a significant interaction between PL and n-3 HUFA.

Effect of dietary PL and cholesterol and their potential interaction

Gong *et al.* (2000a) evaluated dietary requirements of juvenile *L. vannamei* for PL and cholesterol, and their potential interaction in a 6-week experiment. A significant interaction between dietary PL and cholesterol on growth of shrimp was observed. Growth was enhanced as the level of PL increased from 0 to 5% of diet, but as the level of dietary cholesterol increased from 0 to 0.4% of diet, the growth-promoting effect of PL diminished. The cholesterol requirement was estimated to be 0.35% of diet in the absence of supplemental PL. At 1.5% and 3% PL, dietary cholesterol requirements were reduced to 0.14% and 0.13% of diet, respectively. When PL were provided at 5% of diet, 0.05% dietary cholesterol was needed for optimal growth. A higher dietary PL also resulted in higher total lipid in hepatopancreas and lower total lipid in muscle. Gong *et al*. (2000b) also investigated the active components of soybean lecithin for juvenile *L. vannamei*. No dietary

PC requirement was evident based on shrimp growth and survival. Increasing purified PC in the diet decreased total lipid, free FA, and other PL levels in hepatopancreas and increased PC in muscle. However, other PL, mainly phosphatidylethanolamine (PE) and phosphatidylinositol (PI) showed significant effects on shrimp growth when PC was provided at 0.35% or 0.52% of diet. Additionally, no interaction between PC and cholesterol on shrimp growth, survival and feed conversion ratio (FCR) was observed. Compared with the apparent growth-enhancing effect of cholesterol, the effect of purified PC was negligible. With PC at 1.4% of diet, the presence of other PL from lecithin or 0.1% cholesterol significantly enhanced shrimp growth and FCR. Purified soybean PC showed different effects from lecithin on shrimp growth, lipid composition and relationship with dietary cholesterol. Beneficial effect of soybean lecithin was attributed to the presence of PL other than PC.

CONCLUSIONS

Through experimental research we have learned that HUFA, PL, and other lipids are required to achieve maximum growth, feed efficiency, and survival of shrimp. However, the establishment of quantitative requirements for essential lipids by many species currently cultured throughout the world is still under way. Such is the case of *L. vannamei*, whose nutritional requirements during early development, juvenile stage, and adulthood are not completely defined.

When studying dietary requirements for EFA, emphasis should be made in the need for investigating their interactions and metabolism; for instance, competitive interactions between the n-3 and n-6 series of FA for Δ-6 desaturase, or between FA for a given elongase. Because of these competitive interactions among and between FA, the need for investigating appropriate dietary ratios of FA arises, since feeding inadequate proportions of FA to shrimp may result in biochemical and metabolic imbalances and less fit animals. It is relevant to mention that biochemical studies can provide further insight into nutrients' metabolic pathways of shrimp, and supply helpful tools to estimate their requirements. Another interaction that should be addressed is that between EFA and other nutrients such as PL. Research using known PL sources, for example, purified PL of a particular class and known composition of FA, will help understand their metabolic role, and their importance in contributing EFA to the organism. The optimal level of neutral (e.g., TAG) and polar (e.g., PL) lipids in shrimp diets and their digestibility also should be addressed in further investigations. Developing standard purified or semi-purified experimental diets is necessary to approach these investigations; this would allow fair comparisons of experimental results. These investigations can be pursued while searching for alternative lipid sources to exploit in the aquaculture industry.

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