CHAPTER II

LITERATURE REVIEW

Penaeus monodon

Life history of *P. monodon* is classified into six phases: embryo, larva, juvenile, adolescent, subadult and adult. Its color is dark brown to blackish. Their food consist mainly of small crustacean, mollusk and annelids. The adult is predator of slow moving benthic microinvertibrates. The tiger prawn is relatively eurythermal and euryhaline and grows rapidly to a marketable size. Its life span is approximately one and a half to two years (Motoh, 1979)

Larva development of *P. monodon*

Development of larval stages of *P. monodon* consists of 4 substages including 6 nauplius, 3 protozoea, 3 mysis and 3-4 megalopa substages or postlarvae **(Figure 1).** Time of development for each stage requires about 1.5 days, 5 days, 4-5 days and 6-15 days, respectively (Motoh, 1979, 1984). Larva exhibit planktonic offshore behavior with antennal propulsion for swimming in nauplius, antennal and thoracic propulsion in mysis and abdominal propulsion in postlarvae. Normally, there is no feeding requirement in the nauplius stage because they utilize yolk granule in their body. Phytoplankton, such as *Chaetoceros* sp., *Skeletonema* sp. and *Tetraselmis* sp. are introduced from the first zoea to the second mysis. Zooplankton such as, rotifer and

Artemia sp. are fed from the third mysis to postlarva (Motoh, 1979). Sometimes, it is necessary to provide artificial food to the larva when natural food is insufficient or the quality of natural diet is poor.
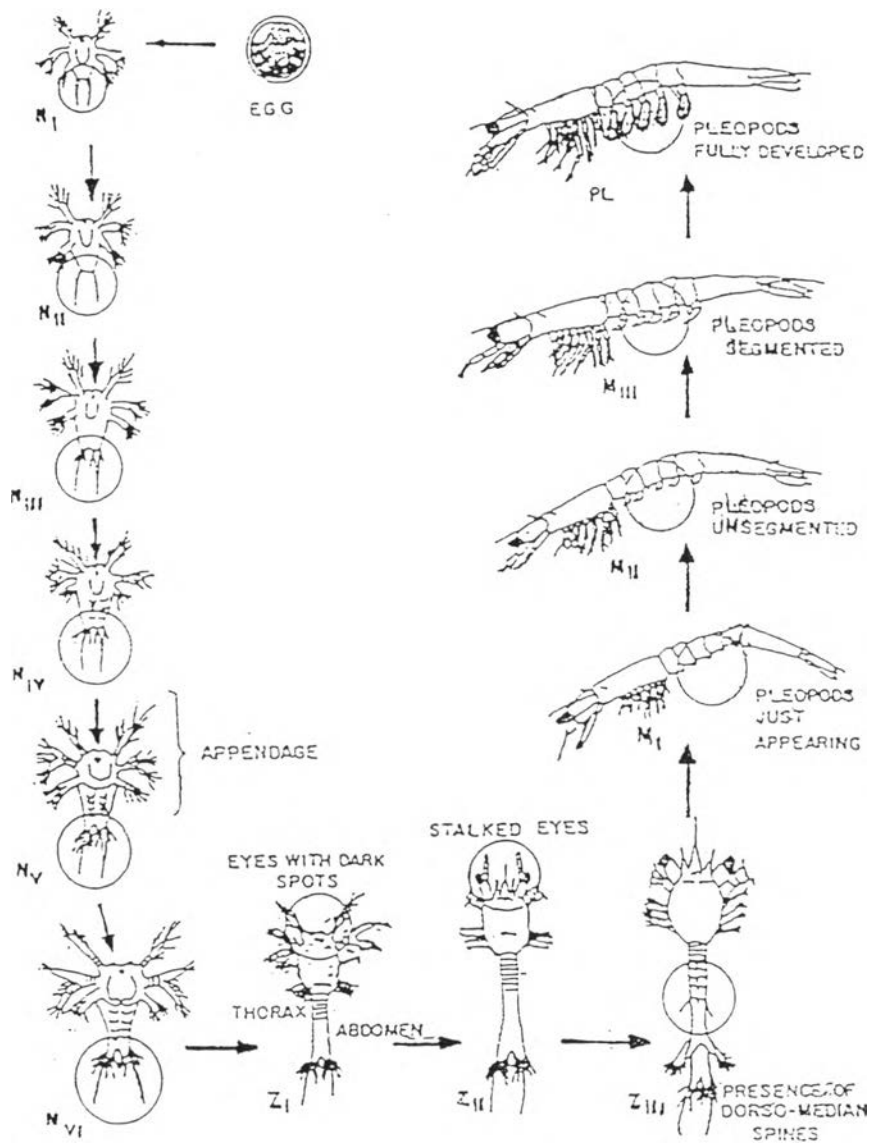
Figure 1 Larval stages of *P. monodon* Motoh, 1979

N = Nauplius stage, Z = Zoea stage, M = Mysis stage

and PL = Postlarva

Artificial feed for shrimp larvae

Many natural food especially algae eg. phytoplankton and zooplankton are suitable in size and quality of nutrition for shrimp larvae. However, they still have several disadvantages. The production of natural foods depend much on environmental factors especially light and temperature. Furthermore, It is quite difficult to control environmental factors for mass culture of algae, the major natural food for shrimp larvae. It also needs space for culture and stock maintenance. Artificial food is thus an alternative. It is easy to produce, to control the quality and to store. In order to standardize the artificial food for shrimp culture, the knowledges on nutritional requirements of larval stages of shrimp have been widely investigated. Several purified particulate diets have been developed and used as standard reference diets in many nutritional studies. Good attempts in this respect for crustaceans have been made by Kanazawa and Castell in 1990 (Lavens et al., 1992).

Microparticulated artificial diets as partial or complete replacements for live algae or zooplankton have been tested in marine cultured animals (Chu et al., 1987; Jones, Kurmaly and Arshad, 1987; Laing, 1987). The rationales are that the nutritional composition can be controlled, feed costs and maintenance can be fixed and/or reduced. In prawn larvae, an artificial diet is proven to successfully promote growth from zoea to postlarval stage but yet their survival rate was lower and more variable (Jones et al., 1987). The authors commented that the artificial diet could not be completely substituted for live algae or *Artemia* sp.

The continuing effort concerning artifficial food for improving animal performance (growth, survival and feed efficiency) in aquaculture has stimulated a search for new supplements for shrimp feeds. A dietary requirement for essential fatty acids and phospholipids have been evaluated for most penaeids (Kanazawa, 1982). It

has been recognized that penaeids express their inability to synthesize *de novo* specific fatty acids of the n-3 and n-6 series as well as sterols from acetate and have the slow rate of phospholipids biosynthesis. Hence those mentioned lipid nutrients have to be presented in the diet (Tacon, 1987). Fats and oils are known as accessible sources of those nutrients and their level of inclusion in diets is directed to meet the requirements for those specific nutrients (D' Abramo, 1989).

In diet formulations, an optimum dietary ratio between the n-3 type essential fatty acids (generally marine oils) and other fatty acids, notably those of n-6 type essential fatty acids (most abundant in plant oils) has been emphasized. Unsaturated oils with less energy values than saturated ones are generally included in penaeid diets (Wiseman, 1991). It has been suggested that food energy is partitioned into survival, molting and growth, with priority placed on survival (Knowlton, 1974). Most nutritional research in penaeid larvae has focused on lipid levels particularly highly unsaturated fatty acid (HUFA) with increased survival and growth abserved after feeding with n-3 HUFA-enriched diets (Sorgeloos and Le'ger, 1992; Coutteau et al., 1996; Chen, 1993). The phosphatidylcholine or lecithin derived from soybean containing high proportions of n-6 and n-3 fatty acids (in form of C18:3n-3) as constituents are thought to be effective in improving growth and survival of shrimp larvae (Kanazawa et al., 1985).

Lecithin

The name "lecithin" was obtained from the phosphatides were first observed in egg yolk in 1846 by Maurice Gobley (hence the name, from the Greek *lekithos*, meaning egg yok which later was renamed to lecithin). Actually, lecithin has a variety of meanings in both general and scientific literature. In scientific definition, lecithin is

the common name of phosphatidylcholine **(Figure 2)** which was the natural mixture of neutral and polar lipid from animal or vegetable sources. Neutral lipids are mainly triglycerides whereas polar lipids consists of glycolipids (lipid containing sugar) and phospholipids (those containing phosphorus) which phosphatidylcholine is the major phospholipids.

In principle, lecithin can be obtained from all kinds of living matter, as their constituents are essential component of cell membrane. This means that lecithins can be produced from vegetable, animal or even microbial sources. But in practical commercial terms, they are made basically from vegetable products like soybean, sunflower or rapeseed, with corn and groundnuts are among minor importance (Schneider, 1992)
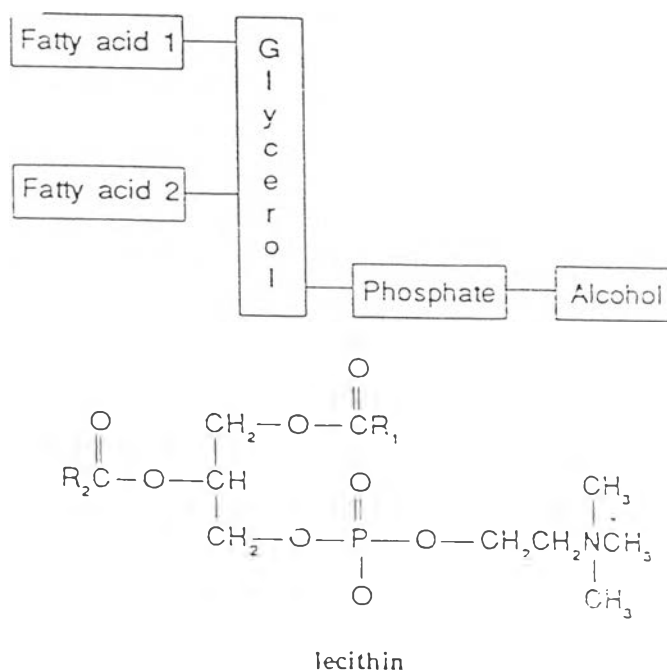
lecithin

Figure 2 Structure of lecithin (phosphatidylcholine) which has a glycerol molecule

as the basic component to which phosphoric acid is esterified at C-3

position and two long chain fatty acids are esterified at C-1 and C-2

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A. Physical Properties

Commercial lecithin is brown to light yellow in color, depending on whether it is unbleached or bleached. When properly refined, it has practically no odor and has bland taste. In consistency, it may vary from plastic to fluid. It is soluble in aliphatic and aromatic hydrocarbons, including the halogenated hydrocarbon. However, it is only partially soluble in aliphatic alcohols. Pure lecithin, phosphatidylcholine, is soluble in ethanol. In water, a particle of lecithin exhibits myelin growths, i.e., cylindrical sheets that are formed by bilayers and are separated by water and which may break up into liposomes (vehicles with a single bilayers of lipid enclosing an aqueous space). In some circumstances, phosphatides form multilamellar vesicles spontaneously. They are converted to the thinner, bilayer structure only upon treatment, e.g.., sonication. Like other antipolar, surface-active agents, the phosphatides are insoluble in polar solvents, e.g., ketones and, particularly, acetone. Acetone does, however, dissolve the triglyceride carrier and this difference in solubility furnishes a convenient means of separating, purifying, and estimating the phosphatides from neutral lipids.

Commercial lecithin is insoluble but infinitely dispersible in water. When commercial lecithin is mixed with water, it readily hydrates to a thick yellow emulsion (Othmer, 1981).

B. Functionality of phospholipids in crustacean

1. Source of membrane phospholipids

Several authors have suggested that larval stages are not capable of synthesizing phospholipids (PL) at a rate sufficient to meet the requirement for the formation of new cell components during the initially short period of rapid larval growth (Kanazawa, 1993; Geurden et al.,1995c). The difference in the activity among PL, with phosphatidylcholine (PC) generally being the most active compound, may then be explained by the specific need for incorporation into membranes, which consist mainly of PC. The present understanding of the metabolism of PL in fish and shrimp is mainly based on extrapolations from the knowledge of mammals (Sargent et al., 1993). It remains unclear whether essential fatty acids (EFA), which are preferentially esterified to the *sn*-2 position in PL, are assimilated as a single entity or separately from PL molecule. The latter may have important implications for the possible direct effect of the fatty acid composition of dietary PL on the composition and function of tissue PL.

2. Provision of choline, inositol, EFA and energy

Preferential catabolism of PC during embryonic and yolk sac stages has been observed in various species of marine fish with the PC presumed to be mainly a source of inorganic phosphate and choline (herring : Tocher et al., 1985), DHA (cod : Fraser et al., 1988) or metabolic energy (cod, halibut, and plaice : Rainuzzo et al., 1992).

Similarly, it has been postulated that dietary PL may serve as a direct source of nutrients for early feeding stages of fish and crustacean.

Various forms and concentrations of dietary choline were not as effective as lecithin in reducing molt death syndrome in juvenile lobsters fed casein-based diets (Conklin et al., 1980). Similarly, the beneficial effect of dietary PC and phosphatidylinositol (PI) on growth and survival of first-feeding crap larvae could not be replicated by the supplementation of choline or inositol (Geurden et al., 1995a; 1995c).

In one of their earlier works, Kanazawa et al. (1979) showed that the growth-promoting effects of 1% dietary clam phospholipids on juvenile *P. japonicus* could not be replicated by including an equal level of the fatty acids derived from this PL source in the diet. Similarly, the beneficial effects of dietary soybean lecithin on growth and survival of larval ayu could not be attributed to the content of EFA, which was low in soybean PL, whereas EFA were supplied in sufficient amounts in the diet (Kanazawa et al., 1981). The latter authors concluded that the growth-promoting effect of PL was due to certain effects of the molecular form of the PL rather than the EFA provided by them.

3. Improvement of diet properties

One hypothesis for the effectiveness of dietary supplementation of soybean lecithin in preventing molt death in lobsters fed casein-based diets is related to the reduction of leaching of water soluble nutrients, in particular manganese and vitamins B (Castell et al., 1991). The limit water stability of many semipurified diets which have been used to study PL requirements in larval and postlarval stages should inspire further exploration of this hypothesis in other species that have been reported to

require PL. The loss of total dry matter in casein-based microbound diets used for studies with postlarval penaeid shrimp was amounted to 40% after 10 min of exposure to water, and was not influenced by the addition of PL (Camara, 1994). Nevertheless, more detailed studies are needed to evaluate the effect of PL on the leaching rate of micronutrients.

Phospholipids have been shown to exert antioxidant (King et al., 1992; McEvoy et al., 1995) and feed attractant properties (Harada, 1987). It remains to be investigated whether these may account to some extent for the beneficial effects of PL supplementation to larval and juvenile diets.

4. PL emulsifying properties

Several authors have suggested that lecithin may be required as a surfactant for efficient lipid emulsification and digestion in early stages of crustaceans and fish larvae (Kanazawa et al., 1979; Conklin et al., 1980; Koven et al., 1993). In this way, Koven et al. (1993) reported a 7 fold increased of ^{14}C-oleic acid in lipids of 22-day-old *Sparus aurata* when fed a PL- or lecithin supplemented diet compared to a PL-free diet. They attributed this increased lipid uptake to the emulsifying function of the lecithin. However, various lines of evidence indicate that dietary PL improves growth and survival by other effects than the enhancement of emulsification and absorption of dietary lipid in the digestive tract. In a recent study with start-feeding crap larvae, it was shown that PL added to a casein-based diet as the sole lipid source enhanced growth in the same way as when added to the same diet supplemented with 4% neutral lipid (Geurden et al., 1995c). In the same study, it was shown that the *in vivo* emulsifying properties of dietary soybean lecithin could not explain the fish performance: Ca^{2+} enrichment of the soybean lecithin, which decreases its emulsifying

properties, did not affect its positive effect on growth and survival, whereas *sn-1* acyl lysolecithin, being a better oil / water emulsifier, gave poorer culture results than untreated soybean lecithin (Geurden et al., 1995c).

5. Role in lipid transport

Various findings support the hypothesis that dietary PL enhance the transport of lipid in crustacean, i.e., the export of absorbed lipids from the gut epithelium into the hemolymph, and the mobility of lipids between the various tissues and organs. The lack dietary lecithin in casein diets for juvenile lobster was associated with lower hemolymph levels of phospholipids and cholesterol (D' Abramo et al., 1982). Using ^{3}H-cholesterol, D' Abramo et al. (1985) found relatively higher levels of cholesterol in the midgut gland and lower hemolymph levels in lobsters fed diets without lecithin, presumably due to a decrease of the transport rate of cholesterol out of the midgut gland into the hemolymph. Lobsters fed lecithin-supplemented diets exhibited higher levels of serum and lipoprotein cholesterol than those fed lecithin-deficient diets regardless of whether crab protein and casein was used as protein source or not (Baum et al., 1990).   The latter finding showed that the impairment of cholesterol transport due to a shortage of lipoprotein-phospholipid-cholesterol complexes was not a possible cause of molt death syndrome observed in juvenile lobsters fed casein diets, but confirmed the role of lecithin in cholesterol transport. Using radioactively labelled tripalmitin and cholesterol, Teshima et al. (1986c; 1986d) showed that dietary PL improve the mobilization of cholesterol, and to a lesser extent, of triglycerides from the gut to the hepatopancreas, hemolymph and muscle in *P. jaconicus*. The latter authors hypothesized that the dietary PL, especially PC, may act as an acyl donor for the lecithin : cholesterol acyltransferase (LCAT) which

converts free cholesterol into sterol ester (Teshima et al., 1986d). In contrast to the above suggested enhancement of cholesterol availability by dietary PL, several researchers failed to demonstrate an interaction between lecithin and cholesterol requirement (*P. japonicus* : Teshima et al., 1982; *P. penicillatus* : Chen and Jenn, 1991; *M. rosenbergii* : Briggs et al., 1988).

Teshima et al. (1986c) assumed that dietary PL may provide specific lipid class as substrate for the formation of lipoproteins which are the main mediators of lipid transport in the hemolymph of shrimp and contain polar lipids as the main lipid component (Teshima and Kanazawa, 1980). Lipoproteins also play an essential role in lipid transport in the circulatory system of fish, and PC is the predominant polar lipid class in fish lipoproteins (Henderson and Tocher, 1987; Sheridan, 1988). Nevertheless, it remains to be shown to what extent dietary PL supply may influence the pathways for the synthesis of lipoproteins responsible for the transport of absorbed lipids.

6. Effect of PL on body lipid composition

The inclusion of PL in the diet affected lipid deposition, resulting in increased lipid retention and levels in the animal (Teshima et al., 1986a; Chen and Jenn, 1991; Takeuchi et al., 1992). Some controversies remain regarding the influence of the level of dietary PL on the lipid class composition of the animal. Larval *P. japonicus* feeding on a diet containing 3% soybean lecithin showed higher tissue level (% of wet weight) of sphingomyelin (SE), phosphatidylserine (PS), PC and PI compared to larvae fed on a PL deficient diet (Teshima et al., 1986c). In a similar study with juvenile *P. japonicus* , Teshima et al. (1986a) found increased body levels of PL, in particular PC, and cholesterol due to the supplementation of 3% soybean

lecithin in the diet. Increased total lipid levels in hepatopancreas and hemolymph in juvenile *P. japonicus* due to PL supplementation were found to be due to an increase of both neutral lipids, i.e. triglycerides and cholesterol, and polar lipids, mainly PC (Teshima et al., 1986b).

An interaction between the incorporation efficiency of dietary EFA and PL supplementation has been documented in various species. The proportions of n-3 HUFA in larval *P. japonicus* varied with the type of PL supplemented to the diet, with the highest and lowest proportion occuring in the larvae receiving soybean PC and soybean PI, respectively (Teshima et al., 1986e). Also, a higher proportion of EPA and DHA was observed in juvenile *P. japonicus* due to the addition of 3% of soybean lecithin in the diet (Teshima et al., 1986a). The better efficiency of dietary EFA due to PL supplementation is likely the basis for the interaction between EFA and PL requirements, as was demonstrated for larval *P. japonicus* by Kanazawa et al. (1985a).