

Nutritional Requirements of Commercially Important Shrimps in the Tropics

Mali Boonyaratpalin

Feed Quality Control and Development Division
National Inland Fisheries Institute
Jatujak, Bangkok 10900, Thailand

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Abstract

The culture of tropical shrimps has gained greater economic importance particularly among Southeast Asian countries. As shrimp farming moves from low-input, low-technology operation to the intensive and even highly intensive scales of operation, farmers are increasingly seeking ways to achieve more efficient means of production. Feed takes up the major portion of the cost of production. It also has a significant effect on the health of cultured species. Therefore, nutritional adequacy and cost-effectiveness of feeds are critical to the growth of the aquaculture industry. This paper discusses the nutritional requirements for growth as well as for health of some commercially important shrimp species in the tropics with emphasis on *P. monodon*.

Introduction

Techniques for the artificial propagation and culture of tiger shrimp, *Penaeus monodon*, have been established. In conjunction with the high market price and demand, the number of tiger shrimp farms and the production of the species have increased tremendously in the last few years in various Asian countries. However, problems still remain to be solved. Water quality is closely related to feed and feeding management. A less polluting diet which is low in nitrogen and phosphorus has to be developed. Also, nearly half of the factors that can be attributed to tiger shrimp diseases seem to be directly or indirectly related to nutritional adequacy of diets.

Penaeus monodon is the most important aquaculture shrimp species in the world. The technology to develop mature spawners, propagate larvae and post larvae, and grow juveniles to commercial size in relatively high density has been well established. Accordingly, artificial feeds of different forms and composition have also been developed and widely used in every phase of culture from larval rearing to broodstock maturation and spawning. However, when we review our understanding

of the nutritional requirements of this species and other marine shrimps, there is considerable limitation in the existing knowledge which hinders the successful development of nutritionally complete and cost-effective diets. Most of what is known about the nutritional requirements of tiger shrimp have been derived from the studies of *P. japonicus* which is believed to be more carnivorous than *P. monodon*. Dietary discrepancy due to species differences is obvious. However, until more studies are conducted to determine the nutritional requirements of *P. monodon*, feed formulations for this rapidly expanding shrimp culture industry would still be based largely on intuition and unknown premises from secret sources rather than nutritional science.

Protein

It has been reported that protein is an essential nutrient for prawns (Andrews *et al* 1972; Balazs *et al* 1973; Shewbart *et al* 1973; Venkataramiah *et al* 1975; Alava and Lim 1983). Protein, which is required for growth and maintenance, is an expensive component in a diet. Excessive protein levels will increase feed cost and nitrogenous waste. Therefore, knowledge of the optimum level of protein and the protein-sparing effects of non-protein nutrients such as lipids or carbohydrates would be effective in reducing feed costs and water pollution.

Various studies have been carried out in the last two decades to evaluate the protein requirement of *P. monodon* (Table 1). The variations are mainly due to differences in research methodologies such as shrimp size, physiological status, diet

Table 1. Suggested optimum protein levels for *Penaeus monodon*.

Shrimp size (g)	Protein source	Protein requirement (%)	Reference
0.5 -1.8	Casein and egg albumin	16	Lee 1971
0.5-0.6	White fish meal	35-40	Lin et al. 1982
0.6 - 0.8	Casein and gelatin	40-50	Bautista 1986
0.9	Casein	40-44	Shiau <i>et al</i> 1991
		36-40	Shiau and Chou 1991
1.3	Soybean meal	40	Alava and Lim 1983
	Squid meal		
	Fish meal		
	Shrimp meal		
	Casein		
Broodstock (60-120)	Fish meal	50-55	Millamena <i>et al</i> 1986
	Squid meal		
	Shrimp head meal		
	Soybean meal		

form and composition, and protein source. Studies using casein as the main protein source have shown optimum dietary protein levels close to those obtained using fish meal. Although there is a huge difference between the highest (55%) and the lowest (35%) suggested requirement levels, 40% seems to be a generally accepted optimum level.

Salinity is among the factors known to influence the protein requirement of shrimp. A recent study comparing the optimum dietary protein level for *P. monodon* reared in seawater and brackishwater gave an inverse relation between protein level and salinity. The optimum protein level for juvenile *P. monodon* raised in full-strength seawater (40%) has been studied by Shiau *et al.* (1991). When the energy content was increased, the optimum protein level for the shrimp raised in seawater could be effectively lowered to 36% (Shiau and Chou 1991). From previous results, we know that shrimp growth under high salinity is lower than that under low salinity. Although shrimp culture using seawater will benefit from a reduced protein requirement, thus a lower feed cost, its overall results will still have to be decided by the balance between reduced feed cost and decreased shrimp growth, plus other related factors. Manipulation of dietary protein alone may not improve the growth of shrimp raised in seawater. Increased supplementation of lipid may be helpful in supplying metabolic energy to enhance growth.

Digestibility of feed ingredients is the key factor in judging how the protein in ingredients can be properly incorporated in shrimp feeds. A study by Akiyama *et al.* (1988) using *P. vannamei* a relatively herbivorous species, indicated that dry matter and protein digestibility of feedstuffs used in purified or semi-purified test diets (such as casein) are usually better than those of feedstuffs used in commercial feed (such as fish meal) (Table 2).

Table 2. Apparent dry matter and protein digestibility for *Penaeus vannamei*

Major feedstuff in diet	Apparent dry matter digestibility	Apparent protein digestibility
Purified feedstuff		
Casein	91.4	99.1
Wheat gluten	85.4	98.0
Soy protein	84.1	96.4
Gelatin	85.2	97.3
Corn starch	68.3	81.1
Practical feedstuff		
Squid meal	68.9	79.7
Fish meal	64.3	80.7
Shrimp meal	56.8	74.6
Soybean meal	55.9	89.9
Rice bran	40.0	76.4

Akiyama *et al.* (1988)

Despite much discussion on protein requirement or optimum dietary protein level for shrimp or other animals, there is one basic concept in nutrition that can not be neglected: animals have requirements for amino acids rather than proteins. Unless we know the exact requirements for indispensable amino acids, trying to manipulate feedstuff proteins to achieve an efficient diet will be most likely fruitless. While relatively abundant knowledge concerning protein nutrition are available for *P. monodon* and other shrimp, there is very little data available concerning amino acid nutrition for successful commercial feed formulation.

Amino acid requirements

Substitution of high quality proteins with low quality proteins supplemented with crystalline amino acid does not enhance the growth of some fish (Cowey and Sargent 1979) and crustaceans (Deshimaru 1982) including penaeid shrimp. Deshimaru (1982) reported that the incorporation of arginine originating from dietary protein into muscle protein of *P. japonicus* was as high as 90.5%, whereas the assimilation of crystalline arginine into muscle protein was only 0.6% after six days of feeding. More than 50% of the arginine in an amino acid test diet (all amino acids in the diet were from a mixture of crystalline amino acids) was absorbed in the posterior part of the alimentary tract of *P. japonicus* (Deshimaru 1976). These results indicate that *P. japonicus* and probably other species of shrimp can effectively absorb crystalline amino acids from the alimentary tract, but do not effectively utilize them in muscle protein synthesis.

Among the possible explanations suggested for the failure of crystalline amino acids to enhance growth is the comparatively low efficiency of utilization of free amino acids (Cowey and Sargent 1979). It was hypothesized that crystalline amino acids used to supplement a protein are assimilated much more rapidly than those linked by peptide bonds in the dietary protein (protein-bound amino acids) (Cowey and Sargent 1979). Tissue concentration of these supplemental amino acids may thus be temporarily elevated and may be immediately catabolized rather than used for protein synthesis. If the hypothesis is true, reducing the rate of release of supplemental amino acids should be advantageous in promoting protein synthesis and thus growth.

In a recent study on juvenile *P. monodon*, Chen *et al.* (1992a) demonstrated that L-arginine microencapsulated with either cellulose acetate phthalate (CAP) or glycerol monostearate (GM) could be effectively added to the diet of the shrimp. Test diet containing supplementary CAP- or GM-arginine resulted in a significantly better growth and food conversion than diet supplemented with crystalline arginine alone. These results indicate that microencapsulation of arginine may have delayed the release of arginine in the gastro-intestinal tract of the shrimp. The delayed release, and thus absorption, may have increased the effective provision of arginine to shrimp juveniles. With the use of microencapsulated L-arginine, the quantitative requirement for the amino acid was determined (Chen *et al.* 1992b). The arginine level required for optimal growth, determined by a broken-line analysis of weight gain, was 25.0 g/kg diet or 54.7 g/kg protein.

The success with CAP-arginine in the diet of *P. monodon* means that this technique may be applied to study requirements for other essential amino acids for this and other shrimps. The simple process to microencapsulate crystalline amino acid with CAP also makes it possible to effectively supplement pure amino acids when low-quality proteins are used to replace fishmeal in shrimp feed. Based on the Chinese National Standard, the minimum arginine and methionine in *P. monodon* feed formulation are 2.15% and 0.8% of the diet, respectively.

Carbohydrates

Carbohydrate is the most economical dietary energy source (cost/kcal). There is little information on the carbohydrate nutrition of prawns (New 1976, 1980; Kanazawa 1985). The types and levels of carbohydrate in the diet have been shown to affect the growth of *P. japonicus* (Deshimaru and Yone 1978; Abdel-Rahman *et al* 1979), *P. aztecus* (Andrews *et al* 1972) and *P. duorarum* (Sick and Andrews 1973). For juvenile *P. monodon*, Pascual *et al* (1983) observed a significant influence of different types and levels of carbohydrate in the diet on survival. Alava and Pascual (1987) indicated that *P. monodon* utilize trehalose and sucrose better than glucose.

Shiau (1992) reported that among *P. monodon* fed glucose, dextrin and starch diet, those fed starch showed the best weight gain followed by those fed dextrin and glucose (Table 3). Poor utilization of glucose by *P. monodon* found in the study is in general agreement with Alava and Pascual (1987). Furthermore, Shiau (1992) found that as the dietary starch level increased from 20 to 30% and dietary protein levels decreased from 40 to 30%, protein digestibility and dry matter digestibility were not affected ($P > 0.05$) but protein deposition significantly increased (Table 4). In contrast, weight gains were reduced ($P > 0.05$) when dextrin and glucose were used as carbohydrate sources (Table 3). It is reasonable to suggest that the optimal dietary protein level for *P. monodon* would be lower if starch were chosen as the carbohydrate source instead of glucose or dextrin. The poor ability to utilize glucose has also been reported for other penaeid species such as *P. japonicus* (Kitabayashi *et al* 1971b; Deshimaru and Yone 1978; Abdel-Rahman *et al* 1979), *P. setiferus* (Andrews *et al* 1972) and *P. duorarum* (Sick and Andrews 1973).

The mechanism of the poor utilization of glucose is not yet fully understood. Abdel-Rahman *et al* (1979) reported that serum glucose levels in *P. japonicus* increased rapidly after administration of glucose and remained at high levels for 24 hours. When the diet contained disaccharides, serum glucose increased to a maximum level at 3 hours and then decreased to a low level. The authors suggested that dietary glucose was quickly absorbed from the alimentary canal and released into the hemolymph resulting in a physiologically abnormal elevation of serum glucose concentration thereby impairing its utilization as an energy source. It was also found that serum glucose levels in *P. monodon* fed the diets containing glucose peaked faster than in shrimps fed the dextrin and starch diets.

Table 3. Means for weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival rate of *P. monodon* fed diets with different carbohydrate sources and levels.

Carbohydrate source	Initial wt. (g)	Final wt. (g)	Weight gain (%)	FER	PER rate	Survival (%)
40% Protein						
20% Glucose	0.51	1.57	207.52 ^d	0.38 ^d	0.71 ^e	55.77 ^c
20% Dextrin	0.59	2.73	370.99 ^{ab}	0.47 ^{bc}	1.05 ^{bc}	65.39 ^b
20% Starch	0.57	2.86	408.17 ^a	0.50 ^{ab}	1.11 ^b	64.42 ^b
35% Protein						
25% Glucose	0.51	1.69	232.38 ^d	0.35 ^d	0.91 ^{cd}	47.29 ^c
25% Dextrin	0.53	2.27	328.99 ^{bc}	0.44 ^{bc}	1.16 ^b	75.00 ^{ab}
25% Starch	0.56	2.73	388.71 ^{ab}	0.48 ^{ab}	1.25 ^b	80.81 ^a
30% Protein						
30% Glucose	0.49	1.23	152.44 ^e	0.26 ^e	0.76 ^{de}	55.94 ^c
30% Dextrin	0.56	2.09	272.68 ^{cd}	0.40 ^{cd}	1.20 ^b	71.55 ^{ab}
30% Starch	0.58	2.80	387.36 ^{ab}	0.54 ^a	1.51 ^a	74.36 ^{ab}

Column means having a common superscript are not significantly different ($P > 0.05$).
From: Shiau 1992

Table 4. Apparent digestibility (%) and protein deposition (%) of *P. monodon* fed different levels and sources of carbohydrates.

Carbohydrate source	Protein digestibility	Dry matter digestibility	Protein deposition
40% Protein			
20% Glucose	78.77 ^b	60.82 ^c	12.09 ^f
20% Dextrin	83.78 ^{ab}	68.01 ^{ab}	18.14 ^{cd}
20% Starch	86.30 ^a	72.68 ^a	20.33 ^{bc}
35% Protein			
25% Glucose	78.07 ^b	65.99 ^b	16.13 ^d
25% Dextrin	83.83 ^{ab}	67.62 ^{ab}	20.44 ^{bc}
25% Starch	81.46 ^{ab}	67.42 ^{ab}	23.22 ^b
30% Protein			
30% Glucose	77.60 ^b	64.85 ^b	13.17 ^{ef}
30% Dextrin	80.55 ^{ab}	70.94 ^{ab}	20.90 ^{bc}
30% Starch	79.85 ^{ab}	66.84 ^{ab}	28.69 ^a

Column means having a common superscript are not significantly different ($P > 0.05$).
From: Shiau 1992

Another possible factor that may relate to the poor growth of prawns fed glucose-containing diet is the inhibition of amino acid absorption in the intestine by the presence of glucose (Alvarado and Robinson 1979). Hokazono *et al.* (1979) reported that the presence of 10M glucose reduced the uptake of L-lysine from 26.64 to 12.34% in the mid-intestine and from 23.24 to 5.4% in the posterior intestine of the rainbow trout.

Lipids

Lipids refer to a group of fat-soluble compounds found in the tissues of plants and animals and are classified as fat, phospholipids, sphingomyelin, waxes, and sterols. Lipids are important sources of metabolic energy. In this respect, dietary lipids may be used to spare the more valuable protein for growth. In particular, free fatty acids derived from triglycerides are the major energy source for metabolism in the muscle.

Lipids provide essential fatty acids which are essential for the maintenance of the integrity of cellular membranes required for optimal lipid transport (bound to phospholipids as emulsifying agents) and are precursors of the prostaglandin hormones. They are also a source of essential steroids, which in turn perform a wide range of important biological functions. From a viewpoint of feed technology, lipids act as lubricants for the passage of feed through pellet die, as substances which reduce the dustiness of feeds, and as enhancer of feed palatability.

Factors such as salinity, temperature, dietary lipids, and sexual maturity affect the fatty acid composition of fresh fish or shrimp. The fatty acid patterns among crustaceans in freshwater and marine environments tend to be similar to those of fish. Linoleic acid (18:2n-6) and n-6 polyunsaturated fatty acids tend to be the predominant fatty acids in freshwater shrimp while linolenic acid (18:3n-3) and 20 and 22 carbon, n-3 highly unsaturated fatty acids predominate in marine crustaceans (Table 5). Crustacean lipids exhibit a similar response to changes in environmental temperature. Both desaturation and chain elongation increase with a decrease *in* temperature.

The influence of dietary lipids on fatty acid composition of *P. setiferus* fed a diet high in 18:2n-6 and low in 20 and 22 carbon HUFA was apparent after one month (Table 6). Specific retention of n-6 fatty acids was evident. The n-6/n-3 ratio changed from 1:1.8 to 1.6:1 after feeding shrimp diet with n-6/n-3 ratio of 4.5:1.

Hayashi (1976) reported higher concentration of n-3 fatty acids in the ovary than in the body of adult crustaceans (Table 7). These indicate the trend of dietary n-3 HUFA requirement for marine shrimp and for broodstock.

Lipid requirement

A study showed that growth of *P. monodon* was significantly enhanced by cod liver oil at 12%, compared with 4% and 8%. At the same dietary levels tested, soybean oil and corn oil were good sources of lipid next to cod liver oil, while coconut oil, pork lard, and beef tallow were poor lipid sources (Catacutan 1991). Further, Sheen and Chen (1992) reported that *P. monodon* fed a diet containing 8, 10, or 12%

Table 5. Fatty acid composition of marine and freshwater crustaceans (Hayashi 1976).

Fatty acid	Freshwater	Marine		
	<i>M. rosenbergii</i>	<i>P. japonicus</i>	<i>P. merguensis</i>	<i>P. monodon</i>
Sat	24.4	26.9	27.5	35.2
Mono	20.7	25.1	25.9	25.8
n-6	32.2	17.4	6.5	11.2
n-3	12.7	42.3	20.1	19.8
18C	60.0	35.0	28.0	33.0
20C	9.4	25.7	17.7	18.5
22C	3.2	14.8	14.6	9.8
n-6/n-3	2.56	0.41	0.23	0.53

Table 6. Effect of diet on fatty acid composition of *P. setiferus* (Bottino *et al.* 1980).

Fatty acid	Composition of <i>P. setiferus</i> lipid after		Diet
	0 month	1 month	
Sat	26.6	22.6	25.0
Mono	18.2	25.2	30.1
n-6	13.9	27.6	33.4
n-3	24.5	17.1	7.3
n-6/n-3	0.57	1.61	4.5

Table 7. Comparison of fatty acid patterns of whole body lipids with ovary lipids of several adult crustaceans (Hayashi 1976).

Fatty acid	<i>Pandalus borealis</i>		<i>Heptacarpus pandaloides</i>		<i>Spirontacaris</i> sp.	
	Whole	Ovary	Whole	Ovary	Whole	Ovary
Sat	42.0	25.7	334.8	26.6	31.0	24.1
Mono	51.0	59.5	50.3	57.8	54.1	54.6
n-6	1.1	2.5	3.6	2.7	3.5	4.3
n-3	5.8	12.2	11.2	12.9	11.3	17.0

Sheen and Chen (1992) reported that *P. monodon* fed a diet containing 8, 10, or 12% cod liver oil/com oil (2:1 w/w) mixture had significantly higher weight gain than those fed lower lipid levels. This shows that juvenile tiger shrimp do not require an absolute level of dietary lipid. Rather, the optimum amount of dietary lipid depends on the amount and quality of dietary protein, the level of available dietary energy, and satisfaction of specific fatty acid requirement. Millamena *et al.* (1986) found that cod liver oil was a better lipid source for the broodstock than soybean lecithin or a combination of cod liver oil and soybean lecithin at 1:1 ratio.

Essential fatty acid requirement

Kanazawa *et al.* (1979) have shown the absence of *de novo* synthesis of linoleic (18:2n-6), linolenic (18:3n-3), eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids from ¹⁴C acetate or ¹⁴C palmitic acid in *P. monodon*. This suggests that *P. monodon* require some of these fatty acids as essential nutrients. Catacutan (1991) reported that 2.6% dietary n-3 HUFA enhanced growth of juvenile *P. monodon*, but levels of 18:2n-6 higher than 5% have a negative effect. Kontara (1986) found that *P. monodon* larvae which received *Artemia* enriched with n-3 HUFA had higher growth and survival rate than shrimp larvae fed *Artemia* nauplii of the same strain. Millamena (1989) showed that the fatty acid composition of broodstock diet influenced the tissue fatty acid patterns and hatchability of eggs from pond-reared *P. monodon*. Females fed a diet high in 20:4n-6, 20:5n-3, and 22:6n-3 highly unsaturated fatty acid and n-3/n-6 fatty acid ratio gave higher percentages of fertilized eggs and higher hatching rate than females fed diets with high phospholipid and lower n-3 HUFA.

Catacutan (1991) and Millamena (1989) indicated that n-3 HUFA was an essential fatty acid for *P. monodon* and the requirement was estimated (from 15.03% n-3 HUFA in a 12.14% dietary lipid) to be 1.8-2.6% of the diet.

Cholesterol and phosphatidylcholine requirement

The effects of dietary phospholipids and cholesterol on growth and survival of juvenile *P. monodon* (initial, mean weight, 0.45 ± 0.14 g) were examined using purified soy phosphatidylcholine (80% purity) as the phospholipid. Three levels of supplemental cholesterol 0, 0.5, and 1% were tested in combination with four levels, (0, 1.25, 2.5 and 5%) of phosphatidylcholine. The results of the 4-week growth experiment indicated that dietary supplementation of phosphatidylcholine or cholesterol significantly improved weight gain but not survival of the shrimp. The effectiveness of cholesterol was not affected by the dietary inclusion of phosphatidylcholine and vice versa. The optimum supplemental levels for *P. monodon* juveniles were estimated to be 0.5% cholesterol and 1.25% phosphatidylcholine (Chen 1993).

Bile acids

Bile acids are formed by the combination of the amino acid glycine or taurine with cholic acid. They are concentrated in the liver, excreted by the liver into the bile, and passed on to the gastrointestinal tract (via the bile duct) where they act as important

soluble enzymes or lipase can act on the fat molecules and split them to facilitate fat absorption. Bile acids also facilitate the major excretion route of cholesterol.

There is no evidence of production of bile acids by crustaceans. This suggests that the metabolic processes of emulsification, digestion, and transport of lipids in crustaceans are unique. Lipid transport in shrimp is accomplished primarily by high density lipoprotein (HDL).

Carotenoid

Shrimp, like other crustaceans, entirely depend on their dietary supply of carotenoid. The major carotenoid found in crustacean tissues and responsible for typical color of *P. monodon* is astaxanthin (Latscha 1989; Boonyaratpalin *et al.* unpubl.; Howell and Matthews 1991). Nutritional deficiency with respect to carotenoids has been suggested as the cause of blue disease in farmed *P. monodon*. Although *P. monodon* can convert canthaxanthin to astaxanthin, the efficiency of conversion was only 2-3 to 1 or lower (Boonyaratpalin *et al.*, unpubl.). Distinction of color was developed in 15 days of feeding a diet containing 50-75 ppm astaxanthin.

Vitamins

Overall knowledge on vitamin requirements of *P. monodon* remains limited. The possible reasons for the gap are the relative newness of *P. monodon* culture and the absence of a well-defined deficiency syndrome for almost all vitamins probably due to the cannibalistic nature of the species enabling them to withdraw nutrients from their prey.

Factors that affect vitamin requirements are size, age, growth rate of shrimp, environmental conditions, and nutrient interrelationships. It has been reported that there are considerable losses of water-soluble vitamins during diet processing, storage, and exposure to seawater during feeding. Due to the slow eating behavior of shrimp, feed pellets are processed at a high temperature so that they remain stable in water for hours.

Vitamin requirements

All of the eight B-complex, water-soluble vitamins are known to be required by fish although the requirement for each has not been established for every cultured species (NRC 1981,1983). For *P. japonicus*, the suggested dietary requirement for thiamin is 60 mg/kg and pyridoxine, 120 mg/kg of diet (Deshimaru and Kuroki 1979). Requirements were estimated on the basis of differences in growth and a decline in whole animal tissue levels of the vitamin. Deficiencies of thiamin and pyridoxine in fish are often characterized by nervous disorders. Over a 12-week period, the growth rate of juvenile shrimp reared on the thiamin-deficient diet was only slightly reduced and survival was similar to those groups reared on the supplemented control diet. Total body thiamin content in shrimp reared on the deficient diet was 0.4 mg/kg. Shrimp provided with 60 mg thiamin/kg diet had a

deficient diet was 0.4 mg/kg. Shrimp provided with 60 mg thiamin/kg diet had a total body content of 1.0 mg thiamin/kg; tissues became saturated at 1.4 mg thiamin/kg as feed levels were increased to above 120 mg/kg diet. Lack of pyridoxine led to a decrease in both survival and growth. Tissue levels of shrimp grown on the deficient diet after 12 weeks was only 0.08 mg pyridoxine/kg of whole animal. Survival and growth were best at a pyridoxine-HCl level of 60 mg/kg feed. At this level, the vitamin content of the shrimp increased to 0.43 mg/kg shrimp. Doubling the dietary pyridoxine level saturated the total body level at 0.54 mg/kg, but growth did not improve.

In a 15-week feeding trial, juvenile *P. monodon* (initial mean weight, 0.13 ± 0.05 g) fed purified diets containing six levels (0, 12, 16, 20, 40, and 80 mg/kg diet) of supplemental riboflavin showed no significant differences in weight gains, feed conversion ratios, and survival. The riboflavin levels in (whole) shrimp body increased with increasing dietary levels and the reverse is true for hemolymph glutathione reductase activity coefficients. The dietary riboflavin required for optimum growth in *P. monodon* was found to be 20.3 mg/kg diet based on the broken-line analysis of body riboflavin data. Shrimp fed unsupplemented diet (riboflavin content of 0.48 mg/kg diet) did not show apparent deficiency signs, except for reduced ratios of shrimp head length to body length (Chen and Hwang 1992).

Kanazawa *et al.* (1976) reported that the provision of choline chloride at 600 mg/kg clearly improved growth and survival of *P. japonicus* juveniles. However, Deshimaru and Kuroki (1979) found that choline is not required even though they were working with similar size animals and somewhat similar diets. These different results suggest that shrimp may be able to synthesize choline under certain conditions. Many vertebrates which can synthesize choline require an additional dietary source during periods of rapid growth and when fed diets high in lipids. Some crustaceans are able to synthesize limited amounts of choline most likely via methylation of dietary ethanalamine as in the case of invertebrates (Bilinski 1962; D'Abramo and Baum 1981). Ultimately, understanding the choline requirement in penaeid shrimp will require an examination of the impact of dietary lipids as well as other dietary factors like methionine which may be involved in choline synthesis.

Myo-inositol is similar to choline in that it is an important constituent of cell membranes and may have some lipotropic activity. Two groups of Japanese investigators (Deshimaru and Kuroki 1976; Kanazawa *et al.* 1976) agreed on the requirement of the shrimp for substantial amounts (above 400 mg/kg). Inclusion of inositol at 2,000 mg/kg of diet maximized both growth and survival of *P. japonicus* (Kanazawa *et al.* 1976). One uncertainty with regard to inositol is its availability. Plant materials, particularly seeds, contain a high level of inositol but it tends to be complexed as phytic acid, an indigestible form for non-ruminant farm animals. In contrast, Vanderzant (1963) found that phytic acid is readily utilized as a choline source by boll weevil larvae. Insects have also been used to examine the value of inositol as a carbohydrate energy source. Even though closely related to glucose,

It would be of value to know how closely shrimp resembles insects with respect to the digestion and utilization of this nutrient. While the dietary requirements for choline and inositol have yet to be confirmed, it is clear that addition of phospholipids containing choline (phosphatidylcholine) as well as inositol (phosphatidylinositol) in shrimp diet is beneficial.

Vitamin C is particularly known for its involvement in hydroxyproline and collagen formation in shrimp. While collagen is not the predominant structural element in shrimp which is an invertebrate, many of the vitamin C deficiency symptoms in *P. monodon* may also be related to insufficient synthesis of this protein. Evidence for impaired collagen formation resulting from a lack of dietary vitamin C was observed in *P. monodon* by Boonyaratpalin and Chaimongkol (1992). Lack of vitamin C in the diet caused black spot, opaque muscle, soft shell, unclosed antennal plate due to weak connective tissue, flipped gill covers, slow growth, incomplete molt, and high mortality in juveniles. The dietary ascorbic acid requirement was 12.6 mg/kg when ascorbyl phosphate (30 mg/kg) was used as vitamin source (Boonyaratpalin and Phongmaneerat, unpubl.).

Shiau and Jan (1992) estimated the optimum supplemental level of ascorbic acid for juvenile *P. monodon*. Seven test diets were prepared containing 0, 100, 250, 500, 1000, 2000 and 3000 mg L-ascorbate per 100 g diet. Weight gain was highest in shrimp fed the diet containing 250 mg. By broken-line analysis, the estimated dietary ascorbic acid requirement for maximal growth was 200.11 mg/100g diet.

The chemical nature of ascorbic acid contributes to the need for a very high level of vitamin C in *P. japonicus* feeds, 10,000-20,000 mg/kg diet (Guary *et al.* 1976) or 3,000 mg/kg (Deshimau and Kuroki 1976) and 1,000-2,000 mg/kg diet for *P. californiensis* and *P. stylirostris* (Lightner *et al.* 1979). Shigueno and Itoh (1988) found that normal growth of *P. japonicus* juveniles could be achieved with as little as 215 mg/kg diet of Mg-L-ascorbyl-2-phosphate. Therefore, vitamin C levels in the diet can be maintained if one of the stable ascorbic acid forms is used. The vitamin C requirement of shrimp is also affected largely by environmental conditions. An 8-week experiment in good environmental condition and stress condition showed that growth was not significantly different in shrimp fed diet with or without vitamin supplementation, but high mortality occurred in shrimp fed diet without vitamin C in stress condition. Therefore, the other possible role of ascorbic acid is related to stress and disease resistance.

Information on dietary or tissue ascorbic acid is not a reliable index for ascorbic acid status in feed or shrimp. At present, most assay methods are inaccurate and hardly replicable due to the instability of ascorbic acid in liquid. Moreover, most assays do not include evaluation of the possible contribution by ascorbate-2-sulfate. Kanazawa *et al.* (unpubl.) examined the requirements of larval *P. japonicus* for various vitamins by using microparticulated diets with carrageenan as binder. As a result, the shrimp larvae were found to require vitamin E, nicotinic acid, choline, pyridoxine, biotin, folic acid, ascorbic acid, cyanocobalamin, vitamin D, inositol, riboflavin, thiamine, and β -carotene. The deficiency of one of these vitamins resulted in the cessation or retardation of metamorphosis and high mortality during

larval development. Further studies have been done on the quantitative requirements of larval *P. japonicus* for several vitamins. The requirements for some vitamins such as ascorbic acid were apparently higher for *P. japonicus* larvae than for juveniles. It is conceivable, however, that some vitamins may have leached into the water before feeding. This means that the vitamin requirements of larval *P. japonicus* should be regarded as practical demand for rearing of the larvae.

Knowing the exact requirement for each of B-vitamins would be of little advantage to the shrimp culturist because the cost of these vitamins is relatively low compared with the overall cost of the feed. It is economically feasible to continue the present practice of providing generous amount of supplemental B-vitamins. The vitamin premix formulation for *P. japonicus* was successfully used in commercial rearing of *P. monodon*. The same can be applied to high-density culture systems in which the shrimps have limited access to other sources of these vitamins. There is no indication that levels should be increased above present amounts.

A potential problem with the above approach is the tendency to provide excessive amounts (just to be on the safe side). Although conventional wisdom suggests that toxicity is a concern only in fat-soluble vitamins, there are cases where decreased growth rates of shrimp and prawns were noted as dietary levels of water-soluble vitamins were raised beyond an optimum. Kitabayashi *et al* (1971) reported that an excess of vitamin C inhibited growth of *P. japonicus*. Doubling the recommended amount (120 mg/kg) of pyridoxine decreased growth performance of *P. japonicus* (Deshimaru and Kuroki 1979). Deletion of riboflavin from a vitamin mixture led to increased growth rates in juvenile freshwater prawn *Macrobrachium rosenbergii* (Heinen 1984).

Further research is needed in the case of choline and inositol and their interrelationship with lecithin because the requirements are not clear. Knowing the exact requirement and deficiency signs will provide flexibility in diet formulation and development of least-cost ration.

Minerals

It appears that shrimp can absorb some minerals from the water by drinking and by direct absorption via the gills, skin or both (Deshimaru *et al* 1978). Minerals serve as structural components of hard-tissue matrices (e.g., bone, fin, rays, scales, teeth, and exoskeleton) and components of soft tissues (e.g., sulfur in proteins, phosphorus in phospholipids, and nucleic acids). They are also components of metalloproteins (e.g., iron in hemoglobin, copper in hemocyanin, zinc in carboxypeptidase), and serve as cofactors and/or activators of a variety of enzymes (e.g., zinc activation of alkaline phosphatase). The more soluble minerals (calcium, phosphorus, sodium, potassium, and chloride) function in osmoregulation, acid-base balance, and production of membrane potentials. With the exception of osmoregulation, the maintenance of osmotic balance between body fluids and the water in which the animals live, the biochemical functions of minerals in aquatic species appear to be similar to those in terrestrial animals (Lovell 1989).

Calcium and phosphorus

The calcium requirement may be totally or partially met through absorption of calcium from the water (Deshimaru and Yone 1978). The low concentration of phosphorus in freshwater and seawater makes a dietary source of phosphorus potentially more critical. Dietary phosphorus requirement of 1% (Kitabayashi *et al.* 1971a), 1-2% (Kanazawa *et al.* 1984), and 2% (Deshimaru *et al.* 1978) have been recommended for *P. japonicus*. A dietary Ca:P ratio of 1:1 with 1% dietary Ca and 1% dietary phosphorus has been recommended for *P. monodon* (Bautista and Baticados 1989). The large disparity between dietary requirements of shrimp may be due to differences in the bioavailability of phosphorus from various sources and the dietary calcium levels.

It appears that calcium affects phosphorus availability and calcium levels in excess of 2.5 should be avoided. In general, bioavailability of minerals specially phosphorus has been found to be positively correlated with the solubility of the mineral in water. Monobasic phosphates of sodium or potassium are highly available (90-95%) sources of phosphorus for all species of fish and shrimp (Lovell 1978; Ogino *et al.* 1979; and Sakamoto and Yone 1979). Availability of the dibasic and tribasic calcium phosphate differs from species to species. For carp which lacks an acidic stomach, tribasic and dibasic calcium phosphate have an availability of only 13% and 46%, respectively. The optimum pH in shrimp stomach is 7.0-8.0, which suggests that utilization of di- and tricalcium phosphate is likely to be poor. Care should be taken to minimize the over supplementation of dietary phosphorus. Considering that there is a great concern on the deleterious effects of phosphorus from effluents of aquaculture facilities, minimizing phosphorus supplements would not only be economical but also a practical approach in reducing phosphorus pollution.

Sodium and chloride

In general, fish and shrimp are able to obtain enough Na and Cl from most feedstuffs in practical diets and seawater; therefore, sodium and chloride deficiency has not been demonstrated. However, in the red drum, adding dietary sodium chloride at low salinities may increase the absorption of amino acids and satisfy other metabolic needs which result in increased growth (Holsapple 1990). This draws attention to a study on the mineral requirement of *P. monodon* reared in low salinity.

Potassium

Kanazawa *et al.* (1984) reported that diets containing 0.9% potassium improved growth of *P. japonicus* compared with diet containing 1.8% potassium. Deshimaru and Yone (1978) recommended dietary supplementation of 1% potassium, based on the comparative growth of shrimp fed a diet without magnesium and potassium supplementation.

Magnesium

Deshimaru and Yone (1978) found that supplementation of 0.3% magnesium did not improve the nutritive value of semi-purified diet for *P. japonicus*. Kanazawa *et al.* (1984) re-evaluated the magnesium requirement of *P. japonicus* and reported that dietary supplementation of 0.1-0.5% magnesium improved the nutritive value of the diet. Davis (1990), utilizing semi-purified diets, found that the individual deletion of potassium did not result in a significant depression in tissue potassium or growth of *P. vannamei*. However, tissue level of magnesium was affected. This indicates a potential interaction between potassium and magnesium which has to be evaluated.

Copper and iron

Species utilizing copper as a component of their respiratory pigment may have an increased copper requirement over species utilizing iron-based respiratory pigments. Depledge (1989) estimated that, on a fresh-weight basis, 40% of the whole-body copper load in shrimp is found in hemocyanin. This suggests a considerable increase in the physiological demand for copper above the requirement of vertebrates. However, Kanazawa *et al.* (1984) found that the dual deletion of iron and copper had no significant effect on the growth and survival of *P. japonicus*. It should be noted that in this series of experiments, percent weight gain was very low (40%) and survival was very poor (57%); hence, the nutritional stress or quality of the diet may not have been adequate to induce a dietary deficiency. In addition to the physiological functions of copper, high levels of environmental copper have been found to be toxic to a variety of marine species (Bryan 1976).

Iron deficiencies in shrimp have not been observed (Deshimaru and Yone 1978; Kanazawa *et al.* 1984; Davis 1990). Excessive dietary supplementation of iron appears to have potential adverse effects on growth of *P. japonicus* (Deshimaru and Yone 1978; Kanazawa *et al.* 1984). In addition, iron-catalyzed lipid oxidation increases with iron supplementation which in turn adversely affects feed stability (Desjardins *et al.* 1987). Crustacean diets primarily contain polyunsaturated fats; therefore, the supplementation of ferrous iron to the diet could affect the stability of the diet through increased lipid oxidation (rancidity) and reduced stability of ascorbic acid (Hilton 1989).

The physiological necessity of iodine, manganese, selenium and zinc has not been evaluated in shrimp. In conclusion, the mineral requirement of shrimp might be higher than that of fish due to the repeated loss of certain minerals during molting. However, supplementation of practical diet with microminerals is generally not necessary.

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