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Vitamin requirements of juvenile Penaeid shrimp

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Abstract. — *The results of supplementing crustacean feeds with vitamins are examined specifically from the standpoint of shrimp culture. Micro-nutrients selected for discussion include : water-soluble vitamins of the B-complex, choline and inositol, vitamin C and the fat-soluble group of vitamins; A, D, E and K. Ways in which utilization of vitamins and ultimately dietary demand are altered by physiological state, conditions of culture, as well as factors which impact on feed levels, are explored.*

INTRODUCTION

Considering the commercial importance of penaeid shrimp culture there are surprisingly few specifics on the value of supplementing formulated feeds with vitamins. One possible reason for this lack is the relative newness of the field. While there was some scattered early work on general aspects of crustacean nutrition (see Beerstecher 1952; Fisher 1960), nutritional research focusing on shrimp and other large benthic crustaceans didn't start until the early 1970's with the advent of appropriate test diets (Kanazawa et *al.* 1970; Meyers and Zein-Eldin 1972; Castell and Budson 1974). A second, and probably more significant, reason for this lack of research is that it was unwarranted. In spite of early concerns that development of effective shrimp feeds would require a sophisticated understanding of crustacean vitamin requirements (New 1976), a variety of formulated feeds are available for commercial culture of juvenile shrimp (New 1987; Lim and Persyn 1989). Typically, these feeds use vitamin premixes derived directly from formulations used in vertebrate feeds and which are of unknown efficacy for shrimp. However, this uncertainty has not hindered the progress of commercial culture. Over the last few years, intensive pond culture of penaeid shrimp, which is reliant on addition of formulated feeds, has become major industry in several parts of the world. Specific problems which conceivably might have been related to vitamin deficiencies have not been noted and there was no sense of urgency about

detailed studies on requirements. As a consequence, overall knowledge regarding vitamin needs of shrimp and related crustaceans remains limited.

While reliance on vertebrate nutritional patterns has worked reasonably well, some caution is appropriate in that significant differences in metabolic capability do exist between these two groups of animals. With this in mind, the present review on vitamin requirements of juvenile shrimp has two goals. The first of these goals is to set the sparse details of shrimp vitamin requirements within the general vertebrate model and to add appropriate insights from studies with other arthropods, primarily insects. The second goal is to attempt to prioritize research thrusts needed to sustain future growth of the penaeid culture industry.

DEFINITION OF A VITAMIN

While once it may have been fairly easy to define a vitamin, this is no longer true. Originally these nutrients were identified by their ability to alleviate specific deficiency symptoms when fed to various mammals and birds. Although absolutely vital for various aspects of growth, health or reproduction, only minute amounts were required in the diet. This was explained by the idea that the role of these essential nutrients was limited to serving as co-factors for enzymatic reactions. A total of 15 compounds are typically considered vitamins. Most of these factors : thiamin, riboflavin, niacin, vitamin B6, pantothenate, folate, vitamin B12, biotin, choline, myo-inositol (inositol) and vitamin C (ascorbic acid) are water-soluble. The remaining four vitamins; A, D, E and K, are fat-soluble.

While they can still probably be classed as either water-soluble or fat-soluble, little else regarding the traditional view of vitamins remains uncomplicated as more and more is gleaned about their biochemical action. Originally, essentiality was thought to be a consequence of an early evolutionary loss leading to a total absence of synthetic ability in multicellular animals. It has been found, however, that the situation is a great deal more complex. Some vitamins, such as niacin, can be synthesized by a number of animals, although typically in insufficient quantities to meet physiological demand without some dietary input. For most terrestrial vertebrates ascorbic acid (vitamin C) is not a dietary requirement at all in that synthesis is more than sufficient to meet typical physiological demand. The role of these factors is also being expanded as some vitamins have been found to act in ways other than as co-factors. Vitamins such as choline and inositol have been shown to have an important function as structural elements of membranes, while vitamin D acts primarily as a hormone in regulating calcium levels in birds and mammals.

As these new aspects of vitamin nutrition have been elucidated it has become harder and harder to define this collection of compounds so as to exclude other nutrients. For example, in the case of shrimp cholesterol might easily be considered a vitamin in the traditional sense. An inability to synthesize the basic ring structure of this fat-soluble compound is characteristic of all crustaceans, insects, and other arthropods which have been tested. Because of this metabolic lesion, a dietary requirement for cholesterol characterizes the entire phylum Arthropoda (see Dadd 1983).

While it is not known to act as a co-factor, cholesterol is an important constituent of biological membranes in an analogous fashion to choline and inositol. This sterol also is needed in arthropods as a precursor of ecdysone, the molting hormone. In this respect the role of cholesterol is very similar to the relationship between cholecalciferol (vitamin D) and the various active metabolites of this molecule which actually regulate mineral levels in terrestrial vertebrates. While one might quibble that the dietary level of cholesterol needed by crustaceans is not minute enough to include it with the vitamins, this distinction weakens considerably if one considers the levels of ascorbic acid or the amount of choline containing phospholipid presently being used in some crustacean diets. True, there isn't a well defined deficiency syndrome for cholesterol but this is unfortunately true for almost all vitamins when it comes to crustaceans.

NUTRITIONAL UNIQUENESS OF SHRIMP

In the above context, it can be seen that the collection of dietary compounds commonly discussed as vitamins in relationship to crustacean nutrition are delineated in large part by tradition based on studies with vertebrates. Thus, it becomes important to consider how useful is this model in understanding nutrient needs of animals in the invertebrate phyla Arthropoda. One important question would be; are there other nutrients, like cholesterol, which are unique to the nutritional needs of shrimp and other crustaceans.

In answering the above question it is particularly useful to look at nutritional studies with other arthropods. While what is known about the nutrition of shrimp and related crustaceans may be somewhat limited, a host of insect species have been studied. In all the studies with various arthropods there is no hint of an additional unique nutrient requirement characteristic of the phylum. This is not to suggest that the potential for improving shrimp growth by focusing on crustacean metabolic needs should be ignored. For example while it is likely that crustaceans are able to synthesize glucosamine, the primary organic component of the exoskeleton, addition of shell waste, chitin or glucosamine to shrimp feeds generally has proven to be beneficial for growth. This boost in growth rate most likely reflects the advantages of sparing metabolic effort for the biosynthesis of glucosamine (see Conklin, 1983).

One point that is seen in examining work with insects is the possibility that individual species or somewhat larger taxonomic groupings can have unique nutrient requirements. For example Dadd (1983) pointed out that, in addition to the 10 amino acids which are typically essential for all animals, a number of other amino acids are either essential or growth promoting in the diet of many insect species. Dadd (1983) notes the crayfish *Astacus leptodactylus*, like some species of mosquito, is unable to synthesize the amino acid asparagine. Provasoli and D'Agostino (1969) found the brine shrimp *Artemia salina* required the amino acid putrescine. Other fairly unique requirements of at least some strains of *Artemia* are nucleic acids, a requirement shared with some species of flies (see Hernandorena 1983). Other possible nutrients which may be of interest in

this regard are; p-amino benzoic acid, ℓ-lipoic acid, glutathione, spermi-dine, and carnitine.

At the moment, there is no indication that any of the above possible requirements are required by penaeid shrimp. Fairly purified diets already exist for crustaceans (Kanazawa et al. 1970; Conklin et al. 1980; Kean et al. 1985) which support juvenile growth and survival. The required amount of any such unidentified nutrients would have to be quite low in order to be masked by unidentified contaminants. In that no requirement is seen with purified diets it is also unlikely that such a requirement would impact animals reared on practical diets containing a variety of complex feeds-tuffs. As will be discussed, however, shrimp and other marine decapods may be unique in their need for dietary phospholipids.

Tab. 1. — Recommended individual vitamin levels (mg/kg dry diet) for chick, trout, and shrimp feeds plus a summary of known dietary requirements for the shrimp (SDR).

Vitamin	Chick[a]	Trout[b]	Shrimp[c]	SDR
Thiamin	15	10	120	R[d]
Riboflavin	15	20	40	—
Pyridoxine	6	10	120	R
Pantothenic	20	40	100	—
Niacin	50	150	150	—
Folic acid	6	5	5	—
Biotin	6	1	1	—
Vit B—12	<0.1	0.1	< 0.1	—
Choline	1,000	3,000	600	?
Inositol	R	400	2,000	R
Vit C	R	100	10,000	R
Vit E	50	30	200	—
Vit A (IU)	4,500	2,500	5,000	—
Vit D (IU)	400	2,400	1,000	—
Vit K	1.5	10	40	—

[a] Standard purified reference diet for chicks (p. 48, NRC 1984)
[b] Recommended feed levels for coldwater fishes (p. 41, NRC 1981)
[c] Mix ≠4 developed for marine shrimp research (Kanazawa, see p. 156, New 1987)
[d] R = required; R = not required; ? = data contradictory; — = requirement not determined; see text for discussion.

While shrimp may share with insects a common requirement for cholesterol, in some other nutritional respects insects may not be a good model for these marine crustaceans. Dadd (1983) has pointed out that insects lack the heavily mineralized exoskeletons characteristic of crusta-ceans. Recent research also indicates that insects may be uniquely different from other invertebrates as well as vertebrates in that they do not use vitamin B12 dependent metabolic pathways (Halarnkar et al. 1987). Thus, in this case, the vertebrate model may be closer to that of crustaceans than the insect model. For many nutrient requirements, however, there may be little difference among animal phyla. This is particularly true of most of the so called B-complex vitamins : thiamin, riboflavin, niacin, vitamin B6, pantothenate, folate, and biotin. This is undoubtedly due to the fact that most central metabolic pathways are common to all multicellular orga-

nisms and as a consequence, there is a similar interaction between vitamin-dependent co-enzyme systems and cellular functions. This commonality of qualitative B-complex vitamin requirements of vertebrates and invertebrates was suggested quite early (Beerstecher, 1950) and has seemed to hold up. Certainly the similarities outweigh differences when comparing the nutritional needs of insects, the arthropod group which has been studied in the most detail, to those of vertebrates (Dadd 1983).

While useful in anticipating qualitative requirements of many micronutrients, the vertebrate model is limited when predicting quantitative needs. In general much higher levels of some vitamins are added in crustacean feeds compared to those levels recommended for other animals (see Table 1). How much of this difference in premix vitamin levels is related to metabolic demand in these two groups is unknown. There are a host of factors which are known to impact on metabolic demand in vertebrates, and probably in crustaceans as well, although the magnitude has yet to be defined. Even for normal metabolic needs within a species there is variation among individual animals in response to nutrient levels. In addition to normal biological variation, physiological state most certainly will modify demand. In many animals nutrient needs increase dramatically during periods of rapid growth. In shrimp and other crustaceans this will involve not only the building of tissue mass but uniquely the periodic process of molting. Reproduction in crustaceans, as in other animals which deposit extensive nutrient reserves into eggs for later use during embryonic development, also undoubtedly increases demand. Other changes in metabolic demand can be induced by changes in the diet of the animal. For example, increasing the lipid level in the diet increases the demand for vitamins that have an antioxidant function. The culturist can also impact the demand for vitamins by stressing the animals. Some of this stress may be purposeful such as increasing density while at other times this may be due to an inadvertent deterioration of optimal culture conditions. Infectious diseases and parasites have been shown to increase vitamin needs in many animals probably as part of a physiological response attempting to overcome these agents. Because of the variety of interaction impacting on metabolic demand, differences between vertebrates and crustaceans can not be evaluated as yet, however, studies with other animals suggests overall changes in physiological demand would be relatively minor.

A more likely explanation of the relatively greater need for vitamins in shrimp feeds would lie in the area of delivery. While nutritive levels added to the test feeds of terrestrial animals tend to be fairly close to actual metabolic demand this is not the case for crustaceans. In all animal culture systems there will be some delivery losses in attempting to provide nutrients which are both stable and yet readily available biologically. While actual, as opposed to formulated, levels of vitamins in feeds may vary depending on manufacturing processes and storage conditions such losses can be controlled to some extent through the use of proper temperature and humidity. However, even with freezing there is some loss of very labile nutrients such as ascorbic acid (Slinger et *al.*, 1979; Soliman et *al.*, 1987). Depending on the method of manufacture, temperature and moisture regimes necessary to develop stable pellets are such that large amounts of labile vitamins can be lost even though processing time is

relatively short. As these losses can be predicted based on experience, supplemental levels of nutrients high enough to compensate are added to the feed. In addition, a so-called extra margin of safety is usually included which increases the levels of vitamins two or three times above what is likely to be lost during processing. This final increase allows stated minimums on bag labels to be met with confidence even though analysis is not carried out on each lot.

Unique to aquaculture are the large losses from formulated diets resulting from leaching of water-soluble compounds (Goldblatt et al., 1979; Slinger et al., 1979). In that fish share the aquatic environment with crustaceans they face many of the same challenges in acquiring nutrients. Also in contrast to birds and mammals, fish lack any nutritional complexity that might be associated with maintaining a stable internal body temperature. Thus although there is a variety of information available to the crustacean nutritionist from studies with other animals, that derived from studies with fish is of particular value. Information on feed parameters such as leaching of water-soluble vitamins which relate directly to the aquatic environment can be quite useful to the shrimp nutritionist.

Tab. 2. — Vitamin levels (mg/kg dry weight) of selected organisms applicable as penaeid shrimp feeds.

Vitamin	Clams			Brine shrimp	
	a	b	c	adults[d]	cysts[e]
Thiamin	3	1	—	27	8
Riboflavin	12	14	12	17	25
Pyridoxine	4	—	—	8	—
Pantothenic	27	—	20	68	77
Niacin	65	49	98	130	116
Folic acid	2	3	—	1	—
Biotin	—	—	—	1	—
Vit B-12	0.5	0.6	3	3	—
Choline	2,900	—	—	6,100	—
Inositol	—	—	—	1,200	—
Vit C	550	350	—	49	—
Vit E	5	—	—	—	—
Vit A (IU)	3,700	7,500	17,000	14,000	37
Vit D (IU)	44	—	—	—	—
Vit K	—	—	—	—	—

[a] presented in Sidwell et al. 1975 (p. 3 + 9; clams, miscellaneous species) values converted assuming 80 % H_2O.
[b] presented in Sidwell 1981 (p. 379; Clam, Venus, Littleneck, Japanese) valves converted assuming 80 % H_2O.
[c] presented in Exler 1987 (p. 172; clam, mixed species, raw) values converted to dry weight[d]presented in Simpson et al. 1983 (p. 191; Gallagher and Brown 1975 and M. Gallagher, personal communication 1982).
[e] from Stults 1974 (p. 65) values converted to dry weight.

Losses due to leaching are of course proportional to the time it takes an organism to devour its food. Shrimp and other crustaceans, which are slow benthic feeders, require large amounts of water-soluble vitamins to be added to the feed in order to offset these heavy delivery losses. The magnitude of these losses can be seen by comparing the recommended feed

levels (Table 1) with levels representative of natural prey items. While direct quantification of the diet of shrimp in the wild has yet to be done, vitamin analyses for some live food organisms appropriate for shrimp rearing can be found in the literature (Table 2). Two important food items are clams and brine shrimp. Chopped pieces of fresh bivalve mollusks such as the short-necked clam *Venerupis philippinarum* have been found to provide an excellent source of nutrition for *Penaeus japonicus* juveniles (Deshimaru and Shigeno 1972) and some information on vitamin content is available. Brine shrimp, the small filter-feeding crustacean *Artemia salina* is used as food in rearing a wide variety of crustaceans as well as fish (Leger et al. 1986). Brine shrimp nauplii are used extensively in the rearing of larval shrimp. Unfortunately, specific information on nauplii is not available but the data from analysis of cysts is probably indicative of vitamin content at hatching.

In the following discussion it must be remembered that the amount of a vitamin added to experimental feeds must be sufficient to cover all the various avenues of loss in addition to meeting the metabolic needs of shrimp. These complexities which are seldom measured in conjunction with differences in culture techniques between laboratories probably explain the apparent wide range of requirement found in the case of some vitamins. This variability where several individual studies exist should serve as a note of caution in the cases where only a single study exists for a particular vitamin.

B-complex vitamins

All eight of the B-complex water-soluble vitamins are known to be required by fish although a requirement for each has not been established in every cultured species (NRC 1981, 1983). For the shrimp *Penaeus japonicus* vitamin requirements as indicated by studies with purified test diets have been suggested only in the case of thiamin, 60 mg/kg of diet and pyridoxine, 120 mg/kg (Deshimaru and Kuroki, 1979). Requirements were estimated on the basis of differences in growth and a decline in whole animal levels of the vitamin. Deficiencies of thiamin and pyridoxine in fish are often characterized by nervous disorders, however, no behavioral changes in shrimp were reported. Confirming studies by other investigators or studies with other species have yet to be carried out.

Over a 12 week period, the growth rate of juvenile shrimp reared on the thiamin-deficient diet was only slightly reduced and survival was equal to those groups reared on the supplemented control diet. Total body content of thiamin in shrimp reared on the 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 above 120 mg/kg diet.

A 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 feed level the vitamin content of the shrimp increased to 0.43 mg/kg shrimp. Doubling the feed levels saturated the total body level at 0.54 mg/kg but growth was not improved.

Requirements for the other B-complex vitamins have not been established. While it would be interesting to have this information in order to compare vitamin requirements among various groups of animals, its value in formulating practical shrimp feeds is limited. Even if the level of supplementation for some of these vitamins is marginal, deficiencies are unlikely because of other sources available to the animal.

Bacterial synthesis of vitamins in the gastrointestinal tract of some animals can result in substantial vitamin contributions. The level of bacterial synthesis in crustaceans has not been examined with regard to vitamin production. In general, compared with most mammals and birds, the gastrointestinal tracts of shrimp and other decapods of commercial interest are relatively simple and microbial synthesis may not provide appreciable quantities of vitamins. On the other hand, whatever amount produced is likely to be efficiently utilized in that delivery losses are eliminated. For aquatic organisms, microbiological growth on pelleted feed and other surfaces also can contribute nutrients even in relatively clean intensive systems. In pond growout systems extensive input of vitamins may be obtained through natural productivity. In the case of catfish no requirement for vitamin C could be established in pond fed fish until the density reached 4,000 kg/hectare (Lovell and Lim, 1978).

Even if the source of vitamins is limited to the feed, knowing the exact requirement for each of the B- complex vitamins would be of little advantage to the shrimp culturist. The role of these vitamins is primarily limited to that of co-factor and, although requirements can vary somewhat, the absolute amounts are still minute. In that the cost of these vitamins is relatively minor considering the overall cost of the feed it is economically feasible to continue the present practice of providing generous supplemental levels. Experience with fish suggests that this approach will work for shrimp feeds as well and there is no real concern with defining requirements any closer than what we have now. At least in the case of the B-complex vitamins the lack of further research would not appear to have any appreciable effect on future developments of the shrimp culture industry.

A potential problem with the above approach may be the tendency to provide excessive amounts -- « just to be on the safe side ». Although conventional wisdom would suggest toxicity is a concern only with fat-soluble vitamins it should be noted there are a number of cases in shrimp and prawns where decreased growth rates are noted as dietary levels of water-soluble vitamins are increased beyond an optimum. Kitabayashi et *al.*, 1971 reported 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). Heinen (1984) reported deletion of riboflavin from a vitamin mixture led to increased growth rates for juvenile freshwater prawns *Macrobrachium rosenbergii.*

Choline and Inositol

As most animals are able to synthesize either one or both of these nutrients, the possibility that shrimp require both choline and inositol is intriguing. This possibility, however, should be viewed with some caution

as information on these two vitamins is both limited and, in some cases, contradictory. The question of need is further confused by a possible dietary requirement for phospholipids containing choline or inositol rather than the vitamins by themselves.

Compared to other vitamins, the amount of choline found in biological tissue is relatively substantial. While there is no known co-factor function (Zeisel 1981), this trimethylated compound can serve as source of labile methyl groups in a number of enzymatic reactions and it serves as a precursor of acetylcholine, the neurotransmitter. As an element of phospholipids, lecithin and sphingomyelin, it is an important structural component of biological membranes. In crustaceans, phospholipids also serve as the primary lipid moiety of the hemolymph lipoprotein transport system (Lee and Puppione 1978; Teshima and Kanazawa 1980a).

Kanazawa and coworkers (1976) reported the provision of choline chloride at 600 mg/kg clearly improved growth and survival of *Penaeus japonicus* juveniles. However, later studies by Deshimaru and Kuroki (1979) found no need for choline even though they were working with similar sized animals and somewhat similar diets. These different results suggest shrimp may be able to synthesize choline under certain, as yet, undefined 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 at least limited amounts of choline most likely via methylation of dietary ethanolamine as is the case in vertebrates (Bilinski 1962; Shieh 1969; D'Abramo and Baum 1981). While the synthetic potential of shrimp has yet to be examined it remains a possible explanation for the variable results to date. 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 its synthesis.

Myo-inositol is similar to choline in that it is an important constituent of cell membranes and may have some lipotropic activity. In this case, both groups of Japanese investigators (Deshimaru and Kuroki 1976; Kanazawa et al. 1976). agreed on the need for substantial amounts (above 400 mg/kg) of this cyclic sugar in the diet of the Japanese shrimp. Inclusion of inositol at 2,000 mg/kg of diet maximized both growth and survival of *P. japonicus*. One unique uncertainty with regard to inositol and practical feeds is its availability. Plant material, particularly seeds, contain high levels of inositol, however, it tends to be complexed as phytic acid, an indigestible form for non-ruminant farm animals. On the other hand, Vanderzant (1963) found 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 this sugar is not utilized to any great extent by various insect species (see Dadd 1985). It would be of value to know how closely shrimp resemble insects with respect to the digestion and utilization of this nutrient.

While there is doubt as to the dietary requirement for choline and the requirement for inositol has yet to be confirmed, it is clear that additions of phospholipids containing choline (phosphatidylcholines) as well as inositol (phosphatidylinositols) to shrimp diets are beneficial.

Improved growth was first noted by Kanazawa and coworkers (1979) when they added a small amount (1 %) of the lecithin fraction obtained from the short-necked clam. Soy lecithin (commercial sources contain a mixture of phospholipids; predominantly phosphatidylcholines, phosphatidylethanolamines and phosphotidylinositols) has been found to be a useful source of these needed phospholipids for both larval and juvenile shrimp (Teshima et al. 1982, 1986a) as well as juvenile lobsters (Conklin et al. 1980; D'Abramo et al. 1981; Conklin et al. 1983). It is assumed that the beneficial effect of dietary phospholipids for penaeid shrimp and juvenile lobster reflects some intrinsically rate limited step in the synthesis of phospholipid number of studies have now been carried out detailing the role of these phospholipids in penaeid shrimp (see Teshima and Kanazawa 1980b; Kanazawa et al. 1985; Teshima et al. 1986a,b,c and d for details) and in homarid lobsters (see D'Abramo et al. 1982 and 1985). Basically, in these two marine crustaceans, dietary phospholipids have been shown to enhance the serum level of both phospholipid and cholesterol. While there is a slight improvement in uptake, work with radioisotopically labelled cholesterol suggests that the primary role of phospholipids is associated with serum transport of cholesterol from the midgut gland to other body tissues. The most effective phospholipids seem to be phosphatidylcholines and phosphatidylinositols containing polyunsaturated fatty acids. Lack of these particular phospholipids is thought to result in a cholesterol deficiency at the tissue level even though it may be present in the diet.

While research has highlighted the effect of certain phospholipids in enhancing cholesterol transport in shrimp and lobsters there are a number of aspects to their dietary role which remain unclear. To date, the dietary need for phospholipids appears unique to these two crustaceans, other vertebrates and insects being able to synthesize needed phospholipids. Also it should be noted that dietary lecithin has little or no effect on the growth, survival or cholesterol levels of freshwater prawn Macrobrachium (Hilton et al. 1984; Briggs et al. 1988). Recently it has been found that the phospholipid requirement varies depending on the protein component of the juvenile lobsters diet (Kean et al. 1985). When using casein as the primary protein component, absence of dietary phospholipids leads to greatly reduced serum cholesterol titer and to death of early juveniles at the time of molting. However, lack of lecithin has no effect on juvenile survival when purified crab protein is used as the primary amino acid source in place of casein. Addition of lecithin to the diets regardless of protein source still enhances cholesterol levels (Baum et al. submitted for pub.) indicating dietary lecithin in the lobster has several roles.

Vitamin C

Generally ascorbic acid synthesis has been thought to be characteristic only of higher vertebrates (amphibians through mammals). This traditional viewpoint developed as a result of enzymatic surveys (see Chatterjee et al. 1975) which extended results from dietary trials with man as well as laboratory and domestic animals. More recent work, however, challenges this paradigm and suggests a number of fish and invertebrates may also be capable of synthesis.

Ikeda and coworkers were the first to suggest fish could synthesize ascorbic acid following radioisotopic labelling experiments with common carp, *Cyprinus carpio* (Ikeda and Sato 1964) and later supported these results with enzymatic analysis. The enzyme gulonolactone oxidase (GLO) is the terminal enzymatic step in ascorbate synthesis and thus indicative of synthetic ability (Burns et *al.* 1956; Sato et *al.* 1976; Sato and Undenfriend 1978). Not only was GLO activity demonstrated in the carp but several other species as well including the goldfish *Carassius auratus* (Yamamoto et *al.* 1978). GLO activity in both carp and goldfish has been independently confirmed by other investigators (Thomas et *al.* 1985; Soliman et *al.* 1985). GLO activity among invertebrates has been noted by Wallace and coworkers (1985) in the horseshoe crab and synthesis of ascorbic acid has been indicated in *Homarus* using C-14 labelled glucose (Desjardins et *al.* 1985). Lightner and colleagues (1979) suggested limited synthesis was possible in penaeid shrimp. While it is premature to speculate on distribution of ascorbic acid synthesis, since relatively few finfish and shellfish species have been examined, this potential can no longer automatically be dismissed. Importantly, it should be noted that large scale phylogenetic patterns may not exist in that Soliman et *al.* (1985) found the ability to synthesize ascorbic acid could vary among species within a genus.

Although originally presence of GLO was thought to indicate vitamin C would not be required at all by a species, this premise should also be viewed with caution. Hanssen et *al.* (1979) demonstrated that even though the willow ptarmigan has high levels of GLO activity significant quantities of vitamin C are required in the diet of this bird to prevent deficiency symptoms. Although Sato and coworkers (1978) found the rate of synthesis in carp was sufficient to meet physiological demand under their conditions of culture this is not true for others (Kitamura et *al.* 1965; Dabrowski et *al.* 1988). The fact that *Tilapia aurea* requires vitamin C in its diet (Stickney et *al.* 1984) as does *Oreochromis mossambius* (Soliman et *al.* 1986b) suggests the reported GLO activity noted by Soliman and coworkers (1985) in these species also does not fulfill normal physiological requirements. Lightner et *al.* (1979) in discussing the potential for synthesis in *P. californiensis* and *P. stylirostris* suggested while physiological demands of adults could be met it was inadequate for rapidly growing juveniles.

Vitamin C is particularly known for its involvement in collagen formation. While collagen isn't the predominate structural element in shrimp it is in vertebrates, many of the vitamin C deficiency symptoms in shrimp may also be related to insufficient synthesis of this protein. Evidence for impaired collagen formation resulting from a lack of dietary vitamin C was seen in *P. californiensis, P. aztecus* and *P. stylirostris* juveniles by Lightner and colleagues (1979). Lack of collagen synthesis caused affected juveniles to suffer « black death », melanized lesions distributed throughout the collagenous tissue underlying the exoskeleton. Deshimaru and Kuroki (1976) reported symptoms of decolorization and development of an abnormal grayish-white color on the carapace margin, lower abdomen and tips of walking legs. A somewhat similar syndrome of black lesions along with deaths at the time of molt were reported for *Macrobrachium rosenbergii* juveniles (Heinen 1984).

Outside of the important role ascorbic acid plays in collagen formation, most of the mechanisms by which ascorbic acid acts in biological systems are poorly known (for a recent review see England and Seifter 1986). It is known that ascorbic acid is a co-factor in the synthesis of carnitine which is needed for effectively utilizing lipid stores for energy. Lack of carnitine may be involved in the reduced growth resulting from anorexia as a consequence of general lethargy. Recent work with fish has suggested that ascorbic acid is important in resistance to stress of various types including infection and in reproduction (see Halver et al. 1975; Hilton 1984; Lovell 1984; Sandnes 1984; Sandnes et al. 1984).

There is limited information on whole animal and tissue levels of ascorbic acid in shrimp (Guary et al. 1976; Magarelli and Colvin 1978). In examining the data of Guary et al (1976) it appears that hepatopancreatic levels below approximately 5.0 mg ascorbate/100 g wet tissue may be indicative of a deficiency as judged by reduced growth in P. japonicus juveniles. How much this might vary among different species is unknown. While this amount appears reasonable when compared to levels found in wild-caught crustaceans (blue crabs Callinectes sapidus and grass shrimp Palaemonetes pugio) as reported by Coglianese and Neff (1981) much higher levels have also been noted (Sinha and Mooswi 1978).

Information on tissue levels of ascorbic acid is further complicated by the fact that most assays did not include evaluation of possible contributions by ascorbate-2-sulfate (A2S). While this form is not efficacious for vertebrates requiring vitamin C, some fish species are able to utilize it. It is also used as a storage form of ascorbic acid in brine shrimp cysts (Mead and Finamore 1969; Golub and Finamore 1972). In salmonids, A2S was as effective as ascorbic acid itself in preventing the symptoms of vitamin C deficiency (Halver et al. 1975; Tucker and Halver 1984a and b, 1986). However, while salmonids can readily cleave the sulfate from ascorbic acid enzymatically (Benitez and Halver 1982), this may not be true of all fish (Tsujimura et al. 1981).

Suggested dietary levels for ascorbic acid in shrimp feeds are astounding compared to other animals. While the optimal amount of vitamin C in the diet of fish will of course depend on species, size, growth rate and culture conditions, generally a level of around 50 mg ascorbic acid/kg of diet is considered adequate (see Milliken 1982). Feed levels several orders of magnitude greater 10,000-20,000 mg/kg diet have been suggested for penaeid shrimp P. japonicus (Guary et al. 1976). Other results for this species are difficult to interpret. Deshimaru and Kuroki (1976) found, although best growth occurred with no added ascorbate, levels at least 3,000 mg/kg diet were needed to prevent mortalities. Lightner and coworkers (1979) found levels of 1,000-2,000 mg/kg diet were sufficient for P. californiensis and P. stylirostris but felt these species could be synthesizing part of their requirements.

The chemical nature of ascorbic acid undoubtedly contributes to the need for the very high levels of vitamin C in shrimp rations. Ascorbic acid is highly soluble in water and easily oxidized when in solution to dehydroascorbic acid. In biological systems such oxidation is easily reversed enzymatically with a glutathione dependent reductase (Hughes 1964; Yamamoto et al. 1977). This reductase allows for alleviation of

deficiency symptoms through the provision of either the oxidized or reduced from ascorbic acid. If dehydroascorbic acid is not reduced, however, further degradation results in the irreversible formation of diketogulonic acid and loss of vitamin activity. Such degradation is common under conditions routinely associated with the manufacture and storage of formulated feeds. In that ascorbic acid is both chemically labile and highly water-soluble provision of adequate dietary levels of vitamin C for cultured shrimp and other crustaceans has been of continuing concern. Loss of vitamin C activity in feeds due to processing, storage, and leaching can be a significant problem (Guary et al. 1976; Goldblatt et al. 1979; Hilton et al. 1977; Sandnes and Utne 1982; Slinger et al. 1979; Soliman et al. 1987). A number of approaches have been tried to reduce ascorbate loss (Murai et al. 1978; Brandt et al. 1985; Soliman et al. 1986a; Albrektsen et al. 1988), however, the general but not very satisfactory answer to this concern has been to supplement formulations used for intensive culture of fish and crustaceans with large amounts of ascorbic acid hoping a small percentage will reach the animals.

Vitamin C levels in the diet can be maintained much easier if one of the stable derivatives of ascorbic acid are used. While the potential advantages of ascorbate-2- sulfate have been pointed out by Halver and coworkers (Halver et al. 1975; Tucker and Halver 1984a and b, 1986) this form is not readily available commercially. Phosphates of ascorbate, however, seems to have a lot of potential (Shigueno and Itoh 1988; VTI 1988). Shigueno and Itoh (1988) found normal growth of *P. japonicus* juveniles could be achieved with as little as 215 mg/kg diet of Mg-L-ascorbyl-2-phosphate.

Fat-soluble vitamins

Predicting the probability of shrimp needing the fat-soluble vitamins; A, D, E and K is the most difficult task of all. Although the water flea *Moina macrocopa* was shown to require some fat-soluble vitamins when reared on an artificial media (Conklin and Provasoli 1977), little is known about the possible need for these vitamins in other crustaceans. Only a few insect species studied have required fat-soluble vitamins, however, the vast majority of insect studies have focused only on early juvenile growth and requirements for fat-soluble vitamins may have been missed (Dadd 1983). The vertebrate model may be of little value with regard to this group in that often these fat-soluble vitamins are involved in highly evolved regulatory systems for which there is no analog in crustaceans (Conklin 1983). Several authors have speculated that i-carotene or derived carotenoid may be an important dietary component of aquatic animals (Gilchrist and Lee 1972; Tacon 1981). Addition of β- carotene to the diet of the water flea *Moina macrocopa* enhanced production slightly (Conklin and Provasoli 1977) and Kanazawa (1985) has stated it is beneficial in larval diets for penaeid shrimp, on the other hand, no benefit has been noted with juvenile lobsters (D'Abramo et al. 1983). In that subtle behavior differences in feeding or sexual interactions expressed as reduced growth or reproduction would be difficult to distinguish from metabolic differences in response to a dietary lack of carotenoid interpretation of experiments involving these pigments is difficult. Firm evidence that carotenoids have

a vitamin function other than as a vitamin A precursor is lacking, however, carotenoids are clearly needed to maintain normal market appearance of shrimp and other aquatic animals (see Goodwin 1986).

It is likely that vitamin A is involved in the visual cycle of crustaceans. As most of vitamin A in arthropods is concentrated in the eyes, however, physiological demand to meet this need is likely be inconsequential (Conklin 1983). Marine fish oils are a good source of vitamin A and practical shrimp rations which incorporate these oils as a lipid source should meet this demand. The use of shrimp and other meals to enhance final pigmentation of the shrimp will provide another source of vitamin A.

Several authors have asserted that vitamin D is important in the diet of crustaceans (larval shrimp, *P. japonicus*; Kanazawa 1983, 1985 and juvenile lobsters, *H. americanus*; Stewart and Castell, 1979), however, details are not yet available. In mammals and birds, vitamin D metabolites regulate calcium levels through hormonal regulation of bone stores in order to maintain homeostatic tissue levels in the rest of the body. In that shrimp (Deshimaru et *al.* 1978) and other crustaceans (Hayes et *al.* 1962) apparently have ready access to significant amounts of dissolved calcium through the gills, the mammalian-avian model in the case of vitamin D would seem to provide little insight into the possible role of this vitamin in shrimp or other commercial decapods (Conklin 1983). Even in species of fish in which a dietary need has been shown (channel catfish, Lovell and Li, 1978; trout, Barnett et *al.* 1979) feed levels are quite low. In that marine fish oils and other marine feedstuffs used in practical crustacean feeds will have appreciable levels of vitamin D, additional supplementation is most likely to be unnecessary or at least superfluous.

Presently, there is no evidence that vitamin E has a specific vitamin function in shrimp, however, because of the importance of polyunsaturated fatty acids in the diets of crustaceans, it is assumed that vitamin E will be important as a metabolic antioxidant. With fish the requirement for vitamin E has been noted to increase when diets contain higher levels of polyunsaturated fatty acids (Watanabe et *al.* 1981 a and b; Cowey et *al.* 1983; Boggio et *al.* 1985). However, it is not always clear in these experiments if the diets were appropriately protected from oxidation during processing and storage. Fish oil rapidly undergo autoxidation if not protected with a synthetic antioxidant (Fritsche and Johnston 1988) and rancid oil may lead to a loss of vitamin E (Hung et *al.* 1981). In that natural levels of vitamin E in fish oils may not afford enough antioxidant protection, synthetic antioxidants such as ethoxyquin (Hung et *al.* 1981) or t-butylhydroquinone (Fritsche and Johnston 1988) should be added to these lipid sources upon receipt. Information as to the need for vitamin E in shrimp diets containing practical feedstuffs which have been appropriately protected with synthetic antioxidants would be of value particularly as changes are made in formulations with regard to ascorbic acid, another antioxidant source in feeds.

Finally, there is no evidence to date that the final fat-soluble factor, vitamin K is needed in the diet of crustaceans nor in any insect (see Conklin 1983). One amino acid absent from crustaceans is å-carboxyglutamic acid. Interestingly, the formation of this amino acid in vertebrates is uniquely vitamin K dependent. While most likely this vitamin is not

required by shrimp and other crustaceans, it apparently does no harm and is often included in the vitamin premixes.

CONCLUSION

Shrimp nutrition has clearly benefited from the information gained with other organisms particularly vertebrates. Effective diets have been formulated for commercial growout of juvenile shrimp even though specific requirements for vitamins are not known in any detail. While further research is indicated for several vitamin requirements studies detailing B-complex vitamins are not a high priority. Even for shrimp in high density culture systems which have limited access to other sources of these factors there is no indication levels should be increased above present amounts.

Further research is indicated in the case of choline and inositol. While it can be concluded that the use of soy lecithin which contains these two nutrients in shrimp diets enhances cholesterol utilization and growth rates, it is not clear that this is the only effect of this added phospholipids. Because of the uncertainties surrounding these two possible vitamins it is not possible to confidently replace all or part these phospholipids with an appropriate combination of other nutrients. This lack of flexibility in diet formulation remains a hinderance to future ration development.

Use of more stable forms of ascorbic acid will undoubtedly bring recommended feed levels more in line with other animal feeds as well as ensuring more reliability. Such changes may also inadvertently reduce antioxidant protection during diet manufacture and storage. Studies aimed at determining appropriate levels of synthetic antioxidants are important in insuring sensitive lipids as well as key vitamins are not degraded prior to consumption. There is increasing evidence that a number of vitamins such as C and E have a vital antioxidant role in protecting lipids in the tissue of aquatic animals (Cowey et *al.* 1985; Cowey 1986; Soliman et *al.* 1986; Sato et *al.* 1987). As interest of shrimp nutritionists moves on form juveniles to sexually mature adults and then larvae these antioxidant factors are likely to become even more important.

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