Use of Soybean Meal in the Diets of Marine Shrimp

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Introduction

Shrimp aquaculture presently produces approximately one million metric tons of shrimp annually. While some 20 species are cultured in various parts of the world, the majority of production is based on eight species (Table 1). For the eastern hemisphere, the fast growing giant tiger shrimp *Penaeus monodon* is the most important, while in the western hemisphere, the white shrimp *Litopenaeus vannamei* is the leading production species.

Shrimp have a complicated life cycle (Figure 1). Eggs from the female are broadcast into the marine environment. Hatching from the egg, the larvae pass through three distinct stages, nauplius, zoea and mysis, before assuming the distinctive adult morphology as post-larval or juvenile shrimp. The distinction between post-larva and juvenile is slight. Generally, the term post-larva is used for the first month and juvenile thereafter.

Depending on one's focus, shrimp aquaculture either started as trapping and holding of wild seed (Ling et al., 1977), or with the development of modern production techniques arising out of the research of the Japanese scientist Motosaku Fujinaga (Fast, 1992). The "trap and hold" approach requires little effort on the part of the farmer, but yields are low and unpredictable. Traditionally with this type of approach, the shrimp feed and grow on available pond organisms. In some cases where additional seed is sourced from the wild, supplementary feed may be added to the pond. Availability of seed is often the limiting factor for shrimp farmers using the "trap and hold" approach.

This bottleneck was partially bypassed by Fujinaga's development of methods allowing captive reproduction of shrimp, starting with gravid females obtained from the wild, and completion of the larval cycle in hatcheries.

Table 1: Major Farmed Shrimp Species

Species Name	Common Name	Former Species Name*
Farfantepenaeus aztecus	Northern brown shrimp	*Penaeus aztecus*
Farfantepenaeus californiensis	Yellowleg shrimp	*P. californiensis*
Fenneropenaeus chinensis	Chinese white shrimp	*P. chinensis*
Fenneropenaeus indicus	Indian white shrimp	*P. indicus*
Litopenaeus stylirostris	Western blue shrimp	*P. stylirostris*
Litopenaeus vannamei	Western white shrimp	*P. vannamei*
Marsupenaeus japonicus	Japanese kuruma prawn	*P. japonicus*
Penaeus monodon	Giant tiger shrimp	*No name change*

*The nomenclature of shrimp was recently revised (Farfante and Kensley, 1977). The previous names of the major farmed species are included for the benefit of the reader looking at older literature.

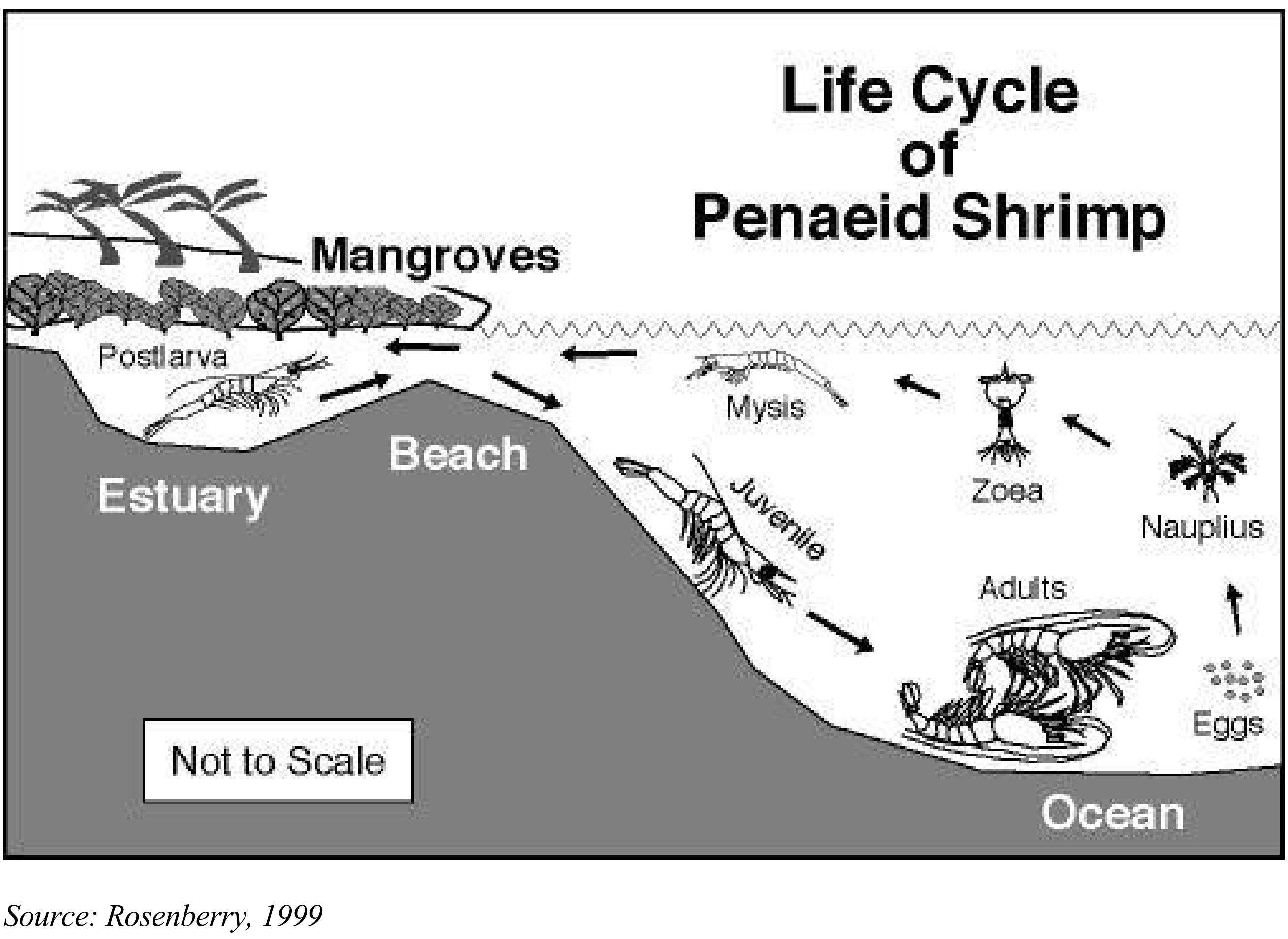
Refinements on Fujinaga's methods in the 1960s and 1970s greatly enhanced the availability of post-larvae and boosted shrimp production in all parts of the world. While this approach still is used today, reliance on broodstock sources from the wild continues to limit shrimp production in these areas.

A further refinement in the production of larvae developed in the 1970s was the ability to induce non-gravid females to undergo egg maturation. It was found that the removal, or ablation, of one of the eyestalks had a striking effect on the development of the ovaries. Ablation of the eyestalk, which contains the hormonal center, reduces the amount of hormones that inhibit gonadal maturation. Thus, non-gravid females could be taken from the wild and after ablation would produce mature gonads. This further increased egg production and importantly allowed hatcheries to produce larvae on demand.

Some species, most notably *Litopenaeus vannamei*, are more amendable to reproduction under culture conditions. As a consequence, pond-reared stock of white shrimp has been cultured over several generations. This has allowed for selection of desirable culture traits, such as disease resistance. However, other species, such as *Penaeus monodon* do not successfully reproduce in captivity and development of selected broodstock is lacking.

The understanding of their nutritional needs is key to the development of hatchery and growout techniques for shrimp. While in the nauplius stage, shrimp survive on yolk reserves from the egg and thus do not feed. In the zoea stage, they feed on algae, and in the mysis stage, shrimp feed on different types of zooplankton. In a hatchery environment, the culturist provides these foods. Algae are cultured for the zoea stage in a number of ways, either separately using specific kinds of algae or by fertilizing filtered seawater. Dried algal powders also can be used although survival rates are not as good.

Figure 1: Shrimp Life Cycle



Source: Rosenberry, 1999

In hatcheries, the mysis stage is often fed on freshly hatched brine shrimp nauplii. Brine shrimp nauplii are of an appropriate small size and are available as dried cysts, which hatch after roughly 24 hours in seawater. Some formulated diets are used to reduce the need for brine shrimp, but complete substitution is not yet possible. Formulated diets are available for post-larval and juvenile stages, enabling the farmer to rear the shrimp to maturity. Diets for broodstock shrimp typically include fresh or frozen supplements to the formulated diets.

The bulk of feed used in the shrimp industry is the formulated feed used in the growout of juveniles to market size. These feeds for growout of shrimp typically contain high levels of protein. Using sources such as high quality fish, shrimp and squid meal, protein levels in the feed range from 30% to 50%, depending on the shrimp species (Table 2) and culture strategy (Table 3). Lower levels of protein are used when shrimp are reared under extensive conditions. Extensive refers to relatively low density of shrimp per surface area of water. While definitions are somewhat inexact (Rosenberry, 1999), extensive can be characterized as using stocking densities of no more than 25,000 post-larvae per hectare and yielding less than 500 kilograms of shrimp per hectare per year. Even species that have an identified high protein requirement can be grown under these conditions, as shrimp can feed on natural prey items growing in the pond in addition to the supplied feed. Due to the low yields, extensive culture carried out in coastal ponds is becoming less prevalent in the shrimp culture industry today.

The majority of shrimp culture today is done under semi-intensive conditions. Stocking rates of post-larvae shrimp are in the range of 100,000 to 300,000 individuals per hectare, and yields can be as high as 5,000 kilograms per hectare per year. As the density of cultured shrimp increases, the relative contribution of natural prey to the diet diminishes and the quality of supplemented feed must be enhanced, including inclusion percentage of protein.

A small fraction of global shrimp production is carried out under intensive culture conditions, producing yields as high as 20,000 kilograms per hectare per year. Intensive culture is conducted typically in small ponds or tanks and requires high stocking rates, the highest quality feed and a variety of other inputs such as aeration or constant water exchange.

Table 2: Suggested Protein Requirements for Various Shrimp Species

Species Name	**Common Name**	**Protein Requirements**
Farfantepenaeus aztecus	Northern brown shrimp	40% – 51% (1)
Fenneropenaeus chinensis	Chinese white shrimp	45% (2)
Fenneropenaeus indicus	Indian white shrimp	34% – 50% (3)
Litopenaeus stylirostris	Western blue shrimp	30% – 35% (4)
Litopenaeus vannamei	Western white shrimp	30% – 40% (5)
Marsupenaeus japonicus	Japanese kuruma prawn	52% – 57% (6)
Penaeus monodon	Giant tiger shrimp	40% – 50% (7)

Source: Shiau, 1998

(1) Venkataramiah et al., 1978, Zein-Eldin and Corless, 1976, (2) Wu and Dong, 2002, (3) Colvin 1976b, Boonyaratpalin, 1998, (4) Colvin and Brand, 1977, (5) Pedrazzoli et al., (6) Deshimaru and Kuroki, 1975, Deshimaru and Yone, 1978, (7) Chen, 1993a.

As the shrimp farming industry has exploded from a minor producer of shrimp to one of global importance, several factors have stimulated efforts to find alternatives for marine protein sources in manufactured shrimp feeds. Undoubtedly, price is the key reason to look for alternatives. The supply and price of high quality fish meal, as well as shrimp and squid meals, can vary dramatically from year to year. There is also a general concern of the potential negative impact that fish meal production might have on natural fisheries (Naylor et al., 2000). Because of its attractive amino acid content, availability and relatively affordable price, soybean meal and soy concentrates have received increasing attention as replacements for marine animal meals.

Soy Products in Shrimp Feeds

Three types of soy products are used in shrimp feeds: protein products, oils and phospholipids. Since the percentage of protein in the diet tends to be related directly to the price of the final feed, most of the focus has been on soy products. However, the phospholipid lecithin also is widely used in shrimp feeds. Also, efforts have been made to incorporate soy oil.

There are a wide variety of constituent products that can be derived from soybeans. To produce dehulled soybean meal, first the hulls are removed and the beans are flaked into a meal by rolling. The oil is removed from the flakes by using a solvent. The flakes are then toasted to make dehulled soybean meal. Dehulled soybean meal containing about 48% protein is low in oil, but also high in complex carbohydrates. This product may be blended with some of the ground hulls to produce soybean meal, which is slightly lower in protein (around 44%). Conversely, carbohydrates can be removed or significantly reduced from dehulled soybean meal through various extraction processes, leaving high protein soy concentrates of various types.

Toasting the soy flakes is important to destroy trypsin, an inhibitor of the digestive enzyme. Removal of the carbohydrate fraction also is desirable. This fraction of soybean meal contains several other anti-nutritional and/or allergenic compounds. In addition, the carbohydrate fraction contains compounds that negatively influence the palatability of the meal. Removal of the carbohydrate fraction produces various concentrates with elevated protein content, generally above 65%.

Table 3: Suggested Protein Levels for Various Culture Strategies

Culture System	Protein Requirement
Extensive	25% – 30%
Semi-intensive	30% – 40%
Intensive	40% – 50%

Source: O'Keefe, 1998

Since shrimp do not process plant carbohydrates particularly well, these soy protein concentrates are of particular value to the industry.

The solvent extracted oil from soybean meal can be processed to further yield refined soy oil and lecithin, a phospholipid. Limited testing of soy oil for use in shrimp diets has been carried out, but the bulk of research has focused on soy lecithin. The addition of soy lecithin in the early stages of shrimp production has clear beneficial effects.

Shrimp Protein Requirements

Protein is the primary and most expensive component of shrimp diets. Consequently, research efforts have concentrated on defining effective sources and optimal levels in the diet. Dietary protein in shrimp is used first in the replacement of tissue proteins depleted during normal metabolism, and then if there is excess, in the synthesis of new tissue for growth and reproduction. Limiting dietary protein will result in a cessation of growth, followed by a loss of weight as non-essential body tissue protein is broken down and used to maintain vital body functions. If the protein in the diet is in excess of what the animal can use, it will be metabolized to provide energy stores.

Proteins are large complex molecules made of amino acids. While reference often is made to the protein requirements of shrimp and other animals, in actuality the dietary requirement is for specific amino acids that compose the molecule. Shrimp are unable to synthesize the group of 10 essential amino acids (EAA) in the diet. The amino acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are generally considered essential for animals.

Using radioactive tracers, Kanazawa and Teshima (1981) found that Japanese kuruma prawn were unable to synthesize these 10 amino acids, and then would require a dietary source. The essentiality of these 10 amino acids has also been shown in *Penaeus monodon* (Coloso and Cruz, 1980) and *Farfantepenaeus aztecus* (Shewbart et al., 1972). Two additional amino acids, cystine and tyrosine, which are not essential, spare methionine and phenylalanine respectively, and are considered pairs.

Until the need for all the EAAs are filled, other amino acids are extraneous and metabolized for energy. Identifying the exact requirements of each EAA in shrimp diets has been problematic. One general approach has been to assume that an optimum amount of EAAs would profile that of the whole body or tail muscle, which makes up the bulk of protein in shrimp. In theory, the closer the amino acid composition of the protein coming from the diet matches the composition of the tissue of the animal, the more effectively the dietary protein would be used (Wilson and Poe, 1985). The use of high quality protein sources, i.e., those having EAA profiles close to shrimp, should support good growth at relatively low dietary inclusion levels.

One problem with the above approach is the variation in the amino acid composition of shrimp from various published studies (Table 4). Variation among the different studies is likely a result of differences in species, age, tissues used, as well as the analysis itself. The resulting uncertainty of which amino acid standard to use complicates the selection of dietary protein sources.

This approach also assumes several conditions. First, that the protein is highly digestible, and second, that the total energy levels of the diet are appropriate. Fortunately, most of the high quality protein sources used in shrimp diets are highly digestible, 90% or better. If the energy levels are too high, shrimp tend to limit dietary consumption resulting in slow growth. On the other hand, if the dietary energy levels are too low, some of the protein will be used for metabolism to support vital physiological

Table 4: Comparison of Various Essential Amino Acid Profiles Used to Formulate Shrimp Diets

Essential Amino Acids	**Percentage (%) of Protein**						
	Tissue Essential Amino Acid Profile						
	F. aztecus[1]	*M. japonicus*[2]	*P. monodon*[3,4,5,6]				*L. vannamei*[7]
Arginine (Arg)	5.17	5.57	6.57	6.34	5.32	7.60	6.10
Histidine (His)	3.15	1.64	2.04	2.31	1.57	1.80	1.62
Isoleucine (Iso)	4.47	3.13	3.66	4.60	3.01	4.04	2.65
Leucine (Leu)	9.75	5.49	6.29	7.76	5.25	9.09	4.69
Lysine (Lys)	6.09	5.78	6.23	6.54	5.53	4.04	4.84
Metionine (Met) and Cystine (Cys)	4.85	2.75	2.39	2.80	2.55	3.51	2.67
Phenylalanine (Phe) and Tryptophan (Try)	8.43	6.17	4.37	5.75	5.85	8.28	5.39
Threonine (Thr)	5.38	2.98	3.25	4.76	2.87	4.11	2.52
Tryptophan (Try)	1.01		0.92			0.90	0.69
Valine (Val)	5.07	3.03	4.24	5.69	2.90	4.63	3.10

Source: O'Keefe, 1998

(1) Shewbart et al., 1972, (2) Deshimaru and Shigeno, 1972, (3) (4) Dy-Penaflorida, 1989, Marsden et al., 1991, Tacon, 1990, (5) Marsden et al., 1991, (6) Sarac et al., 1994, (7) Forster et al., 2002.

functions, leaving fewer amino acids to be incorporated into tissue. In contrast to digestion, this is more of a problem because there is a paucity of information on the energy requirements of shrimp, especially since energy requirements may change with a variety of physiological and environmental factors.

Other investigators have attempted to look more directly at the dietary requirement for key essential amino acids. The idea is to add increasing amounts of a single amino acid to the diet to determine when growth is maximized. Since shrimp are relatively slow feeders and individual amino acids are quite water soluble, the leaching of amino acids has frustrated this direct approach. Generally, attempts to substitute or supplement dietary protein with crystalline amino acids have resulted in poor growth and survival (Teshima et al., 1986). One way this has been overcome is by encapsulating amino acids before adding them to the diet. Chen et al. (1992) utilized this approach with microencapsulated arginine with *Penaeus monodon*, and suggested the requirement for arginine was 5.5% of dietary protein.

In another experiment with *P. monodon* using encapsulated arginine, Millamena et al. (1998) found the requirement was estimated to be 5.3% of the dietary protein intake. These amounts are only slightly less than what could be estimated from tissue analysis (Table 5). Millamena et al. (1998) also used encapsulated amino acids to estimate the requirement for lysine as 5.2% of dietary protein. Using various combinations of intact proteins and lysine supplements, Fox et al. (1995) suggested the lysine requirement for *Litopenaeus vannamei* was only 4.7% of the protein when using a diet containing 45% protein. The estimates for lysine from both groups, particularly for shrimp *Litopenaeus vannamei*, are below what has been recommended in the past (Table 5). More experiments will be required to determine if the divergence between species reflect actual differences in biology or differences in methodology. However, it should be noted that the total dietary protein level for *Litopenaeus vannamei* is thought to be lower than that required for *Penaeus monodon* (Table 2). Millamena et al. (1996a) suggested the methionine requirement for *P. monodon* was 2.4% of dietary protein. In combination with cystine in the diet, methionine plus cystine was suggested to be only 3.5% of dietary protein. Again these values are near the average values obtained from tissue analysis and at the recommended inclusion level for shrimp diets. In the results of Millamena et al. (1997) for threonine, the estimated requirement of 3.5% of dietary protein is only slightly below what might be expected from tissue analysis (Table 5).

Slightly differing results were obtained in a study examining valine requirements (Millamena et al., 1997). It was estimated that valine as 3.4% of dietary protein should be sufficient, which is lower than what is seen in shrimp tissue and below the general recommended level in Table 5. In their most recent study, Millamena et al. (1999) reported dietary amino acid requirements, expressed as a percentage of protein, as histidine 2.0%, isoleucine 2.5%, leucine 4.3%, and phenylalanine plus tryptophan 4.0%. With the exception of histidine, all the rest are lower than anticipated from tissue analysis (Table 5).

Teshima et al. (2002) examined the requirements for amino acids of the prawn (*Marsupenaeus japonicus)* by measuring the daily increase of each EAA in the whole body. In an attempt to avoid any dietary limitation, the shrimp were fed a high quality diet containing 50% protein based on casein and squid. Their assessment of EAA requirements (expressed as a percentage of protein) was: arginine (2.9); histidine (1.1); isoleucine (2.3); leucine (4.3); lysine (3.2); methionine (1.3); phenylalanine (2.6); threonine (2.3); tryptophan (0.6); and valine (2.4). These estimates considerablely are lower than previous estimates.

Clearly, the research aimed at determining the requirement for specific amino acids still is limited and the inconsistencies are not understood. Consequently, it will be some time before unambiguous requirements can be established for the EAA requirements of shrimp. However, in spite of the uncertainties, suggested guidelines exist to aid in the formulation of diets (Table 5).

With their balanced amino acid profile, soybean products have been found to be a relatively good source of protein in shrimp diets. Colvin and Brand (1977) were able to substitute 50% of a 1:1 menhaden fish meal and shrimp meal in the diet of *Farfantepenaeus californiensis* with 42% soybean meal supplemented with 0.8% DL-lysine. Lim and Dominy (1990) tested diets where soybean meal replaced up to 100% of the animal protein, in this case anchovy fish meal, in the diet of *Litopenaeus vannamei*. While growth rates declined when the substitution was more than 28%, the authors suggested that poor pellet stability was the cause. Based on protein utilization, a level of 56% substitution was possible.

Using a soy protein concentrate, Paripatananont et al. (2001) found that 50% of the fish meal could be replaced in the diet of *Penaeus monodon* without reduction of growth. Forster et al. (2002) found they could substitute 75% with a soy protein concentrate in the diet of *Litopenaeus vannamei*. Trials with the diets carried out indoors in aquaria required supplementation with arginine, methionine and phenylanine. Supplementation with lysine alone allowed for substitution of soy protein concentrate up to the 50% level. The soy protein concentrate diets, when used in outdoor trials where some natural food items were available, required no amino acid supplementation, even with up to 100% substitution of the fish meal. Piedad-Pascual et al. (1990) also found good growth could be achieved even when defatted soybean meal was the sole protein source for rearing of *Penaeus monodon* in ponds.

While the data is limited somewhat, it appears that soy protein concentrates can be used at somewhat higher levels than soymeal. This may be due to a number of factors. Akiyama (1988, Akiyama et al., 1989) found that purified protein sources, such as isolated soy protein (apparent protein digestibility 96.4%), were more digestible than practical

Table 5: Comparison of Essential Amino Acids (EAA) Tissue Profiles to Recommended Dietary Profile and Selected Protein Sources

Includes a typical fish meal along with dehulled solvent extracted soybean meal for comparison.

Essential Amino Acids	**Percentage (%) of Protein**					
	Tissue and Meal EAA Profile			**Diet Profile**		
Amino Acid	**Average for Shrimp**[1]	**Menhaden Fish Meal**[2]	**Dehulled Solvent Extracted Soybean Meal**[2]	**Suggested Guidelines**[3]	***P. monodon***[4,5] **Single Amino Acids Studies**	
Arginine (Arg)	6.10	6.1	7.4	5.80	5.5	5.3
Histidine (His)	2.02	2.4	2.5	2.03		2.0
Isoleucine (Iso)	3.65	4.7	5.0	4.24		2.5
Leucine (Leu)	6.90	7.3	7.5	8.16		4.3
Lysine (Lys)	5.58	7.7	6.4	6.14		5.2
Metionine (Met) and Cystine (Cys)	3.07	3.8	2.9	3.45		3.5
Phenylalanine (Phe) and Tryptophan (Try)	6.32	7.2	8.3	7.21		4.0
Threonine (Thr)	3.70	4.1	3.9	4.36		3.5
Tryptophan (Try)	0.94	1.1	1.4	0.80		
Valine (Val)	4.09	5.3	5.1	4.00		3.4

(1) Averaged from the data in Table 4., (2) Lim et al., 1998, (3) Akiyama et al, 1992, (4) Chen et al., 1992, (5) Millamena et al., 1997, Millamena et al., 1996a, Millamena et al., 1996b, Millamena et al., 1998, Millamena et al., 1999. Note: Data from Millamena et al. (1999) was reported as the percentage of amino acid in the diet; values were recalculated assuming a dietary level of 40% protein.

feedstuffs, such as soybean meal (apparent protein digestibility 89.9%). Akiyama also found there were some subtle differences in digestibility with regard to specific amino acids, but in general digestibility coefficients of the individual amino acids followed the apparent protein digestibility.

Since soy products used in aquaculture are derived from a roasted product, the trypsin inhibitor found in raw soybeans is not a major concern. In a study using lightly roasted soy flours and derived soy protein concentrates in the diet of *Litopenaeus vannamei*, Sessa and Lim (1992) found no significant correlation with the residual trypsin inhibitor levels. However, within the carbohydrate fraction of soybean meal there are a host of other factors that may negatively influence dietary utilization of the meal. Studies with fish have found that such heat-stable, alcohol-soluble factors as lectins, oligosaccharides, soy antigens and soyasaponins can contribute undesirable tastes or cause intestinal damage in fish (Dersjant-Li, 2002). The influence of these components on consumption has not been studied for shrimp, but in laboratory tests feed intake does decrease with higher levels of soybean meal inclusion. Since soybean meal has little starch, which is an important binding component in shrimp diets, evaluation of feed intake was confounded in this case (Lim and Dominy, 1990).

Inclusion of high levels of soybean meal may decrease shrimp access to the diet if the pellets disintegrate rapidly. Additional binding agents need to be added to insure that shrimp can fully utilize diets with high levels of soybean meal before the influence of taste or digestive inhibitors can be fully assessed.

Shrimp Lipid Requirements

Lipids are a source of essential fatty acids, sterols, phospholipids and fat-soluble vitamins. Lipids are also an important source of metabolic energy. Since shrimp do not utilize carbohydrates particularly well, lipids are often used in rations as a key source of energy. This reduces the protein denaturation (removal of nitrogen from amino acids) for use as energy. For shrimp, optimum levels of lipid inclusion are between 5% and 8% of the diet (D'Abramo, 1997). Higher lipid levels lead to a reduction of growth. Most likely this is because the energy level is too high, leading to reduced consumption. Also, it has been noted that higher lipid levels lead to fat accumulation in the mid-gut gland of shrimp, suggesting a limitation in processing of dietary lipids (Gonzalez-Felix et al., 2002b).

As with protein, shrimp do not have a specific need for lipid, but rather requirements for specific fatty acids, cholesterol and perhaps phospholipids. Saturated fatty acids including those of chain length up to 20 and 22 carbon atoms can be synthesized by animals from simple precursors such as acetate. While shrimp and other animals are able to enzymatically convert these saturated fatty acids to forms that contain a single double bond, they are unable to produce forms containing multiple double bond sites. Fatty acids containing multiple double bonds or polyunsaturated fatty acids (PUFAs) are important in the formation and maintenance of cell membranes and serve as precursors for important regulatory hormones.

Two basic PUFAs are linoleic (18:2n-6)[1] and linolenic (18:3n-3). Abundant in plants but not synthesized by animals, they are thus also referred to as essential fatty acids (EFAs). A dietary requirement for PUFAs has been reported for *Farfantepenaeus aztecus* (Shewbart and Mies, 1973), *Marsupenaeus japonicus* (Kanazawa et al., 1977), *Penaeus monodon* (Kanazawa et al.,1979a), *Fenneropenaeus indicus* (Read, 1981) and *Litopenaeus stylirostris* (Fenucci et al., 1981).

Through a chain elongation process (addition of carbon atoms) and further desaturation (formation of additional double bonds) most animals can use these EFAs to synthesize other important fatty acids.

These derived longer-chained fatty acids with additional unsaturated sites are often referred to in the literature as highly unsaturated fatty acids (HUFAs). Two important HUFAs are eicosapentaenoic (20:5n –3 or EPA) and

[1] *Fatty acids are identified by use of the shorthand designation. The first number is the number of carbon atoms in the molecule. The number following the colon is the number of double or unsaturated bonds and the number n-x (replacing the earlier ω designation) indicates the position of the first double bond from the methyl end of the molecule. Eicosapentaenoic (20:5n-3) and decosahexanenoic (22:6n-3) are also often referred to by the designation EPA and DHA respectively.*

decosahexanenoic (22:6n – 3 or DHA) acids. Differing from most animals, marine shrimp have only limited ability to transform PUFAs into HUFAs (Kanazawa and Teshima 1977; Kayama et al., 1980). Kanazawa and associates, first using radioactive tracers followed by feeding experiments, found that Japanese kuruma prawns were unable to synthesize any of these key fatty acids and dietary sources are needed for maximum growth and health (Kanazawa et al., 1979b, 1979c). Studies using fatty acid additions or oils with varying fatty acid compositions in the diet of *Fenneropenaeus indicus* (Read 1981), *Litopenaeus vannamei* (Lim et al., 1997) and *Marsupenaeus japonicus* (Guary et al, 1976; Kanazawa et al., 1977) indicate that dietary importance increases with increasing chain length and unsaturation of the n-3 family of fatty acids as follows: 18:2n – 6 < 18:3n – 3 < 20:5n –3 < 22:6n – 3. While there appears to be little bioconversion between EPA and DHA (Teshima et al., 1992), analysis of requirements are complicated by the fact that there appears to be some sparing of EPA and DHA by linolenic acid.

There may also be a need for a balance between n-6 and n-3 fatty acids in the diet (Xu et al., 1993). Optimum levels of EPA and DHA are about 1% of the diet, at least in the cases of the kuruma prawn (Kanazawa et al., 1979b) and the Chinese white shrimp (Xu et al., 1994). When using only linolenic acid and DHA, Merican and Shim (1997) suggested a 1.44% requirement for the giant tiger shrimp *Penaeus monodon*. Gonzalez-Felix et al. (2002b), using a mixture of n-3 highly unsaturated fatty acids, suggested the western white shrimp *Litopenaeus vannamei* could fulfill its requirements for HUFAs with a dietary inclusion of around 0.5% of the diet. Deering et al. (1997) have suggested, based on work with *Penaeus monodon,* that to improve dietary formulation, EFA levels should be considered as a proportion of total fatty acids in the diet and not simply as a percentage of the total diet.
Soybean oil is often used in dietary studies with shrimp. Colvin (1976a) found no significant difference when examining the use of soybean, sunflower, linseed or peanut oil in the diet of *Fenneropenaeus indicus*. Lim et al. (1997b) found only linseed oil was superior to soybean oil among vegetable oils tested for *Litopenaeus vannamei*. Since various vegetable oils available for use in shrimp diets, including soybean oil, do not contain the required n-3 highly unsaturated fatty acids, EPA and DHA, they are used in combination with fish oil. The combination of vegetable oil and fish oils generally produces superior results, but experimental results are still contradictory (Lim and Akiyama, 1995).

Among the shrimp species tested to date, *Litopenaeus vannamei* seems to be the most flexible, with regard to fatty acids. Gonzales-Felix and Perez-Velazquez (2002) suggest this species is able to meet its essential fatty acid requirements with highly unsaturated fatty acids from either the n-3 or n-6 family of fatty acids. However, as a practical matter, Lim et al. (1997a) found inclusion of menhaden oil was the preferred source of lipid in the diet of *Litopenaeus vannamei*.

Shrimp Lecithin Requirements

The phosopholipid lecithin is often used in shrimp diets and it appears to facilitate the transport of lipids within the shrimp, serving as a source and promoting the utilization of essential fatty acids. Kanazawa et al. (1979c) suggested the value of lecithins derived from the short-necked clam in the diet of *Marsupenaeus japonicus* was associated with the substantial amounts of EPA and DHA as their constituent fatty acids.

Since it is readily available, soy lecithin is often used in shrimp feeds at 1% to 2% of the diet (Akiyama et al., 1992; Shiau, 1998). A number of species, including *Marsupenaeus japonicus* (Kanazawa et al., 1979c), *Penaeus monodon* (Chen, 1993b; Paibulkichakul et al., 1998), and *Fenneropenaeus chinensis* (Kanazawa, 1993) have been shown to benefit from the addition of lecithin or the constituent purified phosphatidylcholine.

While soybean lecithin does not have significant amounts of EPA and DHA in contrast to the lecithins derived from marine oils, it appears to facilitate the utilization of other lipid components in the diet of shrimp. Both Coutteau et al. (1996) and Gonzalez-Felix et al. (2002a) found that the addition of purified phosphatidylcholine from soybean lecithin increases tissue levels of n-3 and n-6 PUFA in the tissues of *Litopenaeus vannamei* post-larvae at the expense of saturated fatty acids. Kontara et al. (1997), working with *Marsupenaeus japonicus* post-larvae, found that the addition of soybean phosphatidylcholine improved the uptake of dietary HUFAs into shrimp tissue.

The combination of dietary phosphatidylcholine and dietary HUFAs both increased growth rates as well as stress tolerance to osmotic shock. Gonzalez-Felix et al. (2002c) incorporated a variety of lipids, including coconut, soybean, linseed, peanut and menhaden oils, into the diet of *Litopenaeus vannamei*, each with and without lecithin. While in all cases the inclusion of menhaden oil produced the best growth, the addition of lecithin produced better growth when compared to the same dietary oil source without lecithin.

Shrimp are unique in that they are unable to synthesize cholesterol. Consequently, cholesterol is an essential dietary ingredient (Teshima, 1983). The requirement for cholesterol is between 0.5% and 1% of the diet. Phytosterols are relatively ineffective in substituting for cholesterol in juvenile shrimp (Teshima et al., 1989). As with fatty acids, dietary lecithin seems to be useful in the utilization of cholesterol for several shrimp species (Kanazawa, 1993). Gong et al. (2000) estimated the cholesterol requirement of *Litopenaeus vannamei* in the absence of dietary phospholipid was 0.35% of the diet. The addition of soybean lecithin at 1.5% and 5% diminished the requirement of cholesterol to 0.14% and 0.05%, respectively. The interaction between lecithin and cholesterol is not present in all species of shrimp. Chen (1993b) and Paibulkichakul et al. (1998) found no detectable interaction in the diet of *Penaeus monodon*

Future Considerations

Clearly, the use of soy products in shrimp aquaculture will increase substantially in the future. Aquaculture continues to grow at a more rapid pace than farming of terrestrial animals. In addition, the aquaculture industry is shifting away from the traditional extensive approach to more intensive practices. This shift will further increase the need for formulated feeds. Since fish stocks, including those used to make meals and oils, are near or at their limits, other sources will have to fill the upcoming gap in demand. Soy products, which are readily available and attractive from a nutritional standpoint, are an obvious possibility.

For shrimp feeds, the most pressing need is to find alternative protein sources. Although soybean meal is the most nutritive plant protein source, it also contains high levels of complex carbohydrates, which are of concern to aquaculturists. Shrimp and carnivorous fish, as opposed to terrestrial animals, do not utilize these carbohydrates effectively for energy. While energy requirements can be met by inclusion of oils, the carbohydrate fraction of soybean meal also contains a number of anti-nutritional factors that in fish reduce utilization of soybean meal.

Since aquaculture is a relatively young field, feed formulation is still somewhat of an art. Where specific information is lacking, the feed must be formulated using whatever data can be gleaned from work carried out with other animals. In the case of shrimp, where there is a paucity of data with regard to a host of nutritional questions, it may be necessary to use paradigms established for another shrimp species or even across phylum lines of fish to establish basic nutritional guidelines.

There is some evidence, however, to suggest that shrimp aquaculture can use more soybean meal directly than intensive fish farming. Shrimp are typically grown under semi-intensive conditions in ponds where they readily feed on the variety of prey items found there. Interestingly, Piedad-Pascual et al. (1990) found that shrimp that had access to the pond bottom could be grown on defatted soybean meal as their sole protein source. This is a research area that needs to be investigated in more detail.

Demonstrations of the use of soybean meal making up all or most of the protein component of shrimp diets at various levels of culture densities would be valuable in defining the limits to the direct use of soybean meal.

Certainly, it is easier to meet the necessary protein levels in shrimp feeds through the use of soy protein concentrates. Also, since the carbohydrate fraction is removed from soy protein concentrates, concerns about possible anti-nutritional factors are alleviated. Soy protein concentrates however, still have several drawbacks. Echoing the work from soybean meal, it appears that soy protein concentrates can serve as the sole protein source when shrimp are grown in systems where some natural foods are available. In indoor culture systems, supplementation of several amino acids is necessary.

It is somewhat surprising that amino acid supplementation of the protein fraction of soy seems to be necessary. Use of the essential amino acid profile of tissue has been a very useful technique in guiding feed formulation of terrestrial animals. A comparison of the essential amino acid profile of soy protein to that of shrimp tissue would suggest that amino acid supplementation would not be necessary.

This contradiction indicates more specific research should be focused on the utilization and turnover of essential amino acids by shrimp. Key amino acids that should be examined for several species are arginine, methionine and lysine.

A disadvantage of soy protein isolates is cost. Since soy protein isolates cost roughly the same as fish meal, there is little incentive to substitute one for the other. Ultimately, this may change in that natural fish stocks used for fish meal are limited and it may be possible to reduce the production costs associated with soy protein isolates.

As opposed to a likely increase in the use of soybean products to satisfy the protein component of shrimp feeds, use of soy oil and soy lecithin is unlikely to increase dramatically. While soybean oil is roughly as nutritious as the other available plant oils, all are limited in the HUFAs required by shrimp. At the moment, these essential fatty acids are supplied in the shrimp feed by the inclusion of fish oil. While it is now theoretically possible to genetically engineer soybeans to produce oil containing these essential fatty acids, this may decrease the value of the oil for other uses.

A more practical solution might be to focus on value-added products, such as a mixture of soybean oil and fish oil together with appropriate antioxidants that could be readily used by feed manufacturers. Soy lecithin is a minor constituent of shrimp diets. It also appears that a 2% inclusion level of lecithin produces maximum benefits, and for some species higher levels reduce growth.

Soy product utilization will continue to grow as the shrimp industry continues to expand. However, the real growth potential is the use of soy products as a protein source in shrimp diets and it is here that the industry should focus their efforts.

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Managed Aquaculture Program

This technical review paper was created through the Managed Aquaculture Marketing and Research Program (AquaSoy Initiative), funded through the United Soybean Board and American Soybean Association. The AquaSoy Initiative is designed to remove the barriers to soybean meal use in diets fed to aquaculture species. The program has been divided into two components, one focused on awareness, the other on research.

The awareness program initially focuses on Southeast Asia and India, where there are significant opportunities to intensify production within established aquaculture industries with the use of soybean meal-based diets.

The focus of the research component is salmonids, specifically rainbow trout and Atlantic salmon, and commercial crustaceans, all of which are large industries currently underutilizing soybean meal. The highly integrated and collaborative nature of this initial series of projects should result in expansion of soybean meal into new rapidly growing existing markets in North America, Europe and Asia.

This paper is one of a series of four technical review papers prepared by aquaculture specialists that summarize soy product use and potential in the diets for key aquaculture species groups. The technical reviews address the following species groups: 1) freshwater omnivorous fish; 2) marine fish; 3) marine shrimp; and 4) salmonids. All of these papers can be viewed at **www.soymeal.org**.

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