# THE UTILIZATION OF ROASTED FULL-FAT SOYBEAN AND METHIONINE SUPPLEMENTATION IN DIETS FOR JUVENILE FRESHWATER PRAWNS *Macrobrachium rosenbergii*

By

NUMCHAI CHAREONTESPRASIT

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

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## ABSTRACT

Two feeding experiments were conducted on one the utilization of roasted full-fat soybean (FFSB) as the sole plant protein source and the other on methionine supplementation in diets for juvenile freshwater prawns, *Macrobrachium rosenbergii.* In the first experiment, diets containing 0, 7, 14, and 21% FFSB were fed for a 8-week period and then diets containing 0, 14, 28, and 42% FFSB were fed for a second period of 12 weeks duration. In the second experiment, four diets which contained 24-30% protein and 0.16-0.35% supplemental methionine were fed for 16 weeks.

Results from these studies indicated that dietary full-fat soybeans did not adversely affect growth and survival of prawns. The highest dietary FFSB (twenty one percent FFSB for the first period and 42% FFSB for the second period) could be incorporated in prawn diets. There was no advantage conferred by supplemental methionine at any of the protein levels studied here. The lowest level of protein (24%) plus 0.24% methionine was found to be effective for growth and survival of prawns as the highest protein (30%) plus 0.16% methionine. However, the feed conversion ratio of 1.98 in prawns fed the highest protein diet was significantly lower (ie., higher feed efficiency) than in those fed the lowest protein diets with supplemented methionine with ratios of 2.64 and 2.54.

In summary, the highest dietary FFSB and the lowest dietary protein were found to cost the least of the diets examined here, although the cost difference per unit of tail meat was not statistically significant.

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Most importantly, however, this thesis is dedicated to my parents, Banthit and Wanida Chareontesprasit, for their unswerving moral support and encouragement over the past thirty years.

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# 1. INTRODUCTION

Interest in crustacean farming was greatly stimulated in many countries by the widespread decline of natural populations of aquatic species. The decline was a result of increasing industrialization, deterioration of water quality, loss and degradation of habitats and over-exploitation of wild stocks. As a result, the population of crustaceans declined rapidly and prices were driven up to levels which made artificial culture very attractive to investors (Huner and Brown, 1985; Wickins, 1982). In Thailand, attempts to increase prawn resources were initiated during the 1950s by stocking wild-caught juvenile *Macrobrachium rosenbergii* in artificial ponds (Sandifer and Smith, 1985).

*Macrobrachium rosenbergii* is one of the most important freshwater species of cultured prawns in Thailand. This freshwater prawn used to be produced mainly for export, in particular to Japan and the United States, and for the local restaurant market. Subsequently, the export growth potential for this species was limited by competitive marine species. Although, prawn prices have fallen sharply, this prawn is still the most profitable for farmers in inland freshwater areas to harvest and is being extensively domestically marketed.

Rising feed costs have influenced the extent to which the prawn is cultured (Feller and Herrfurth, 1986). To be economically viable, prawn farming must be no more expensive than other methods of production. Therefore, reduction of feed costs is a major objective in prawn nutrition, in order to increase profitability for farmers. In semi-intensive culture of prawns, Vogt et al. (1986) reported that feed represents 30% of the total production costs in the U.S.A., 38% in Ecuador and 35% in Japan. In Taiwan, 45% is spent on feed in an intensive prawn farm. Although these percentage are lower than the feed costs of 60-80% reported for all raised terrestrial animals by New (1976), they are still the largest single expenditure in prawn farming.

For many years, nutritionists have tried to substitute less expensive plant protein for the expensive fish meal component of animal feeds. The high cost of animal protein prohibits its use as the sole protein source in formulating feeds. Therefore, a mixture of animal and plant proteins is desirable in the formulation of prawn diets (Vogt et al., 1986). When defatted soybean meal has been used in animal diets, supplementation of oil, amino acids, vitamins, and minerals (Viola et al., 1983) is needed for normal growth. Therefore, it is economically preferable to use full fat soybean meal which contains high levels of oil, rather than the defatted soybean meal plus soy oil supplementation (Reinitz et al., 1978).

Before soybeans are used in diets, they must be heat-treated in order to destroy toxic factors, particularly trypsin inhibitor, without adversely affecting the nutritional value of the beans. Several procedures have been developed to process full-fat soybean including roasting, expansion through extrusion, autoclaving, and steaming. Small-scale farms require processing techniques which are simple to perform and feed formulations which are simple and practical to prepare (Wee and Shu, 1989). The roasting method may be appropriate to eliminate toxic factors and practical to use, particularly during the rainy season in Thailand. After being roasted soybeans need not be dried before storage.

Various studies have shown that growth of animals fed diets containing cooked full-fat soybean meal was not adversely affected as long as the diets included methionine supplementation. Soybean meal diets are deficient in methionine and this becomes the first limiting amino acid (Halver, 1989). Full-fat soybean meal has been successfully used in diets for swine and rat (Seerly et al., 1974), chicks (White et al., 1967 ; Wood et al., 1971), chicken layers (Latshaw and Clayton, 1976), turkeys (Shen et al., 1970), rainbow trout fingerling, *Oncorhynchus mykiss* (Reinitz et al., 1978), and catfish fingerlings, *Clarias gariepinus* (Balogun and Ologhobo, 1989)

In addition to use of FFSB lowered the feed cost, the protein component (the most expensive nutrient source) of the diet has also been successfully reduced by receiving balanced amino acids without adversely affecting growth and survival of animals. This has been shown for chicks (Najib and Sulivan, 1982; Olson et al.,1969) and swine (Brown et al.,1973; Lunchick et al., 1978). However, evidence is lacking as to the nature of balanced amino acid profile for prawn diets (New, 1976)

The objectives of this study were :

1 to determine the effects on growth and feed utilization of using roasted full-fat soybean meal as a substitute for defatted soybean meal in diets for juvenile *Macrobrachium rosenbergii*.

2 to determine the effects on growth and feed utilization in these juveniles of several levels of methionine supplementation to low protein diets.

These results may be useful to small scale farmers who grow soybeans and can use them directly to produce inexpensive and practical feeds on farms instead of buying either expensive defatted soybean or pelleted feed from the agroindustry.

# 2. REVIEW OF THE LITERATURE

## 2.1 The Biology of *Macrobrachium rosenbergii*

*Macrobrachium* is an important genus of caridean prawn belonging to the Phylum Arthropoda, Class Crustacea, Order Decapoda, Family Palaemonidae. The genus *Macrobrachium* consists of approximately 125 species, of which 49 are of interest to fisheries, and at least 15 (including *M. rosenbergii*) of which are considered suitable for commercial aquaculture. *M. rosenbergii* is a tropical species widely distributed from Australia to New Guinea to the Indus River delta in the Indo-Pacific region (Sandifer and Smith, 1985).

*Macrobrachium rosenbergii* has five pairs of walking legs with the second bearing substantial chelae or claws, typically indicative of a territorial or predatory species. Males of *M. rosenbergii* grow larger than females, regularly reaching 200 g or more in the wild. The largest *M. rosenbergii* have been reported to weigh 654-1,000 g In nature, adult *Macrobrachium* inhabits rivers and lakes where there is direct access to the sea (Sandifer and Smith, 1985).

In mature females, the ovaries are located dorsal to the stomach and hepatopancreas in the cephalothorax. The ovaries extend from just behind the eyes and beneath the rostral crest to the first abdominal segment. Laterally an oviduct arises from each ovary, just anterior to the heart, and extends ventrolaterally to the gonopore on the coxa of the third pereiopod. The ripe ovary is bright orange in colour and is visible through the carapace. During the breeding season, mature females migrate downstream into estuarine areas before the eggs hatch to allow the free-swimming larvae to develop in brackish water (Sandifer and Smith, 1985).

Eleven stages can be observed in larval growth (Malecha, 1983; New and Singholka, 1982; Provenzano, 1985):

| Stage | Size(mm tip of rostrum-telson length) | Prominent characteristic |
|---|---|---|
| I | 1.92 | Sessile eyes |
| II | 1.99 | Stalked eyes |
| III | 2.14 | Uropods appear |
| IV | 2.50 | Two dorsal teeth on rostrum |
| V | 2.84 | Telson narrower and elongated |
| VI | 3.75 | Pleopod buds appear |
| VII | 4.06 | Pleopods biramous and bare |
| VIII | 4.68 | Pleopods with setae |
| IX | 6.07 | Endopods of pleopods with appendices internae |
| X | 7.05 | Three or four dorsal, teeth on rostrum |
| XI | 7.73 | Teeth on half of upper dorsal margin |
| Postlarva | 7.69 | Teeth on upper and lower margin of rostrum, also behavioral changes, mainly in swimming on its back. Postlarva begins at 28 days after hatching and swims upstream to freshwater. |

In culture, *M. rosenbergii* grows best in subtropical and tropical environments under the following conditions: water temperatures of 26-31°C (79-88°F); salinity for larval stages in the range of 12-16 ppt, dissolved oxygen at least 2.1 ppm at 23°C (73°F), 2.9 ppm at 28°C (82°F) or 4.7 ppm at 33°C; pH in the range of 6.0-10.5 (Sandifer et al., 1983; Sandifer and Smith, 1985; Aquacop, 1983).

The natural food of *Macrobrachium* consists mainly of detritus, plant and animal matter (Costa and Wanninayake, 1986). Young males and females (11-20 mm total body length) prefer to consume only detritus and diatoms. Medium sized prawns (21-30 mm) are partly bottom feeders and partly omnivores while the large sized prawns (31-40 mm) are bottom feeders and carnivores (Jayachandran and Joseph, 1989). The food consumed by a prawn is ingested into the foregut and then is digested and absorbed mainly in the digestive gland. The major digestive enzymes that have been found from the foregut and digestive gland are proteolytic enzymes, lipase and esterase, and carbohydrases which are similar to mammalian enzymes (Dall and Moriarty, 1983).

Many different live organisms are fed to larval *Macrobrachium* in culture. These include algae (Liao et al., 1983); rotifers (Lovett and Felder, 1988); brine shrimp, *Artemia* (New and Singholka, 1982). In addition, non-living feeds such as fish eggs, chicken egg custard, squid flesh and compounded feed are also consumed by prawn larvae (Akiyama and Dominy, 1989). However, Corbin et al. (1983) state that very little is known either quantitatively or qualitatively about nutrients required by larval prawn. Of these, *Artemia* nauplii are the most frequently used larval food. They are generally high in protein, lipid, and energy. The levels of essential amino acids and fatty acids present in *Artemia* nauplii vary with the source but appear adequate to promote growth and survival in prawns.

For the production of postlarvae, some ingredients that have been used for commercial prawn feed include fish meal and by-products, squid meal and by-products, shrimp meal and by-products, soybean meal, wheat products, blood meal, and yeast. However, nutrient digestibility may vary depending on ingredient source (Akiyama and Dominy, 1989).

Prawns require energy and nutrients such as protein, vitamins, and minerals for normal metabolic function, maintenance and growth. Proteins utilized by animals for growth and repair of tissues are composed of up to 20 major amino acids. The carbon skeletons of the common L-amino acids can be converted to amphibolic intermediates to synthesize fat, carbohydrate, and protein (Rodwell, 1983; Akiyama and Dominy, 1989). Protein levels of diets for cultured *Macrobrachium* may lie between 27% and 35% (Corbin et al., 1983).

Prawns need energy for growth, muscular activity and reproduction. Their energy comes from utilized protein, lipids, and carbohydrates. Prawns require relatively lower dietary energy than mammals because they do not have to maintain a constant body temperature. They do not have to use energy to maintain their position in the water and they use less energy to excrete their nitrogenous wastes as ammonia than if they excreted urea or uric acid. Lipids include fats, phospholipids, sphingomyelins, waxes and sterols. All animals utilize lipids for normal growth and survival. Lipids can serve as carriers of fat soluble vitamins, as precursors for other compounds (sterols and phospholipids), and serve as attractants in diet (Akiyama and Dominy, 1989). Lipid should not exceed 10% of prawn artificial diet (Corbin et al., 1983). The ratio of utilized protein to non-protein energy has been found to affect the energy level of diets. For maximum growth of *M. rosenbergii*, 40% protein plus 1:2 lipid to carbohydrate ratio is suggested by Sick and Millikin, (1983). Corbin et al., (1983) suggest 25% protein plus 1:4 lipid to carbohydrate ratio in the diet.

Vitamins are required in small amounts for normal growth, metabolism and reproduction as essential components of specific enzyme systems involved in functions such as carboxylation, transcarboxylation and decarboxylation reactions. Minerals function in osmotic balance in body fluids and are constituents of the exoskeleton and of tissues. They are also involved in transmission of nerve impulses and muscle contractions, and provide essential components for enzymes, vitamins, hormones, pigments and co-factors in metabolism, catalysts and enzyme activators (Akiyama and Dominy, 1989).

## 2.2 Full-Fat Soybeans

Soybean (*Glycine max L*) has been one of the most important cash crops in the Thai economy for a number of years due to its numerous domestic and industrial uses. During the last decade, soybean cultivation has been introduced to the northern region and the central plain areas of Thailand. Recently, the cultivation of the crop has been extended to the northeast region due to modernized agriculture, with the aim of improving the quality and quantity of the crop. This project has produced a large crop of soybeans for human and animal consumption containing high quantities of protein, vitamins and oil (Suksri et al., 1990).

Soybeans prior to oil extraction are referred to as full-fat soybeans (FFSB) (Monari, 1991). Full-fat soybean meal is the product resulting from heat-treating whole soybeans without removing any of the constituents. The bean's oil content of about 18% remains intact (Collado, 1989). Raw full-fat soybeans not previously subjected to any kind of heat treatment have relatively low nutritive value and may adversely influence animal health, if consumed. This is a consequence of the presence of biologically-active compounds with an antinutritive or toxic value (Monari, 1991). These include protease inhibitors, haemagglutinins, saponins, goitrogenic factors, rachitogenic factors, metal chelating factors, and urease.

Protease (trypsin and chymotrypsin) inhibitors likely serve a natural function within the bean by protecting it against bird attack or microbial invasion. When the raw soybean is fed to non-ruminants, the factors bind to the trypsin and chymotrypsin enzymes which are secreted by the animal's pancreas, causing loss of digestive efficiency. After ingesting raw soybean, the animal shows a reduced growth rate and a deteriorating feed conversion ratio. The response varies with species but tends to involve the secretion of more digestive enzymes and subsequent pancreatic hypertrophy. Furthermore, since these enzymes contain a high proportion of sulphur-containing amino acids, such hyperactivity may exacerbate the already well-known relative deficiency of these amino acids in soya protein.

There are two principal protease inhibitors present in raw soybeans, the Kunitz factor and the Bowman-Birk factor. The latter is more resistant to the action of heat, alkali and acid. Their average levels in raw soybeans are of the order of 1.4 and 0.6% by weight, respectively.

Haemagglutonins bind with cells of the intestinal mucosae resulting in a reduction in nutrient absorption. Saponins are glycosides present in soybeans at 0.5%. They are associated with a bitter taste and have a haemolytic effect on red blood cells. Goitrogenic factors are also glycosides belonging to the isoflavinic group. Some of these, such as genistin, have goitrogenic activity resulting in thyroid gland swelling and a reduction in the activity of thyroxine secreted by the thyroid itself. Rachitogenic factors (about 0.10% of raw soybeans) are principally associated with genistin and interfere with calcification of bone. Turkeys are particularly sensitive to these factors. Some soybean oil meals interfere with the availability of certain trace minerals, such as manganese, zinc, copper, and iron due to metal chelating factors.

Raw soybeans also have variable urease activity. This is less of significance to nutrition than as a method for assessment of the degree of adequacy of processing. Urease activity is decreased by heating. Roasting is one of the common methods of heat treatment to degrade antinutritional factors and is carried out in a common drier (Monari, 1991).

The quality of protein in soybean meal as in any other protein source is reduced through denaturation if the meal is overheated. Urease activity is a rapid and relatively inexpensive means of measuring protein quality. However, the urease activity test has its limitations. A maximum trypsin inhibitor level of 1-3 mg per g soybean meal is considered acceptable for aquaculture feeds. This trypsin inhibitor level is associated with a urease activity value of 0.00 to 0.23 (Table 1). The problem is that a urease activity of 0.00 may be considered adequate for aquaculture feeds, but it can be associated with reduced protein solubility and quality due to overheating (Akiyama, 1988).

Table 1. The relationship between urease activity protein solubility index and trypsin inhibitor in soybean meal.

| Urease Activity (measured by pH change) | Protein Solubility (%) | Trypsin Inhibitor (mg/gm meal) |
|---|---|---|
| 2.40 | 99.2 | 21.0 |
| 2.04 | 87.7 | 12.2 |
| 0.23 | 79.1 | 3.1 |
| 0.12 | 83.2 | 2.2 |
| 0.00 | 74.9 | 2.1 |
| 0.00 | 71.8 | 1.0 |
| 0.00 | 58.5 | 0.5 |
| 0.00 | 38.0 | 0.1 |

Source : Akiyama (1988).

Holmes (1988) reported that the urease test is not always a good guide to the destruction of trypsin inhibitor. The method can produce erroneous results when organic acids have been used as preservatives or sterilizing agents, particularly where pH change is used as a measure of the urease present. However, he suggested urease activity standards of overcooked as less than 0.05; satisfactory 0.1-0.3; undercooked 0.3-0.5; and raw beans, greater than 0.5 mg N/min at 30 °C.

## 2.3 Full-Fat Soybeans in Animal Diets

Full-fat soybean contains high dietary energy, protein and fat that make FFSB meal desirable as a component in high nutrient diets. NRC (1983) found FFSB to be composed of 10% moisture, 37.9% crude protein, 4.6% Ash, 5.0% crude fiber and 18.0% lipid on a dry weight basis. Full-fat soybean for aquaculture feed contains 4,568 gross energy (kcal/kg) (Lovell, 1987) or 3,640 metabolizable energy (kcal/kg) (NRC, 1983). Additionally, FFSB contains varying amounts of tocopherol, vitamin E, sulphur, choline, lecithin complex (1.5-2.5%) and linoleic acid (9.5-12.4%), all of which make a positive contribution to the diet. In addition to the high nutrient concentration, FFSB offers indirect advantages of a lowering in dust levels during handling, enhancement in pelleting ability, reduction in wear of machinery and improved diet appearance and palatability compared with many other diets (Monari, 1991).

### 2.3.1 Full-Fat Soybeans in Diets of Terrestrial Species

Broilers fed diets with 15% FFSB produced carcass fat with significantly lower contents of palmitoleic and oleic acids, and with significantly higher proportions of linoleic and linolenic acids than those fed diets without FFSB (Porter and Britton, 1974). The FFSB content in the diet could be increased to 41.25% without causing any negative effect on production, mortality or manure condition (Waldroup and Cotton 1974). Latshaw (1974) and Latshaw and Clayton (1976) concluded that 15% FFSB in the diet would promote satisfactory laying performance. Full-fat soybean can contribute 10% of the diet for turkeys without any detrimental effects (Moran et al., 1973; Turner et al., 1973).

Pigs fed FFSB up to 20% of the diet showed body fat, either in internal organs or in the back, that had a lower melting point than that of pigs fed diets based on soybean oil meal (Monari, 1991). Full-fat soybean in dairy cow diets leads to a rise both in milk yield and the length of lactation. The oil in FFSB increases the proportion of long chain fatty acids, particularly the unsaturated fatty acids, oleic and linoleic, in milk which may have benefit for human nutrition (Smith et al., 1980; Baker et al., 1986). Kung and Huber (1983) reported that, although roasted FFSB slightly improved daily milk yield, butter fat decreased in cows on the FFSB diets. However, Owen et al. (1985) obtained improved performance with dairy cows fed roasted FFSB. Roasting increased the dry matter concentration, the butter fat level, the milk yield and the milk solids. In addition, the non-fat solids and lactose fractions were higher.

### 2.3.2 Full-Fat Soybeans in Aquatic Animal Diets

Full-fat soybean products can be used in fish diets because fish digest soy oil easily. For example, trout have a digestibility coefficient of 0.89 for soy oil, which is equivalent to that for cod liver oil and higher than that for hydrogenated fish oil. The value for catfish for soya oil is 0.81. Linoleic and linolenic acids are present in soya oil at levels of 52% and 8%, respectively. These amounts are able to provide the essential fatty acid requirements of trout as well as those of carp, eel, salmon, ayu, and tilapia. In addition, the digestible energy of cooked FFSB is high (Monari, 1991).

Reinitz et al. (1978) reported that rainbow trout fry fed a diet containing 72.7% FFSB had a greater daily increase in length and weight, and an improved feed conversion ratio, than those fed a control diet based on 25% herring meal, 5% fish oil and 20% soybean oil meal. In addition, there was no reported problem of diet palatability nor loss of firmness and flavour of the fish. Monari (1991) reported that a carp diet containing 50% heated FFSB + 10% corn gluten meal produced no differences in performance than one based on 10% fish meal and 24% soybean oil meal.

Balogun and Ologhobo (1989) reported that a diet containing cooked soybean for catfish, *Clarias gariepinus*, was superior in all aspects to all diets containing raw soybean meal. Gradual decreases in growth and nutrient utilization parameters were observed in all groups of fish fed raw soybean meal. Furthermore, the highest percentage mortalities were obtained in groups of fish fed 50%, 75% and 100% raw soybean meals.

Wilson and Poe (1985) reported that fingerling channel catfish, *Ictalurus punctatus* showed the best growth rates when 83% of the trypsin inhibitor activity in the soybean meal was destroyed. Similarly, Wee and Shu (1989) reported that boiling may be appropriate to eliminate trypsin inhibitors since 1 h boiling destroyed approximately 80% of the inhibitor activity in diets for Nile Tilapia.

## 2.4 Amino Acid Supplementation

Various studies show that animals fed diets containing cooked FFSB meal are not adversely affected as long as the diets contain methionine supplementation to compensate for the deficiency of this amino acid in soybeans. An essential amino acid, methionine is the first amino acid to become limiting in soybean meal (Halver, 1989). The best quality of protein for maintenance and growth results when the amino acids are balanced in the animal rations (Cunha, 1957).

The structural formula of methionine is

$$\begin{array}{lllll} CH_2 & - \; CH_2 & - & CH & - \; COO^- \\ | & & & | & \\ S & - \; CH_3 & & NH_3^+ & \end{array}$$

Synthetic crystalline methionine is produced when bacteria convert homoserine and aspartate-ß-semialdehyde in the presence of aspartokinase. In methionine metabolism, three-fifths of the skeleton of methionine is converted to succinyl-CoA in the citric acid cycle. Methionine serves as a methyl group donor, directly utilized by oxidation, in the form of S-adenosylmethionine, the principal source of methyl groups in the body. Methionine also serves as the precursor for 1,3-diaminopropane portions of the polyamines, spermine, and sperimidine. Methionine may undergo oxidative deamination to form the corresponding α-keto acid. This reaction is reversible and conversion of D- to L- isomer is possible (Rodwell, 1983).

### 2.4.1 Amino Acids in Diets of Terrestrial Species

Najib and Sullivan (1982) reported that diets containing 11.5% protein plus 0.447% methionine + cystine fed to chicken layers promoted egg weight, egg production, and feed efficiency compared to diets containing higher protein levels of 14.5% and 13%. Olson et al. (1969) found that methionine supplementation at 0.2% in cassava meal diets for hybrid chickens (New Hamshire x Single Comb White Leghorn) produced the best growth and weight gain.

In addition to methionine, lysine has been added to low protein diets for swine. The protein levels could be decreased to 4% by supplementing approximately 0.04% lysine for each percentage of protein decreased in order to formulate inexpensive pig diets without adversely affecting growth and performance (Brown et al., 1973; Baker et al., 1975; Lunchick et al., 1978).

### 2.4.2 Amino Acids in Aquatic Animal Diets

Reinitz et al. (1978) reported 1% methionine added to 72.7% FFSB meal diets of rainbow trout promoted growth, feed conversion ratio, and daily length increment. Murai et al. (1989) indicated that the basal soy product of 41.2% soy flour in fingerling carp (*Cyprinus carpio*) diets was deficient in methionine and supplementation of 0.25% methionine alone was sufficient to improve growth, protein deposition and feed efficiency. However, a high level of 0.50% methionine was toxic and stimulated methionine degradation to taurine and methionine sulfoxide.

# 3.MATERIALS AND METHODS

## 3.1 Source of Experimental Animals

Juvenile *Macrobrachium rosenbergii* used in this trial were obtained from a postlarval rearing pond belonging to a small-scale farmer in the Supanburi province of Thailand. Available animals were variable in size. The farmer purchased postlarvae after hatching from a hatchery farm in that area where the prawns had already been acclimated to freshwater. The farm-hatched parents were domesticated stock and all the test animals were taken from a group of animals of similar history. For transportation to Khon Kaen University by a truck, 1000 postlarvae were divided into lots of 100 and each lot was placed in 2 l freshwater in a 10 l plastic bag into which oxygen was pumped. After their arrival at Khon Kaen, all postlarvae were released into a concrete tank and were fed the T1 (described below) diet for 3 days in order to allow recovery from stress associated with the 7-hour transportation before being allocated to experimental treatments.

## 3.2 Laboratory Analyses

Raw full-fat soybean was purchased from a local market in Khon Kaen province. Raw full-fat soybean was roasted in a pan of approximately 20 l placed on a wood-fuelled stove. The optimum cooking of roasted full-fat soybean was indicated qualitatively by the urease test as the conversion of urea to ammonia gas in the presence of phenol red indicator (Khajarern et al., 1987).

Urea phenol red solution was prepared by dissolving 0.14 g of phenol red in 7 ml of 0.1 N sodium hydroxide and 35 ml of distilled water. Twenty one grams of urea (reagent grade) were dissolved in 300 ml of distilled water. The entire volum of two solutions were mixed together and titrated to amber-colour with 0.1 N sulfuric acid. Approximately five g samples of roasted FFSB were placed in a Petri dish with 5-8 drops of amber-colored phenol red solution. Phenol red solution was also added to a series of dishes containing standards with known percent raw soybean. Colour matching allowed an estimate to be made of the raw soybean left in the roasted sample. Optimal cooking of roasted FFSB was indicated when approximately 3-5% of the raw soybean was left raw.

Before allotment to experimental treatments, fifty prawns were selected fof sacrifice and analyzed for protein by the macro-Kjeldahl determination of nitrogen. Full-fat soybean was analyzed for protein by the macro-Kjeldahl determination of nitrogen, for lipid by ether extraction, for ash by the portion remaining after water and organic matter were removed by combustion, for dry matter, for Ca and P by titration according to Lovell (1981). Composition of FFSB determined in this study is given in Table 2.

Table 2 Chemical composition of roasted full-fat soybean used to formulate feeds (% wet weight basis)

| | |
|---|---|
| Moisture | 8.81 % |
| Dry matter | 91.19 % |
| Ash | 5.24 % |
| Protein | 37.59 % |
| Lipid | 16.42 % |
| Fiber | 8.97 % |
| Gross energy | 4451.10 kcal/kg |
| Ca | 0.35 % |
| P | 0.58 % |

At the end of both experiments, 50% of prawns of each tank were sacrificed and the tail meat was weighed. Following this, complete carcasses were analyzed for protein, lipid, ash, dry matter, Ca, P, and fiber by the same methods as for full-fat soybean analyses. However, unfortunately, the data from the Experiment 1 were lost from the laboratory.

3.3 Formulation of Feeds

Several ingredients, defatted soybean meal, prawn shell meal, squid viscera meal, fish meal, skim milk, cod liver oil, synthetic methionine and lysine, were purchased locally in Bangkok. Other ingredients corn, rice bran and full-fat soybean were purchased from the local market in Khon Kaen. Vitamins and minerals were obtained and formulated from the non-ruminant nutrition laboratory at the Department of Animal Science, Faculty of Agriculture, Khon Kaen University. The chemical composition of full-fat soybean reported in Table 2 was used in the formulation of test diets for both experiments. Chemical composition of other feed ingredients was taken from NRC (1983). Nutrient requirements of a prawn, such as protein, were obtained from Corbin et al. (1983). Other nutrient requirements were calculated from Akiyama and Dominy (1989). There are two formulations for the test diets because the adult prawns require less protein than the young ones (Corbin et al. (1983).

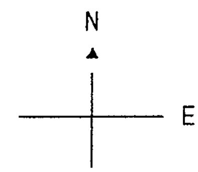
All of the ingredients were ground finely using a grinding machine and mixed together according to the formulae described in Tables 3 and 4. Then the feeds were made into pellets by a pelletor at the rate of 50 kg/h. The pellets were approximately 1 mm radius and 5 mm long. The feeds were produced in a large batch about a week before starting each experiment and were kept for the duration of an experiment in plastic bags at ambient temperature.

## 3.4 Feeding Experiments

The two experiments were conducted during the summer and fall of 1990 and 1991, respectively in the fish farm at the Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. The tests were conducted in small concrete tanks (112 cm wide x 112 cm long x 50 cm high) of approximately 600 l volume. Two contiguous rows of eight tanks were oriented east-west and the tanks were outdoors half in direct of sunlight and half in shade.

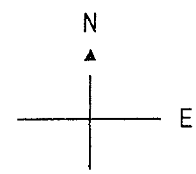
Both experiments were undertaken using a completely randomized design for four replicates. Lay out of experimental design is shown in Figure 1.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| T3R1 | T4R4 | T3R4 | T2R1 | T1R3 | T2R3 | T4R2 | T1R4 |
| T2R4 | T1R2 | T4R1 | T4R3 | T3R3 | T1R1 | T2R2 | T3R2 |




(A)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| T5R4 | T8R2 | T7R1 | T6R2 | T7R2 | T7R4 | T6R4 | T8R3 |
| T7R3 | T6R1 | T8R1 | T5R2 | T6R3 | T5R3 | T8R4 | T5R1 |




(B)

Fig.1 Lay out of experimental design for Experiment 1(A) and Experiment 2 (B).

In Experiment 1, fifty 30-day old juvenile *Macrobrachium rosenbergii* with a mean size ranging from 3.2-3.4 cm (orbit-telson length) and mean weight from 0.6-0.7 g were stocked in each tank at random and reared in 430 l of water contained in each of the 16 concrete tanks. The experiment ran from June to October 1990. In Experiment 2, twenty 60-day old juvenile *Macrobrachium rosenbergii* with a mean size ranging from 4.61-5.0 cm and mean weight from 1.71-2.13 g were stocked in each tank at random and reared in the same concrete tanks as above. The experiment ran from June to October 1991.

For the first four weeks of Experiment 1, four diets containing different ratios of defatted soybean and roasted FFSB meal (Table 3) were fed to the test animals. Then for the last 12 weeks of Experiment 1, four different diets were fed to the animals. For example, animals fed $T1_A$ in the first 4 weeks, were fed $T1_B$ in the next 12 weeks and so on.

Table 3. Diet formulations used in Experiment 1. Chemical composition of these diets is given in Tables 4 and 5.

| Period | Diet | Defatted soybean meal (% of diet) | Roasted FFSB meal (% of diet) |
|---|---|---|---|
| 4 weeks | $T1_A$ (control) | 36 | 0 |
| | $T2_A$ | 30 | 7 |
| | $T3_A$ | 24 | 14 |
| | $T4_A$ | 18 | 21 |
| 12 weeks | $T1_B$ (control) | 36 | 0 |
| | $T2_B$ | 24 | 14 |
| | $T3_B$ | 12 | 28 |
| | $T4_B$ | 0 | 42 |

For Experiment 2, four types of diets using various levels of protein and methionine supplementation described in Table 4 were used.

Each of the four diets was fed to prawns in four of the 16 tanks. Feed was offered twice a day initially at 25% of body weight, then at 20% in the last 3 months of the experiment.

The conditions of the water in Experiments 1 and 2 were monitored every morning (8:00-9:00) and afternoon (15:00-16:00) and were found to vary

Table 4. Chemical composition of four experimental diets (shown in Table 3) containing various levels of roasted full-fat soybean for the first 4 weeks in Experiment 1.

| Ingredient | % Diet | | | |
|---|---|---|---|---|
| | $T1_A$ | $T2_A$ | $T3_A$ | $T4_A$ |
| Corn | 21.27 | 20.27 | 19.27 | 18.27 |
| Rice bran | 10.00 | 10.00 | 10.00 | 10.00 |
| Fish meal | 15.50 | 15.50 | 15.50 | 15.50 |
| Soybean meal | 36.00 | 30.00 | 24.00 | 18.00 |
| Roasted full-fat soybean | - | 7.00 | 14.00 | 21.00 |
| Shrimp shell meal | 10.00 | 10.00 | 10.00 | 10.00 |
| Squid viscera meal | 2.00 | 2.00 | 2.00 | 2.00 |
| Milk | 1.80 | 1.80 | 1.80 | 1.80 |
| Cod liver oil | 0.70 | 0.70 | 0.70 | 0.70 |
| Lysine | 0.08 | 0.08 | 0.08 | 0.08 |
| Methionine | 0.15 | 0.15 | 0.15 | 0.15 |
| Vitamin and mineral mix [1] | 2.50 | 2.50 | 2.50 | 2.50 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Cost[2], Baht/kg | 20.33 | 20.21 | 20.10 | 19.98 |
| Formulated chemical composition | | | | |
| Metabolizable energy (ME),kcal/kg | 3341.03 | 3394.31 | 3447.59 | 3500.87 |
| Crude protein (CP),% | 32.76 | 32.80 | 32.84 | 32.88 |
| Lipid,% | 5.73 | 6.83 | 7.94 | 9.04 |
| Crude fiber (CF),% | 4.08 | 4.11 | 4.14 | 4.18 |
| Ash,% | 11.75 | 11.69 | 11.63 | 11.57 |
| Calcium,% | 2.06 | 2.06 | 2.06 | 2.06 |
| Phosphorus,% | 1.04 | 1.04 | 1.04 | 1.04 |
| Amino acid,% | | | | |
| Arginine | 2.06 | 2.04 | 2.05 | 2.06 |
| Glycine | 1.63 | 1.66 | 1.68 | 1.71 |
| Histidine | 0.80 | 0.80 | 0.80 | 0.81 |
| Isoleucine | 1.45 | 1.48 | 1.51 | 1.53 |
| Leucine | 2.48 | 2.45 | 2.42 | 2.40 |
| Lysine | 2.10 | 2.10 | 2.11 | 2.11 |
| Methionine | 0.74 | 0.75 | 0.75 | 0.76 |
| Cystine | 0.43 | 0.42 | 0.41 | 0.41 |
| Phenylalanine | 1.46 | 1.47 | 1.49 | 1.51 |
| Tyrosine | 1.19 | 1.19 | 1.19 | 1.20 |
| Serine | 1.38 | 1.40 | 1.42 | 1.44 |
| Threonine | 1.24 | 1.26 | 1.27 | 1.29 |
| Tryptophan | 0.39 | 0.39 | 0.39 | 0.39 |
| Valine | 1.56 | 1.58 | 1.59 | 1.61 |

[1] Vitamin and mineral mix described in Table 7

[2] 22 Baht = 1 Can$

Table 5. Chemical composition of four experimental diets (shown in Table 3) containing various levels of roasted full-fat soybean for the last 12 weeks in Experiment 1.

| Ingredient | % Diet | | | |
|---|---|---|---|---|
| | $T1_B$ | $T2_B$ | $T3_B$ | $T4_B$ |
| Corn | 25.45 | 23.45 | 21.45 | 19.25 |
| Rice bran | 12.00 | 12.00 | 12.00 | 12.20 |
| Fish meal | 12.00 | 12.00 | 12.00 | 12.00 |
| Soybean meal | 36.00 | 24.00 | 12.00 | - |
| Roasted full-fat soybean | - | 14.00 | 28.00 | 42.00 |
| Shrimp shell meal | 10.00 | 10.00 | 10.00 | 10.00 |
| Squid viscera meal | 1.50 | 1.50 | 1.50 | 1.50 |
| Cod liver oil | 0.40 | 0.40 | 0.40 | 0.40 |
| Lysine | 0.05 | 0.05 | 0.05 | 0.05 |
| Methionine | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin and mineral mix [1] | 2.50 | 2.50 | 2.50 | 2.50 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Cost[2], Baht/kg | 19.63 | 19.40 | 19.17 | 18.93 |
| Formulated chemical composition | | | | |
| Metabolizable energy (ME),kcal/kg | 3312.00 | 37418.55 | 3525.11 | 3631.79 |
| Crude protein (CP),% | 30.61 | 30.69 | 30.77 | 30.95 |
| Lipid,% | 5.30 | 7.50 | 9.71 | 11.92 |
| Crude fiber (CF),% | 4.28 | 4.35 | 4.41 | 4.48 |
| Ash,% | 11.03 | 10.90 | 10.78 | 10.70 |
| Calcium,% | 1.85 | 1.85 | 1.85 | 1.86 |
| Phosphorus,% | 0.96 | 0.96 | 0.96 | 0.97 |
| Amino acid,% | | | | |
| Arginine | 1.91 | 1.93 | 1.94 | 1.97 |
| Glycine | 1.51 | 1.56 | 1.62 | 1.68 |
| Histidine | 0.74 | 0.75 | 0.76 | 0.77 |
| Isoleucine | 1.34 | 1.40 | 1.45 | 1.51 |
| Leucine | 2.32 | 2.26 | 2.21 | 2.15 |
| Lysine | 1.87 | 1.88 | 1.89 | 1.91 |
| Methionine | 0.63 | 0.64 | 0.65 | 0.66 |
| Cystine | 0.41 | 0.40 | 0.38 | 0.36 |
| Phenylalanine | 1.37 | 1.40 | 1.43 | 1.46 |
| Tyrosine | 1.12 | 1.12 | 1.13 | 1.13 |
| Serine | 1.32 | 1.36 | 1.40 | 1.45 |
| Threonine | 1.15 | 1.18 | 1.21 | 1.25 |
| Tryptophan | 0.37 | 0.36 | 0.36 | 0.35 |
| Valine | 1.44 | 1.47 | 1.50 | 1.54 |

[1] Vitamin and mineral mix described in Table 7

[2] 22 Baht = 1 Can$

Table 6. Vitamin and mineral mix used for the experimental diets

| Vitamin | g | Mineral | g |
|---|---|---|---|
| Thiamin HCl | 0.150 | $K_2HPO_4$ | 10.000 |
| Riboflavin | 0.500 | $NaH_2PO_4.H_2O$ | 8.920 |
| Pyridoxin | 0.150 | $Ca(H_2PO_4).H_2O$ | 26.500 |
| Nicotinic acid | 2.000 | KCl | 16.500 |
| Calcium pantothenate | 0.750 | $MgSO_4.7H_2O$ | 2.800 |
| Inositol | 10.000 | $AlCl_3.6H_2O$ | 0.024 |
| Biotin | 0.015 | $ZnSO_4.7H_2O$ | 0.476 |
| Folic acid | 0.038 | $MnSO_4.H_2O$ | 0.081 |
| Para amino benzoic acid | 1.000 | $CuCl_2$ | 0.020 |
| Choline chloride | 20.000 | KI | 0.023 |
| Ascorbic acid | 25.000 | $CoCl_2.6H_2O$ | 0.140 |
| Cyanocobalamine | 0.001 | | |
| L-tocopherol | 1.000 | | |
| Menadione | 0.100 | | |
| B-carotene | 0.670 | | |
| Ercocalciferol | 0.015 | | |
| Binder | | | |
| Polymethylocarbamide | 100.000 | | |

Source : Piedad-Pascual (1986)

Table 7. Diet formulations used in Experiment 2. Chemical composition of these diets is given in Table 8.

| Period | Diet | Protein | Supplemented methionine | Total methionine |
|---|---|---|---|---|
| 16 weeks | T5 (control) | 30 | 0.16 | 0.72 |
| | T6 | 27 | 0.27 | 0.72 |
| | T7 | 24 | 0.35 | 0.72 |
| | T8 | 24 | 0.24 | 0.61 |

within the following ranges; temperature (26-29.5 °c); pH (8.7-9.2); dissolved oxygen (DO) (9.4-11.8 mg/l); and conductivity (157-159 µmhos/cm at 25 °C) (measured by portable electrical meters). The tanks were cleaned daily by siphoning off fecal matter and left-over feed. In addition, thorough cleaning of the tanks was done once a week. In order to reduce cannibalism, two plastic egg trays were placed on the bottom of each tank for the animals to hide in while molting.

In each experiment, each tank was initially filled with 400 l of city water from aerated for three days to remove the chlorine. Water was replaced in the tanks at the rate of 1l/min for 6 hours every day for 4 months under laboratory conditions. Continuous aeration was provided by an air pump and airstone in each tank.

Total weight of prawns in each of the 16 tanks was measured every 14 days for 4 months. Excessive water was removed by blotting them on cotton fabric before weighing. They were weighed as a group on a piece of thin cotton fabric using an electrical portable scale to within the nearest 0.01 g. Twenty percent of the prawns in Experiment 1 and all the prawns in Experiment 2 were measured from the posterior edge of the orbit to the tip of the telson using a ruler. Whenever mortality occurred the feeding rate

Table 8. Chemical composition of four experimental diets (shown in Table 4) containing various levels of protein and methionine supplementation in Experiment 2.

| Ingredient | % Diet | | | |
|---|---|---|---|---|
| | T5 | T6 | T7 | T8 |
| Corn | 19.24 | 25.93 | 31.25 | 31.36 |
| Rice bran | 12.00 | 12.00 | 12.00 | 12.00 |
| Fish meal | 12.20 | 5.40 | - | - |
| Roasted full-fat soybean | 42.00 | 42.00 | 42.00 | 42.00 |
| Shrimp shell meal | 10.00 | 10.00 | 10.00 | 10.00 |
| Squid viscera meal | 1.50 | 1.50 | 1.50 | 1.50 |
| Cod liver oil | 0.40 | 0.40 | 0.40 | 0.40 |
| Methionine | 0.16 | 0.27 | 0.35 | 0.24 |
| Vitamin and mineral mix [1] | 2.50 | 2.50 | 2.50 | 2.50 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Cost[2], Baht/kg | 18.98 | 18.25 | 17.66 | 17.56 |
| Formulated chemical composition | | | | |
| Metabolizable energy (ME),kcal/kg | 3624.11 | 3627.47 | 3624.06 | 3624.24 |
| Crude protein (CP),% | 30.31 | 27.50 | 24.77 | 24.78 |
| Lipid,% | 11.92 | 11.71 | 11.54 | 11.55 |
| Crude fiber (CF),% | 4.48 | 4.55 | 4.61 | 4.61 |
| Ash,% | 10.70 | 9.21 | 8.02 | 8.03 |
| Calcium,% | 1.86 | 1.51 | 1.23 | 1.23 |
| Phosphorus,% | 0.97 | 0.79 | 0.65 | 0.65 |
| Amino acid,% | | | | |
| Arginine | 1.97 | 1.75 | 1.57 | 1.57 |
| Glycine | 1.68 | 1.42 | 1.21 | 1.21 |
| Histidine | 0.77 | 0.69 | 0.62 | 0.62 |
| Isoleucine | 1.51 | 1.34 | 1.20 | 1.20 |
| Leucine | 2.15 | 1.93 | 1.75 | 1.75 |
| Lysine | 1.86 | 1.55 | 1.31 | 1.31 |
| Methionine | 0.72 | 0.72 | 0.72 | 0.61 |
| Cystine | 0.36 | 0.34 | 0.32 | 0.32 |
| Phenylalanine | 1.46 | 1.33 | 1.22 | 1.22 |
| Tyrosine | 1.13 | 1.03 | 0.94 | 0.94 |
| Serine | 1.45 | 1.33 | 1.24 | 1.24 |
| Threonine | 1.24 | 1.10 | 0.98 | 0.98 |
| Tryptophan | 0.35 | 0.31 | 0.28 | 0.28 |
| Valine | 1.54 | 1.35 | 1.20 | 1.20 |

[1] Vitamin and mineral described in Table 7

[2] 22 Baht = 1 Can$

was proportionately reduced. The prawns were returned individually to their respective tanks after measuring in order to minimize stress to the prawns.

Growth response parameters were calculated as follows :

Percentage weight gain

$$= \frac{\text{(final weight (g/prawn)-initial weight (g/prawn))}}{\text{initial total weight (g)/prawn}} \times 100$$

Specific growth rate (SGR), %/day

$$= \frac{\text{(ln final weight (g/prawn-ln initial weight (g/prawn))}}{\text{days}} \times 100$$

Percentage length gain (orbit-telson length)

$$= \frac{\text{(final length (cm/prawn)-initial length (cm/prawn))}}{\text{initial length (cm/prawn)}} \times 100$$

Feed conversion ratio (FCR)

$$= \frac{\text{total feed fed (g/prawn)}}{\text{total wet weight gain (g/prawn)}}$$

It was not possible to estimate the total food consumption because the prawns were fed *ad libitum* and did not consume all the food. Average food consumption was calculated from a feed conversion ratio of 2.3 (Sandifer and Smith, 1985) and assumed to be the same for all treatments.

Protein efficiency ratio (PER)

$$= \frac{\text{total wet weight gain (g/prawn)}}{\text{amount of protein fed (g/prawn)}}$$

Apparent net protein utilization (%, NPU)

= 100 x (total body protein at the final weight (g/prawn)-total body protein at initial weight (g/prawn))/amount of protein fed (g/prawn)

The total body protein at initial weight (g/prawn) was analyzed using the method given above and was assumed to be the same proportion for all replicates and treatments.

Survival rate (%)

$$= 100 - \frac{(100 \times \text{number of prawns died /tank})}{\text{total number of prawns/tank}}$$

### 3.5 Statistical Analyses

Means of parameters averaged over the four replicate tanks were compared across treatments using one-way analysis of variance (ANOVA). Duncan's multiple rank test was used to compare the treatment means when the ANOVA indicated statistical significance at the $P<0.05$ or $P<0.01$ levels (Kleinbaum and Kupper, 1978).

# 4. RESULTS

## 4.1 The Utilization of Roasted Full-Fat Soybean in Diets by *Macrobrachium rosenbergii* Juveniles

Over the 16 week experiment, it was found that there were no significant differences (P>0.05) in several parameters among the tested diets (T1, T2, T3, and T4) containing various levels of roasted FFSB. The prawns fed the highest level of roasted FFSB (T4) tended to have lower survival rates (higher mortality) (Table 9) than the prawns fed the lower levels of roasted FFSB (T1, T2, and T3) but the differences were not significantly different (P>0.05). The survival rates of prawns in this experiment were 77.50%, 78.00%, 77.00%, and 71.00% of the prawns fed T1, T2, T3, and T4 diets, respectively.

The percentages of weight gained (Table 10 and Fig. 2) were 1153.08%, 914.06%, 999.71%, and 1052.49%; the specific growth rates (Table 11) were 2.25 %/day, 2.06 %/day, 2.13 %/day, and 2.18 %/day and the percentages of length gained (Table 12 and Fig.3) were 117.72%, 104.38%, 105.12%, and 107.00% in prawns fed T1, T2, T3 and T4 diets, respectively.

However, at the end of 10 weeks, the percentage of weight gained (Table 10) by prawns fed T1 diet (576.39%) was significantly higher than those fed T2 (473.79%), T3 (503.05%), and T4 (509.35%) diets (P<0.05). Similarly, the specific growth rate (Table 11) over the 10 weeks showed the same pattern. Length at 10 weeks also showed the same trend but the differences among diets were not statistically significant.

From the size distribution (Table 13 and Fig. 4), the number of prawns in each size group did not differ significantly among diet

Table 9. Survival rate (%) of prawns fed diets containing various levels of roasted full-fat soybeans (FFSB), averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| 0-2 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | 99.50±0.50 |
| 0-4 | 99.50±0.50 | 99.50±0.50 | 100.00±0.00 | 99.00±1.00 |
| 0-6 | 99.50±0.96 | 96.50±1.26 | 97.50±0.96 | 90.50±2.99 |
| 0-8 | 89.00±4.12 | 91.00±4.12 | 90.00±3.16 | 89.00±3.70 |
| 0-10 | 88.00±4.16 | 87.50±4.35 | 88.50±2.06 | 88.50±3.95 |
| 0-12 | 84.00±4.76 | 85.50±2.50 | 87.00±3.00 | 84.50±3.86 |
| 0-14 | 84.00±4.76 | 84.00±4.55 | 83.00±3.42 | 79.50±5.12 |
| 0-16 | 77.50±4.79 | 78.00±7.14 | 77.00±3.87 | 71.00±4.20 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 3.

Table 10. Average weight gained (%/prawn) by prawns fed diets containing various levels of roasted full-fat soybeans (FFSB), averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| 0-2 | 69.50±9.58 | 57.97±9.01 | 69.83±8.07 | 80.34±10.43 |
| 0-4 | 139.85±13.00 | 134.49±13.12 | 125.38±7.01 | 146.02±11.50 |
| 0-6 | 257.26±19.11 | 251.86±19.00 | 263.89±10.88 | 264.23±19.30 |
| 0-8 | 436.64±32.31 | 387.82±33.06 | 398.96±27.41 | 423.74±38.15 |
| 0-10 | 576.39±25.01[a] | 473.79±22.51[b] | 503.05±21.19[b] | 509.35±12.83[b] |
| 0-12 | 790.99±24.28 | 760.44±45.96 | 680.52±30.09 | 698.58±44.06 |
| 0-14 | 994.85±40.05 | 812.23±44.04 | 861.90±53.13 | 867.74±57.63 |
| 0-16 | 1153.08±33.29 | 914.06±36.66 | 999.71±75.09 | 1052.49±54.72 |

[a,b] Means within a row with different superscripts are significantly different at the P=0.05 level.
[1] SE = Standard error of estimate
[2] Diets are described in Table 3.

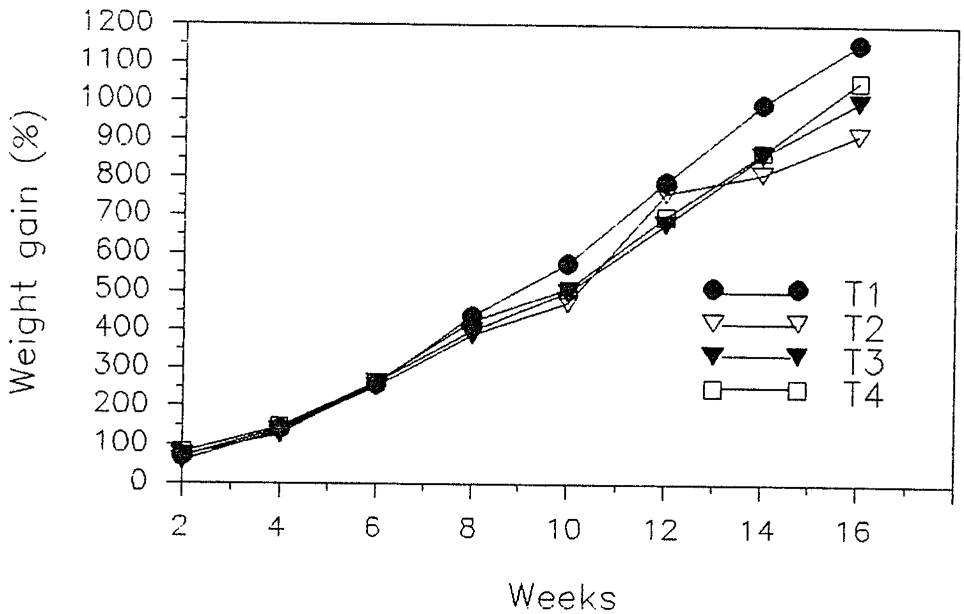



Fig.2 Time course of weight gain of prawns fed diets containing four levels of roasted FFSB. Diets are defined in Table 3.

Table 11. Specific growth rate (%/day) of prawns fed diets containing various levels of roasted full-fat soybeans (FFSB), averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| 0-2 | 3.74±0.40 | 3.23±0.42 | 3.76±0.34 | 4.17±0.43 |
| 0-4 | 3.11±0.20 | 3.03±0.20 | 2.90±0.11 | 3.20±0.17 |
| 0-6 | 3.02±0.13 | 2.98±0.14 | 3.07±0.07 | 3.07±0.13 |
| 0-8 | 2.99±0.11 | 2.82±0.12 | 2.86±0.10 | 2.94±0.12 |
| 0-10 | 2.72±0.05[a] | 2.49±0.06[b] | 2.57±0.05[ab] | 2.58±0.03[ab] |
| 0-12 | 2.60±0.03 | 2.56±0.06 | 2.45±0.04 | 2.47±0.06 |
| 0-14 | 2.44±0.04 | 2.25±0.56 | 2.31±0.06 | 2.31±0.06 |
| 0-16 | 2.25±0.03 | 2.06±0.52 | 2.13±0.06 | 2.18±0.05 |

[a,b] Means with a row with different superscripts are significantly different at the P=0.05 level.
[1] SE = Standard error of estimate
[2] Diets are described in Table 3.

Table 12. Average length gained (%/prawn) by prawns fed diets containing various levels of roasted full-fat soybeans (FFSB), averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| 0-2 | 14.31±3.09 | 10.18±3.17 | 12.04±3.33 | 7.62±2.24 |
| 0-4 | 16.16±3.04 | 22.87±2.90 | 23.03±2.28 | 15.43±0.87 |
| 0-6 | 67.15±2.24 | 65.75±3.34 | 65.61±5.82 | 57.86±5.79 |
| 0-8 | 82.39±4.12 | 73.48±3.83 | 78.07±2.78 | 72.02±4.48 |
| 0-10 | 90.34±1.46 | 81.71±3.68 | 83.03±5.05 | 78.03±4.33 |
| 0-12 | 96.96±5.32 | 98.38±3.73 | 99.93±2.21 | 100.53±7.20 |
| 0-14 | 105.48±6.00 | 90.30±2.99 | 101.16±3.13 | 96.37±2.18 |
| 0-16 | 117.72±2.69 | 104.38±6.48 | 105.12±5.17 | 107.00±6.40 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 3.

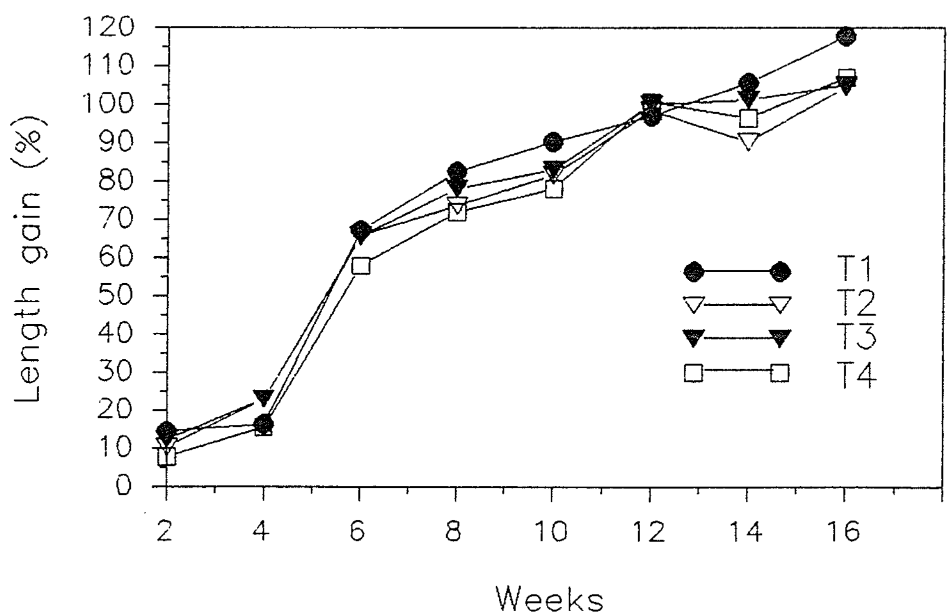



Fig.3 Time course of length gain of prawns fed diets containing four levels of roasted FFSB. Diets are defined in Table 3.

Table 13. Size (orbit-telson length) distribution of prawns fed diets containing various levels of roasted full-fat soybeans (FFSB) for 16 weeks. Values represent the number of prawns in each size group as a % of the total (mean ± SE[1]).

| Size | Diet[2] | | | |
|---|---|---|---|---|
| | T1 | T2 | T3 | T4 |
| Small size (2.5-5.2 cm) | | | | |
| | 6.95±1.46 | 15.68±4.56 | 13.92±2.93 | 10.51±1.46 |
| Medium size (5.3-8.0 cm) | | | | |
| | 72.47±6.01 | 68.78±1.68 | 72.88±2.68 | 70.92±5.45 |
| Large size (8.1-11.0 cm) | | | | |
| | 20.96±1.46 | 15.54±5.06 | 13.20±0.95 | 18.57±4.89 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 3.

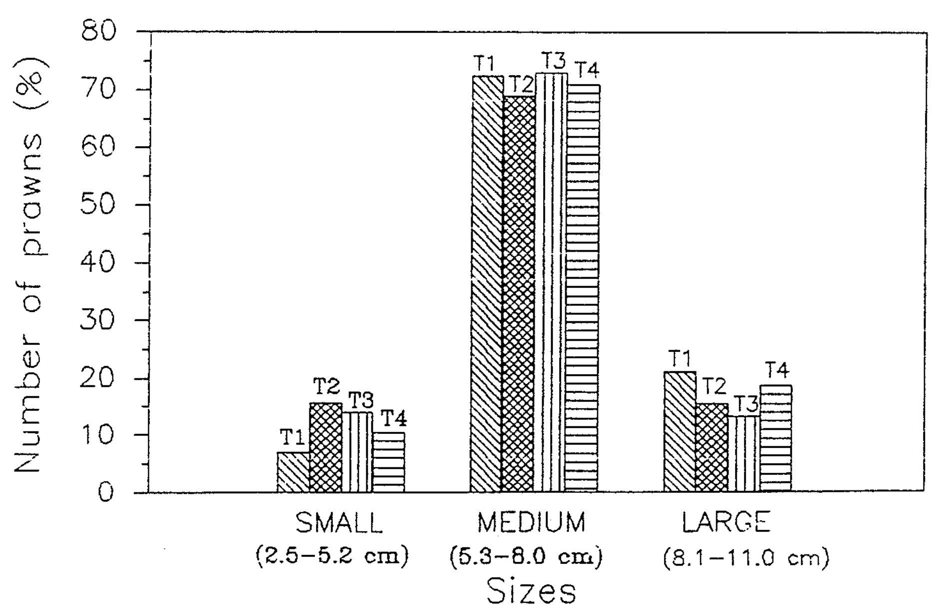



Fig.4 Size distribution of prawns fed diets containing four levels of roasted FFSB for 16 weeks. Diets are defined in Table 3.

treatments, even though prawns fed T1 diet tended have greater numbers of the large size than those fed T2, T3 and T4 diets.

Feed efficiency measured as feed conversion ratio (FCR) was not significantly different among the various levels of roasted FFSB (Table 14). The feed conversion ratio averaged over the 0-16 weeks was 2.00, 2.45, 2.28, and 2.02 for prawns fed T1, T2, T3 and T4 diets, respectively.

### 4.2 The Utilization of Methionine Supplementation in Low Protein Diets by *Macrobrachium rosenbergii* Juveniles

The effects of methionine supplementation in low protein diets on prawn growth were indicated by comparison of survival rate, percentage of weight gain, length gain, and of specific growth (%/day) among the four treatments. The survival rate of prawns (Table 15) fed the low protein (24%) diet with supplemented low methionine (T8) tend to be lower, but not statistically significant, compared with either those fed the high protein (30%) diet (T5) or the low protein (24%) diet with supplemented high methionine (T7). The survival rate after 16 weeks was 47.50%, 57.50%, 56.25%, and 36.25% of prawns fed T5, T6, T7, and T8 diets, respectively.

The percentage weight gains over 16 weeks were 480.82%, 406.33%, 390.67%, and 357.45% (Table 16 and Fig. 5); the specific growth rates were 1.56 %/day, 1.45 %/day, 1.41 %/day, and 1.36 %/day (Table 17) and the percentage of length gained were 61.53%, 52.52%, 52.22, and 56.07% (Table 18 and Fig. 6) for prawns fed T5, T6, T7, and T8 diets, respectively. For none of these three parameters was there a statistical difference among diets, although the data suggested that prawns fed high protein (30%) diet (T5) tended to have a higher percentage weight gain, length gain, and specific growth rate.

Table 14. Feed conversion ratio of prawns fed diets containing various levels of roasted full-fat soybeans (FFSB), averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| 0-2 | 2.52±0.38 | 2.94±0.60 | 2.39±0.26 | 2.13±0.36 |
| 0-4 | 2.39±0.26 | 2.35±0.27 | 2.56±0.19 | 2.22±0.23 |
| 0-6 | 2.43±0.22 | 2.34±0.22 | 2.28±0.10 | 2.32±0.22 |
| 0-8 | 2.27±0.19 | 2.41±0.19 | 2.42±0.17 | 2.30±0.20 |
| 0-10 | 2.13±0.13 | 2.45±0.16 | 2.38±0.09 | 2.34±0.08 |
| 0-12 | 2.20±0.10 | 2.16±0.07 | 2.50±0.11 | 2.45±0.16 |
| 0-14 | 1.99±0.10 | 2.33±0.10 | 2.30±0.12 | 2.09±0.17 |
| 0-16 | 2.00±0.08 | 2.45±0.23 | 2.28±0.18 | 2.02±0.14 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 3.

Table 15. Survival rate (%) of prawns fed diets containing various levels of protein and methionine supplementation, averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T5 | T6 | T7 | T8 |
|---|---|---|---|---|
| 0-2 | 98.75±1.25 | 97.50±1.44 | 98.75±1.25 | 95.00±1.25 |
| 0-4 | 96.25±1.25 | 96.25±1.25 | 92.50±2.50 | 92.50±1.44 |
| 0-6 | 81.25±6.88 | 83.75±4.27 | 81.25±5.91 | 67.50±7.77 |
| 0-8 | 66.25±6.88 | 67.50±2.50 | 65.00±4.56 | 57.50±6.29 |
| 0-10 | 62.50±5.20 | 65.00±2.89 | 62.50±4.33 | 51.25±3.15 |
| 0-12 | 55.00±8.42 | 62.50±3.23 | 63.75±5.54 | 46.25±4.73 |
| 0-14 | 48.75±5.54 | 62.50±3.23 | 58.75±5.54 | 43.75±4.27 |
| 0-16 | 47.50±7.50 | 57.50±4.33 | 56.25±5.15 | 36.25±8.98 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 7.

Table 16. Average weight gained (%/prawn) by prawns fed diets containing various levels of protein and methionine supplementation, averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T5 | T6 | T7 | T8 |
|---|---|---|---|---|
| 0-2 | 34.81±4.56 | 35.84±7.52 | 42.93±4.86 | 29.36±7.54 |
| 0-4 | 70.58±16.16 | 67.45±14.87 | 87.08±22.14 | 63.92±12.96 |
| 0-6 | 96.24±13.36 | 88.47±12.31 | 94.36±19.38 | 73.36±12.51 |
| 0-8 | 115.93±8.40 | 104.56±9.92 | 122.01±24.09 | 86.46±6.77 |
| 0-10 | 184.07±22.58 | 165.58±10.15 | 178.97±16.37 | 161.79±9.10 |
| 0-12 | 262.50±30.15 | 243.25±13.84 | 256.69±40.38 | 234.77±12.69 |
| 0-14 | 321.87±28.31 | 312.87±20.53 | 312.92±40.52 | 257.59±24.57 |
| 0-16 | 480.82±44.03 | 406.33±17.52 | 390.67±47.37 | 357.45±10.64 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 7.

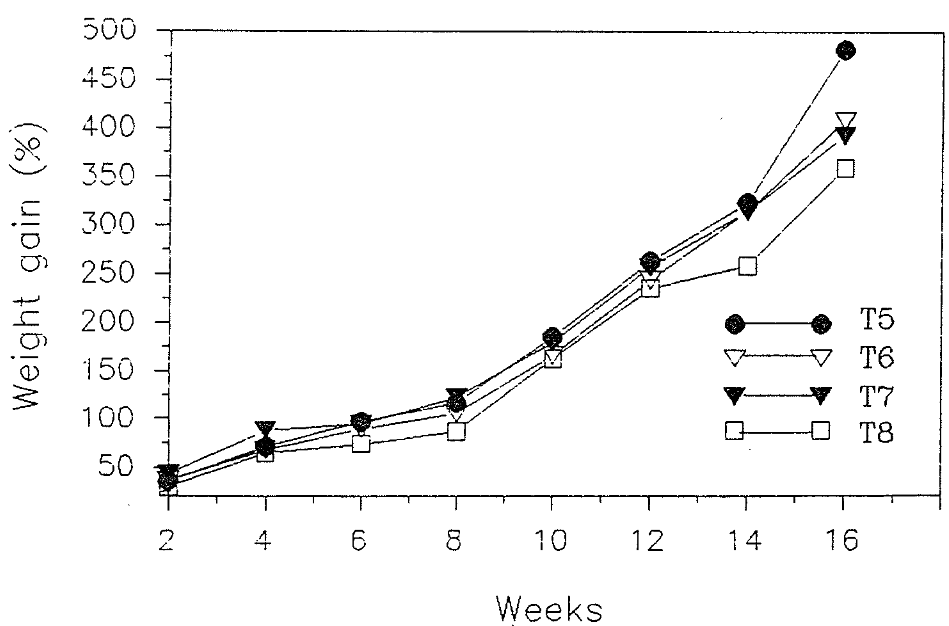



Fig.5 Time course of weight gain of prawns fed diets containing various levels of protein and methionine supplementation. Diets are defined in Table 7.

Table 17. Specific growth rate (%/prawn/day) of prawns fed diets containing various levels of protein and methionine supplementation, averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T5 | T6 | T7 | T8 |
|---|---|---|---|---|
| 0-2 | 2.01±0.57 | 2.14±0.48 | 2.50±0.48 | 1.75±0.66 |
| 0-4 | 1.85±0.37 | 1.79±0.34 | 2.16±0.43 | 1.73±0.29 |
| 0-6 | 1.59±0.17 | 1.49±0.16 | 1.55±0.24 | 1.29±0.18 |
| 0-8 | 1.37±0.07 | 1.27±0.09 | 1.39±0.19 | 1.11±0.07 |
| 0-10 | 1.48±0.11 | 1.39±0.05 | 1.46±0.08 | 1.37±0.05 |
| 0-12 | 1.52±0.09 | 1.47±0.05 | 1.49±0.13 | 1.44±0.05 |
| 0-14 | 1.46±0.07 | 1.37±0.05 | 1.43±0.10 | 1.29±0.07 |
| 0-16 | 1.56±0.07 | 1.45±0.03 | 1.41±0.09 | 1.36±0.02 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 7.

Table 18. Average length gained (%/prawn) by prawns fed diets containing various levels of protein and methionine supplementation, averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T5 | T6 | T7 | T8 |
|---|---|---|---|---|
| 0-2 | 8.51±1.55 | 7.65±3.97 | 9.49±2.72 | 9.09±3.23 |
| 0-4 | 16.44±1.94 | 14.17±4.12 | 14.87±3.25 | 14.31±4.29 |
| 0-6 | 19.67±1.83 | 17.02±3.19 | 16.71±3.57 | 15.89±4.64 |
| 0-8 | 23.21±1.92 | 19.73±2.88 | 20.77±3.57 | 18.73±3.66 |
| 0-10 | 30.91±2.58 | 28.69±2.30 | 30.77±3.50 | 32.90±2.94 |
| 0-12 | 40.50±4.02 | 38.89±2.53 | 38.78±3.88 | 42.49±2.05 |
| 0-14 | 52.12±3.83 | 44.25±3.91 | 46.78±5.56 | 45.36±3.66 |
| 0-16 | 61.53±3.89 | 52.52±1.87 | 52.22±5.68 | 56.07±1.32 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 7.

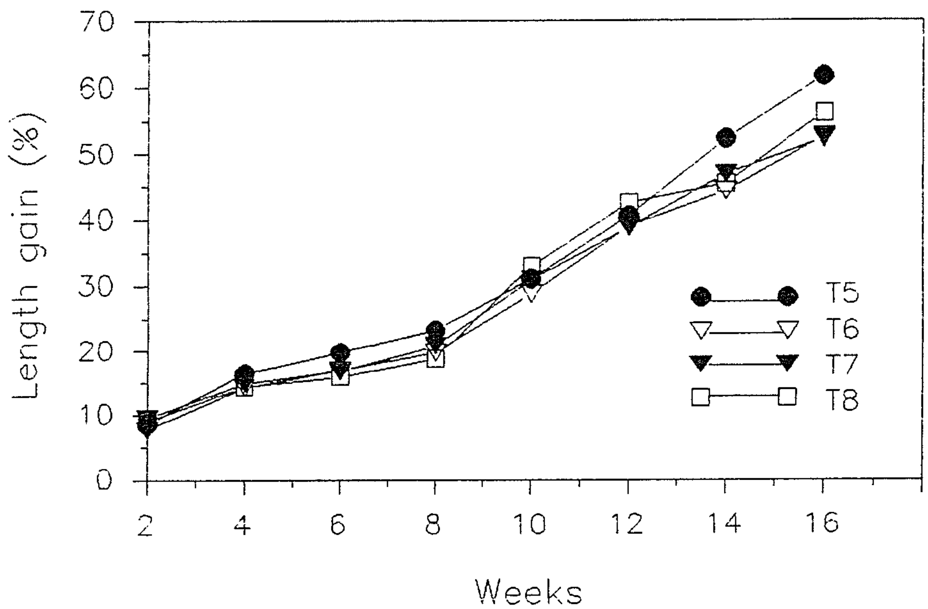



Fig.6 Time course of length gain of prawns fed diets containing various levels of protein and methionine supplementation. Diets are defined in Table 7.

Table 19 and Fig.7 present the feed conversion ratios of prawns fed various protein diets with methionine supplementation for 16 weeks. It was found that the feed conversion ratio of 1.98 in prawns fed high protein T5 diet was significantly lower (i.e., higher feed efficiency) than those fed low protein T7 and T8 diets with added methionine where the ratios were 2.64 and 2.54 ($P<0.05$), respectively. The ratio of 2.26 in prawns fed T6 diet was not significantly different from the other three ratios.

For the protein efficiency ratio (Table 20), it was found that there were no significant differences among the experimental diets containing various protein and supplemental methionine levels. The protein efficiency ratios over the 16 weeks were 1.68, 1.63, 1.57, and 1.61 of prawns fed T5, T6, T7, and T8 diets, respectively.

Feed cost per unit of production (Table 21) of prawns fed the low dietary protein with supplemented low methionine (T8) tended to be lower, but not significantly different from those fed the high dietary protein (T5 and T6) or the low dietary protein with supplemented high methionine (T7). Proximate analysis of prawn carcass (Table 21) showed, on a dry weight basis, that the percent tail meat, dry matter, crude protein, lipid, crude fiber, ash, calcium and phosphorus did not differ significantly among the groups. The carcass composition of prawns averaged over all diets was 42.51% tail meat, 24.59% dry matter, 59.52% protein, 8.81% lipid, 7.70% crude fiber, 14.15% ash, 5.95% Ca, and 1.08% P.

Table 19. Feed conversion ratio of prawns fed diets containing various levels of protein and methionine supplementation, averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T5 | T6 | T7 | T8 |
|---|---|---|---|---|
| 0-2 | 3.67±1.65 | 3.05±1.22 | 2.30±0.44 | 4.28±2.03 |
| 0-4 | 2.93±0.93 | 3.07±1.11 | 2.47±0.65 | 2.89±0.75 |
| 0-6 | 2.19±0.26 | 2.39±0.44 | 2.56±0.49 | 2.92±0.58 |
| 0-8 | 2.13±0.08 | 2.36±0.28 | 2.35±0.35 | 2.78±0.19 |
| 0-10 | 2.21±0.18 | 2.35±0.19 | 2.39±0.11 | 2.37±0.08 |
| 0-12 | 2.22±0.15 | 2.31±0.18 | 2.50±0.29 | 2.36±0.11 |
| 0-14 | 2.12±0.09 | 2.35±0.23 | 2.38±0.23 | 2.60±0.26 |
| 0-16 | 1.98±0.12[b] | 2.26±0.11[ab] | 2.64±0.22[a] | 2.54±0.08[a] |

[a,b] Means with a row with different superscripts are significantly different at the P=0.05 level.
[1] SE = Standard error of estimate
[2] Diets are described in Table 7.

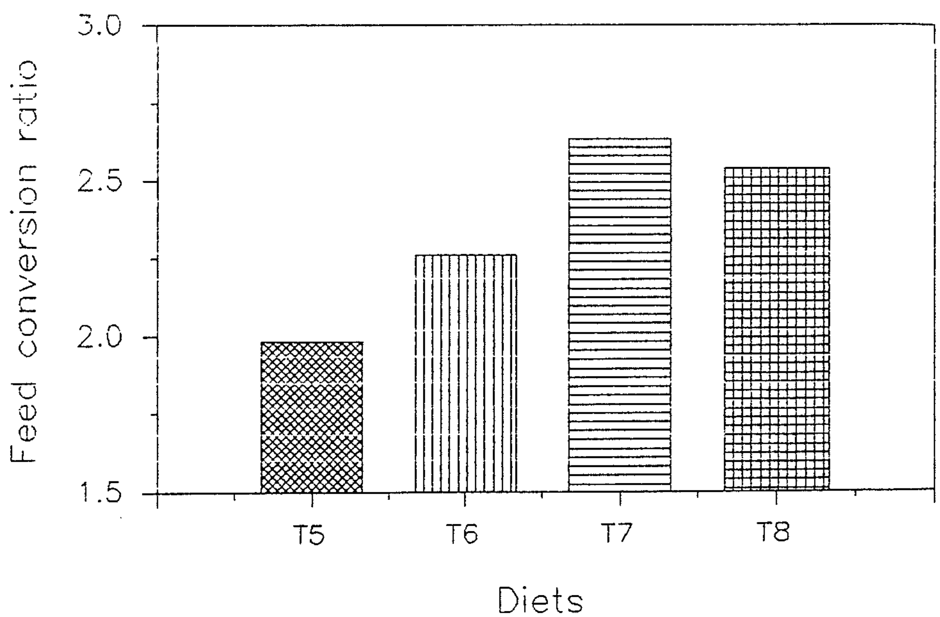



Fig.7 Feed conversion ratio of prawns fed diets containing various levels of protein and methionine supplementation for 16 weeks. Diets are defined in Table 7.

Table 20. Protein efficiency ratio (PER) and apparent net protein utilization (NPU,%) of prawns fed diets containing various levels of protein and methionine supplementation, averaged over four tanks per treatment (mean ± SE[1]).

| Period (Weeks) | Diet[2] T5 | T6 | T7 | T8 |
|---|---|---|---|---|
| Protein efficiency ratio (PER) | | | | |
| 0-2 | 1.38±0.39 | 1.66±0.41 | 1.98±0.38 | 1.57±0.49 |
| 0-4 | 1.40±0.29 | 1.55±0.35 | 1.98±0.45 | 1.63±0.29 |
| 0-6 | 1.57±0.17 | 1.66±0.25 | 1.76±0.31 | 1.53±0.24 |
| 0-8 | 1.56±0.06 | 1.61±0.19 | 1.86±0.29 | 1.48±0.09 |
| 0-10 | 1.52±0.12 | 1.58±0.14 | 1.72±0.09 | 1.72±0.06 |
| 0-12 | 1.51±0.11 | 1.60±0.12 | 1.69±0.20 | 1.73±0.07 |
| 0-14 | 1.57±0.07 | 1.60±0.16 | 1.75±0.16 | 1.61±0.14 |
| 0-16 | 1.68±0.10 | 1.63±0.08 | 1.57±0.13 | 1.61±0.05 |
| Apparent net protein utilization (NPU,%) | | | | |
| 0-16 | 31.49±1.84 | 30.73±2.28 | 31.06±2.58 | 31.02±1.05 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 7.

Table 21. Carcass composition and feed cost per unit of production (Baht/kg tail meat) of prawns fed diets containing various levels of protein and methionine supplementation for 16 weeks, averaged over four tanks per treatment (mean ± SE[1]).

| Analyses | Diet[2] T5 | T6 | T7 | T8 |
|---|---|---|---|---|
| Feed cost per unit of production (Baht/kg tail meat)[3] | 81.38±6.09 | 80.63±4.83 | 83.52±4.97 | 73.06±3.05 |
| Tail meat, % | 41.64±0.61 | 42.63±0.93 | 42.54±0.71 | 43.26±0.87 |
| Dry matter, % | 25.77±1.99 | 24.63±1.12 | 23.69±0.30 | 24.29±0.69 |
| Crude protein, % | 58.68±0.67 | 58.80±1.30 | 61.03±1.07 | 59.58±0.89 |
| Lipid, % | 9.39±0.58 | 8.98±0.60 | 7.60±0.62 | 9.27±0.49 |
| Crude fiber, % | 7.80±0.05 | 7.83±0.34 | 7.40±0.58 | 7.78±0.33 |
| Ash, % | 14.38±0.14 | 14.37±0.36 | 14.27±0.43 | 13.57±0.09 |
| Ca, % | 5.97±0.09 | 6.02±0.34 | 5.99±0.36 | 5.83±0.05 |
| P, % | 0.91±0.04 | 1.06±0.04 | 1.27±0.06 | 1.08±0.09 |

[1] SE = Standard error of estimate
[2] Diets are described in Table 7.
[3] 22 Baht = 1 Can$

# 5. DISCUSSION

## 5.1 The Utilization of Roasted Full-Fat soybean in diets by *Macrobrachium rosenbergii* Juveniles

The composition of roasted full-fat soybean (8.81% moisture, 91.19% dry matter, 5.24% ash, 37.59% protein, 16.42% fat, 8.97% fiber, 4451.10 kcal/kg of energy, 0.58% phosphorus, and 0.35% calcium) determined in this study was similar to that reported by NRC (1983). In the present study, prawns fed partial or complete replacement of soybean meal by roasted full-fat soybean (T2, T3, T4 diets) showed average survival rate, weight gain, specific growth rate, length gain, size distribution, feed conversion ratio and survival rate which were not significantly different from the group fed the T1 diet. The present data suggest that the tested diets containing roasted full-fat soybean were highly palatable to *M. rosenbergii*. A similar observation was made for full-fat soybean diets fed to rainbow trout by Reinitz et al. (1978).

However, the prawns fed the highest level of roasted FFSB tended to have a lower survival rate than those fed the lower levels of roasted FFSB. This may be caused by the high dietary lipid content (11.92%). In fact, the lipid in the diet of *M. rosenbergii* should not exceed 10%. Akiyama et al. (1991) stated that decreased growth and increased mortalities are associated with lipid levels exceeding 10%. Similarly, New (1976) reported that, at 15% lipid in diets, growth rate of prawns was significantly reduced. This may be caused by suppressed metabolism, toxic effect of the lipids or limitations of lipase activity.

The percentage weight gain and specific growth rate at 10 weeks of prawns fed T1 diet were significantly higher ($P<0.05$) than those fed T2, T3, and T4 diets. The reason is still unclear, however the technique of housing prawns individually adopted in this trial was not successful in totally eliminating territorial aggression and cannibalism. This may have produced errors in FFSB effectiveness because prawns may have eaten each other in addition to their food.

The results indicated that the inactivation of trypsin inhibitor activity by roasting full-fat soybean enhanced the nutritive value of this plant protein source for prawns as measured by the above growth parameters. It was established that prawns grew well when fed properly roasted full-fat soybean in which 95-98% of the trypsin inhibitor activity was destroyed by heating even though it contained 3-5% raw full-fat soybean. Similarly, Viola et al. (1983) found that properly heated soybean meal, in which 95-100% of the trypsin inhibitor activity was inactivated, was incorporated into diets without adversely affecting carp.

Wee and Shu (1989) reported excellent growth of Nile tilapia obtained with diets containing wholly boiled soybean as the plant protein source. Boiling not only destroyed 80% of the trypsin inhibitor, it also enhanced the availability of carbohydrate, as indicated by improved total digestibility as the amount of boiled full-fat soybean meal was increased in the tested diets. Nevertheless, the roasting method of raw full-fat soybean was more effective in reducing trypsin inhibitor activity than the boiling method (95% compared with 80%). Compared to other procedures such as autoclaving and steaming, roasting is simpler and more appropriate for the small-scale farmer because the procedure does not require special apparatus and the urease test is easy to perform. In any case, the urease test can be done with any form of cooking.

Full-fat soybean in these experimental diets contained 1.5-2.5% (Monari, 1991) lecithin as a phospholipid which may be utilized by prawns. Crustaceans have long been known to have a limited ability to synthesize phospholipids *de novo*. This biosynthesis is not sufficient to satisfy the metabolic requirements of crustaceans (Briggs et al., 1988). D'Abramo et al. (1981) for *Homarus species* and Kanazawa et al., (1985) for *Penaeus japonicus* reported that dietary soybean lecithin at levels between 0.5-0.8% of the diets proved essential in terms of growth and survival in casein-based purified diets.

However, in this study, there were no significant differences in growth and survival among treatments even when the diets incorporated various levels of roasted full-fat soybean and dietary lipids increased as the FFSB proportion. The result was in contrast to the established lecithin requirements of marine prawns and lobsters as mentioned before. Similarly, Hilton et al. (1984) and Briggs et al. (1988) reported that cholesterol and lecithin supplementation proved unnecessary for adequate growth and survival rate in purified and semi-purified diets fed to *M. rosenbergii* juveniles.

## 5.2 The Utilization of Methionine Supplementation in Low Protein Diets by *Macrobrachium rosenbergii* Juveniles

In this study, there were no significant differences in weight gain, length gain, specific growth rate and survival rate between prawns fed FFSB-based diets containing protein levels ranging from a high of 30% to a low of 24% with methionine supplementation ranging from 0.25-0.36%. This may be due to the multi-ingredient rations and a balanced amino acid profile with methionine supplementation. In contrast, Lee (1971) reported in *Penaeus monodon* that there was a proportional increase in growth rate when protein levels were increased from 18.3% to 45.8%. Alsot, the digestion and absorption of protein in a high protein diet was superior to that in a low protein diet. However, the optimal levels of dietary protein for prawns are 27-35% (Corbin et al., 1983). Dietary protein levels as low as 14-15% have been found to be feasible for penaeids and carideans when the diets include a suitable balance of amino acids (New, 1976).

The supplementation of methionine was effective because a well balanced amino acid profile was present in the squid viscera meal component of the diets. New (1976) noted that a basal diet of squid meal, squid meat extract, and squid liver extract gave improved growth rate with *Penaeus japonicus* when supplemented with methionine. Furthermore, the experimental diet used in this study contained high energy (approximately 3600 ME kcal/kg). New (1976) stated that low protein/high energy and high protein/low energy prawn diets gave better growth rates and poorer survival than either low protein/low energy or high protein/high energy diets.

In this study, the beneficial effects of methionine supplemented between 0.25-0.36% in low protein diets apparently were not affected by any amino acid imbalance or by toxic affect known to result from high methionine dosages (Murai et al., 1989). Wee and Shu (1989) reported that the residual trypsin inhibitor in properly processed soybean meal was not thought to be the limiting factor for the growth of carp but rather that inadequacy of lysine could be limiting. However, lysine is not essential for *M. rosenbergii* (Halver, 1989). Murai et al. (1989) found that addition of more than 1% of methionine to a petroleum yeast diet induced remarkable growth retardation in fingerling rainbow trout. Andrews and Page (1974) were unable to show a beneficial effect when 0.15-0.30% methionine was added to soybean meal diets for channel catfish, *Ictalurus punctatus*, and it may be that the requirement of catfish for methionine is much lower than that for prawns.

Possibly, the supplemented methionine in low protein diets may not be of real benefit because the prawns fed high protein (30%) diet tended, not statistically significantly, to have a higher percentage weight gain, specific growth rate, and length gain than those fed the three lower protein diets. In support of this possibility is the fact that the feed conversion ratio of prawns fed the high protein diet is significantly lower than those fed the two lowest protein diets. Protein efficiency ratio and apparent net protein utilization were not significantly different among treatments.

A factor confounding the results from the two experiments is the extent to which algae present in the tanks contributed to prawn nutrition. Prawns have an omnivorous mode of feeding, which in part consists of meticulous searching and ingestion of various items. Also for a period following final metamorphosis, prawns will at times swim freely through the water column. Filter-feeding may contribute to the prawns' nutrition during this activity.

### 5.3 Significance of the Results of This Study to Small-Scale Prawn Farmers

The final average live weights of prawns in the two experiments were 5.88 g for 5 month-old prawns in Experiment 1 and 9.38 g for 6 month-old prawns in Experiment 2 (Appendices 1 and 2). New and Singholka (1982) showed that *Macrobrachium rosenbergii* grew faster than this in earthen ponds so that after 8 months of culture, they weighed 70 g. However, the results from the present study were similar to those of Foster and Beard (1974). Who reported that juvenile *Macrobrachium rosenbergii* averaging 0.15 g, stocked at densities of 25 and 166 prawns/$m^2$ for 4 months in small laboratory aquaria, grew to prawns averaging 11.9 g at the lower stocking density and 6.7 g at the higher stocking density. Survival rates were 45 and 73%, respectively.

In summary, over a 16 week period the diets containing 4 levels of roasted FFSB in Experiment 1 and the diets with reduced protein level with supplemental methionine in Experiment 2 were as effective as the control diets in promoting growth and survival of prawns. These formulating diets would be acceptable and applicable for farmers to culture prawns and increase profitability. The feed cost per kg (Tables 4,5,8) was the lowest in the highest dietary FFSB and in the lowest dietary protein and supplemental methionine. Furthermore, the feed cost/ kg tail meat (Table 21) was also the lowest in the lowest dietary protein with the low added methionine.

However, further studies on the utilization of roasted full-fat soybean and methionine supplementation in diets for *Macrobrachium rosenbergii* juveniles should be undertaken on the following aspects.

1. The utilization and evaluation of raw full-fat soybeans on the nutritive value and effects of trypsin inhibitor activity in prawn diets.

2. Higher levels of full-fat soybeans than 42% should be determined for effectiveness and their limitations.

3. Total nutrients and energy digestibility of roasted full-fat soybeans in prawn diets need to be investigated.

4. The minimum and maximum levels of methionine which would be sufficient as a supplemental amino acid when full-fat soybean provides the majority of protein.

5. The exact amount of each amino acid requirement of prawns should be quantified.

7. The result of these experiments should be repeated to confirm their effectiveness when cannibalism by *Macrobrachium rosenbergii* is completely eliminated.

8. The observations on individual prawns should be done followed by an ANOVA with blocks in order to increase statistical power to detect differences among treatments.

# 6. CONCLUSIONS

1. Juvenile *M. rosenbergii* fed diets of roasted full-fat soybean as the sole plant protein source achieved similar growth parameters as prawns on control defatted soybean meal diet.

2. Roasted full-fat soybean can partially or completely substitute for defatted soybean meal and comprise up to 42% of prawn diet.

3. Properly roasted full-fat soybean meal, in which 95-98% of trypsin inhibitor activity was destroyed, was incorporated into diets without adversely affecting growth and survival of prawns.

4. Palatability and physical structure of roasted full-fat soybean in diets were acceptable to *M. rosenbergii.*

5. The roasting method of raw full-fat soybean was effective in reducing trypsin inhibitor activity. Since the method does not require particular apparatus, it would be feasible and appropriate for the small-scale farmer to use.

6. The use of high levels of full-fat soybean meal in prawn rations may be limited by lipid content.

7. Prawns fed the high dietary protein had a significantly lower feed conversion ratio than those fed the low dietary protein.

8. Protein levels in full-fat soybean based, methionine-supplemented diets for juvenile *M. rosenbergii* can be lowered from 30% to 24%.

9. Prawns on low protein diets with supplemented methionine at 0.25-0.36% in low protein diets were not affected by amino acid imbalances and methionine toxicity.

10. The reduction of protein levels with methionine-supplemented is a cost-effective diet for prawn production.

# 7.APPENDICES

**Appendix 1.** Raw data from Experiment 1: the utilization of roasted full-fat soybean in diets by *Macrobrachium rosenbergii* juveniles

| Weeks | Number | | | | Av. weight (g) | | | | Av. length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T1 | T2 | T3 | T4 | T1 | T2 | T3 | T4 |
| 0 | 50 | 50 | 50 | 50 | 0.66 | 0.76 | 0.70 | 0.66 | 3.20 | 3.38 | 3.32 | 3.48 |
| | 50 | 50 | 50 | 50 | 0.64 | 0.66 | 0.70 | 0.70 | 3.36 | 3.40 | 3.38 | 3.38 |
| | 50 | 50 | 50 | 50 | 0.66 | 0.70 | 0.68 | 0.66 | 3.30 | 3.34 | 3.22 | 3.28 |
| | 50 | 50 | 50 | 50 | 0.70 | 0.70 | 0.64 | 0.70 | 3.34 | 3.36 | 3.44 | 3.38 |
| 2 | 50 | 50 | 50 | 50 | 1.02 | 1.23 | 1.07 | 1.00 | 3.86 | 3.73 | 3.77 | 3.70 |
| | 50 | 50 | 50 | 50 | 0.97 | 1.16 | 1.34 | 1.36 | 3.87 | 3.64 | 3.58 | 3.66 |
| | 50 | 50 | 50 | 50 | 1.21 | 0.93 | 1.12 | 1.30 | 3.81 | 3.95 | 3.76 | 3.48 |
| | 50 | 50 | 50 | 49 | 1.32 | 1.13 | 1.09 | 1.25 | 3.54 | 3.53 | 3.85 | 3.71 |
| 4 | 50 | 49 | 50 | 50 | 1.36 | 2.03 | 1.58 | 1.40 | 3.98 | 4.12 | 4.26 | 3.82 |
| | 49 | 50 | 50 | 50 | 1.49 | 1.42 | 1.71 | 1.83 | 3.68 | 4.12 | 4.00 | 4.08 |
| | 50 | 50 | 50 | 50 | 1.71 | 1.71 | 1.50 | 1.71 | 3.84 | 4.40 | 4.16 | 3.80 |
| | 50 | 50 | 50 | 48 | 1.83 | 1.48 | 1.35 | 1.76 | 3.82 | 3.92 | 4.00 | 3.90 |
| 6 | 47 | 48 | 49 | 46 | 2.00 | 2.81 | 2.50 | 2.05 | 5.44 | 5.80 | 5.42 | 4.90 |
| | 48 | 48 | 48 | 49 | 2.29 | 2.50 | 2.71 | 2.76 | 5.46 | 5.32 | 6.12 | 5.62 |
| | 49 | 50 | 50 | 44 | 2.55 | 2.54 | 2.29 | 2.39 | 5.42 | 5.66 | 5.32 | 5.36 |
| | 47 | 47 | 48 | 42 | 2.67 | 2.07 | 2.40 | 2.74 | 5.74 | 5.56 | 5.26 | 5.44 |
| 8 | 45 | 46 | 49 | 46 | 3.07 | 3.70 | 3.33 | 3.10 | 6.18 | 5.58 | 6.12 | 5.76 |
| | 45 | 46 | 43 | 49 | 3.24 | 3.80 | 3.88 | 3.26 | 5.84 | 5.80 | 6.00 | 5.94 |
| | 49 | 50 | 46 | 41 | 3.71 | 3.30 | 2.95 | 3.50 | 5.90 | 6.12 | 5.78 | 6.00 |
| | 39 | 40 | 42 | 42 | 4.29 | 2.92 | 3.40 | 4.40 | 6.14 | 5.88 | 5.88 | 5.54 |
| 10 | 45 | 43 | 46 | 46 | 4.44 | 4.74 | 4.02 | 3.95 | 6.18 | 6.36 | 6.06 | 5.80 |
| | 43 | 42 | 43 | 49 | 3.93 | 3.81 | 4.43 | 4.09 | 6.26 | 6.36 | 6.24 | 6.18 |
| | 49 | 50 | 46 | 41 | 4.50 | 4.06 | 3.80 | 4.02 | 6.28 | 5.76 | 5.48 | 5.78 |
| | 39 | 40 | 42 | 41 | 5.15 | 3.60 | 4.13 | 4.51 | 6.40 | 6.02 | 6.70 | 6.30 |
| 12 | 45 | 43 | 46 | 46 | 5.67 | 5.93 | 5.11 | 4.89 | 6.62 | 6.74 | 6.60 | 6.36 |
| | 43 | 42 | 40 | 45 | 5.47 | 6.55 | 6.00 | 5.23 | 6.30 | 6.80 | 6.62 | 6.60 |
| | 45 | 46 | 46 | 38 | 5.89 | 5.76 | 5.00 | 5.13 | 6.78 | 6.88 | 6.64 | 7.04 |
| | 35 | 40 | 42 | 40 | 6.71 | 5.93 | 5.12 | 6.50 | 6.28 | 6.32 | 6.84 | 7.08 |
| 14 | 45 | 41 | 42 | 42 | 6.55 | 7.10 | 5.81 | 5.45 | 6.44 | 6.06 | 6.72 | 6.98 |
| | 43 | - | 38 | 45 | 6.99 | - | 7.26 | 6.45 | 6.58 | - | 7.08 | 6.68 |
| | 45 | 45 | 46 | 33 | 7.30 | 6.63 | 6.27 | 7.04 | 7.36 | 6.38 | 6.30 | 6.24 |
| | 35 | 40 | 40 | 39 | 8.32 | 5.99 | 6.78 | 7.40 | 6.74 | 6.74 | 6.78 | 6.66 |
| 16 | 43 | 38 | 40 | 35 | 7.79 | 8.29 | 6.50 | 6.57 | 6.93 | 7.31 | 6.52 | 6.58 |
| | 39 | - | 34 | 40 | 8.08 | - | 8.97 | 8.13 | 7.13 | - | 7.25 | 6.97 |
| | 41 | 45 | 43 | 30 | 8.12 | 7.78 | 7.79 | 8.17 | 7.17 | 6.78 | 6.88 | 7.08 |
| | 32 | 34 | 37 | 37 | 9.38 | 5.88 | 6.68 | 8.51 | 7.52 | 6.52 | 6.74 | 7.33 |

- missing data.

**Appendix 2.** Raw data from Experiment 2 : the utilization of methionine supplementation in low protein diets by *Macrobrachium rosenbergii* juveniles

| Weeks | Number | | | | Av. weight (g) | | | | Av. length (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T5 | T6 | T7 | T8 | T5 | T6 | T7 | T8 | T5 | T6 | T7 | T8 |
| 0 | 20 | 20 | 20 | 20 | 1.87 | 1.71 | 2.07 | 2.01 | 4.80 | 4.67 | 4.87 | 4.81 |
| | 20 | 20 | 20 | 20 | 2.13 | 1.98 | 1.95 | 1.80 | 5.00 | 4.74 | 4.87 | 4.95 |
| | 20 | 20 | 20 | 20 | 1.63 | 1.94 | 1.90 | 1.71 | 4.61 | 4.67 | 4.87 | 4.89 |
| | 20 | 20 | 20 | 20 | 1.79 | 2.12 | 2.00 | 2.00 | 4.71 | 4.69 | 4.94 | 4.96 |
| 2 | 20 | 19 | 20 | 20 | 2.90 | 2.52 | 2.25 | 2.58 | 5.23 | 5.21 | 5.07 | 5.26 |
| | 20 | 20 | 19 | 19 | 2.92 | 3.05 | 2.18 | 2.53 | 5.39 | 5.62 | 4.92 | 5.09 |
| | 20 | 19 | 20 | 20 | 2.52 | 2.49 | 2.37 | 2.80 | 5.30 | 5.25 | 5.07 | 5.27 |
| | 19 | 19 | 19 | 19 | 2.80 | 2.59 | 2.03 | 2.75 | 5.35 | 5.38 | 4.86 | 5.32 |
| 4 | 19 | 19 | 20 | 19 | 3.60 | 3.36 | 2.62 | 3.35 | 5.57 | 5.70 | 5.54 | 5.48 |
| | 19 | 20 | 19 | 19 | 3.71 | 3.85 | 2.44 | 3.17 | 5.90 | 5.86 | 5.16 | 5.38 |
| | 20 | 18 | 18 | 18 | 3.51 | 3.13 | 2.63 | 3.98 | 5.63 | 5.42 | 5.26 | 5.60 |
| | 19 | 18 | 18 | 19 | 3.47 | 3.50 | 2.61 | 3.33 | 5.81 | 5.61 | 5.06 | 5.43 |
| 6 | 18 | 15 | 19 | 13 | 4.10 | 3.71 | 3.39 | 3.71 | 5.80 | 5.80 | 5.64 | 5.66 |
| | 19 | 15 | 16 | 17 | 4.28 | 4.15 | 3.00 | 3.40 | 5.98 | 5.90 | 5.39 | 5.59 |
| | 18 | 16 | 18 | 13 | 3.56 | 3.33 | 2.89 | 4.02 | 5.74 | 5.50 | 5.30 | 5.72 |
| | 9 | 14 | 16 | 15 | 3.61 | 3.82 | 2.83 | 3.40 | 5.92 | 5.70 | 5.11 | 5.47 |
| 8 | 15 | 10 | 16 | 12 | 4.30 | 3.95 | 4.18 | 4.04 | 5.92 | 6.00 | 5.83 | 5.83 |
| | 15 | 13 | 13 | 13 | 4.73 | 4.34 | 3.53 | 3.52 | 6.11 | 5.98 | 5.51 | 5.80 |
| | 15 | 12 | 14 | 11 | 4.61 | 3.45 | 3.59 | 4.06 | 6.03 | 5.58 | 5.64 | 5.80 |
| | 9 | 11 | 15 | 11 | 3.63 | 3.95 | 3.39 | 3.75 | 5.95 | 5.80 | 5.40 | 5.60 |
| 10 | 13 | 10 | 15 | 12 | 5.75 | 5.75 | 5.15 | 4.90 | 6.26 | 6.46 | 6.19 | 6.14 |
| | 14 | 12 | 14 | 12 | 6.22 | 5.22 | 4.75 | 4.73 | 6.59 | 6.32 | 6.09 | 6.16 |
| | 15 | 12 | 12 | 11 | 5.31 | 4.88 | 4.97 | 4.74 | 6.41 | 6.21 | 6.06 | 6.27 |
| | 9 | 10 | 12 | 10 | 5.16 | 5.49 | 4.93 | 5.07 | 6.39 | 6.56 | 6.19 | 6.33 |
| 12 | 12 | 7 | 15 | 10 | 6.33 | 7.69 | 6.44 | 7.05 | 6.72 | 7.10 | 6.59 | 6.47 |
| | 14 | 12 | 13 | 11 | 7.66 | 7.15 | 5.90 | 6.28 | 6.94 | 6.91 | 6.54 | 6.75 |
| | 15 | 12 | 14 | 10 | 7.47 | 5.91 | 5.31 | 5.56 | 6.87 | 6.51 | 6.46 | 6.64 |
| | 9 | 8 | 12 | 8 | 6.53 | 7.09 | 6.06 | 6.74 | 6.72 | 6.95 | 6.71 | 6.94 |
| 14 | 11 | 7 | 12 | 9 | 7.99 | 8.54 | 8.01 | 7.51 | 7.40 | 7.54 | 6.98 | 7.18 |
| | 14 | 12 | 13 | 11 | 8.89 | 8.37 | 6.81 | 6.29 | 7.34 | 7.30 | 6.71 | 6.84 |
| | 13 | 11 | 14 | 9 | 8.18 | 7.13 | 6.16 | 7.81 | 7.38 | 6.49 | 6.74 | 7.39 |
| | 9 | 8 | 11 | 7 | 7.27 | 7.60 | 7.52 | 5.80 | 6.98 | 7.09 | 7.12 | 6.67 |
| 16 | 11 | 7 | 13 | 7 | 11.01 | 11.70 | 9.71 | 11.69 | 7.76 | 7.99 | 7.40 | 7.76 |
| | 13 | 10 | 13 | 10 | 11.27 | 9.30 | 9.43 | 9.75 | 7.65 | 7.43 | 7.20 | 7.52 |
| | 13 | 10 | 13 | 9 | 9.56 | 7.88 | 7.82 | 9.53 | 7.59 | 6.83 | 6.90 | 7.72 |
| | 9 | 8 | 10 | 2 | 8.44 | 9.35 | 9.59 | 8.75 | 7.24 | 7.49 | 7.45 | 7.75 |

# 8. REFERENCES

Akiyama, D.M., 1988. Soybean meal utilization by marine shrimp In Abstracts from World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs, Singapore, October 1988, vol. 2 AQ 13.

Akiyama,D.M. and Dominy W.G.,1989. Penaeid shrimp nutrition for the commercial feed industry. American Soybean Associaton , MC(P) 15/1/89 Vol.3 AQ 18.

Akiyama,D.M., Dominy W.G., and Lawrence, A.L.,1991. Penaeid shrimp nutrition for the commercial feed industry. American Soybean Associaton , MITA(P) 93/1/91 vol. AQ 32.

Andrews, J.W. and Page, J.W., 1974. Growth factors in the fish meal components of catfish diets. *Journal of Nutrition*, 104:1091-1096.

Aquacop, 1983. Intensive larval rearing in clear water of *Macrobrachium rosenbergii* (de man, Anuenue stock) at the centre Oceanologique du Pacifique, Tahiti. p.179-187. In *CRC Handbook of Mariculture v.1 Crustacean Aquaculture*. Edited by McVey, J.P. CRC Press, Inc. Boca Raton, Florida.

Baker, D.H., Katz, R.S., and Easter, R.A., 1975. Lysine requirement of growing pigs at two levels of dietary protein. *Journal of Animal Science*, 40:851-855.

Baker, J.C., Tomlinson, J.E., and McGee, 1986. The evaluation of soybean meal, roasted whole soybeans or whole cottonseed as a concentrate ingredient for lactation dairy cows. *Journal of Dairy Science*, supplement 1, 221. Abstract.

Balogun, A.M. and Ologhobo, A.D. 1989. Growth performance and nutrient utlization of fingerling *Clarias gyrepinus* (Burcsell) fed raw and cooked soybean diets, *Aquaculture*, 76:119-126.

Briggs, M.R.P., Jauncey, K., and Brown, J.H., 1988. The cholesterol and lecithin requirements of juvenile prawn (*Macrobrachium rosenbergii*) fed semi-purified diet. *Aquaculture*. 70:121-129.

Brown, H.W., Herman, B.G., and Jenson, A.H., 1973. Lysine requirement of the finishing pig for maximum carcass leaness. *Journal of Animal Science*, 37:1159-1164.

Collado, C.M., 1989. Full-fat soybean meal: The alternative to feed energy shortage. Published by American Soybean Association, v. PO6 1989-12.

Corbin, J.S., Fujiimoto, M.M and Iwai, I.Y.Jr., 1983. Feeding practices and nutritional considerations for *Macrobrachium rosenbergii* culture in Hawaii. p. 391-412. In *CRC Handbook of Mariculture v.1 Crustacean Aquaculture*. Edited by McVey, J.P. CRC Press, Inc. Boca Raton, Florida.

Costa, H.H., and Wanninayake, T.B., 1986. Food, feeding, and fecundity of the giant freshwater prawn *Macrobrachium rosenbergii* from natural habitats in Sri Lanka. *The First Asian Forum. Proceedings of the First Asian Fisheries Forum*, Manila, Philippines, 26-31 May 1986. pp. 555-558.

Cunha, T.J., 1957. Swine feeding and nutrition. Interference Publishes Inc., New York.

D'Abramo, L.R., Bordner, C.E., Conklin, D.E., and Brum, N.A., 1981. Essentiality of dietary phosphatidylcholine for the survival of juvenile lobsters, *Homarus*. *Journal of Nutrition*, 111:425-431.

Dall,W. and Moriarty, D.J.W., 1983. Functional aspects of nutrition and digestion. p.215-261. In *The Biology of Crustacea v.5 Internal Anatomy and Physiological Regulation.* Mantel,L.H.,ed. Academic Press, New York.

Feller,M. and Herrfurth, A.G., 1986. Developments in Asian shrimp culture. *Marine Fisheries Review*, 48:50-54.

Forster, J.R.M. and Beard, T.W., 1974. Experiments to assess the suitability of nine species of prawns for intensive cultivation. Aquaculture, 3:355-368.

Halver, J.E., 1989. Fish nutrition. $2^{nd}$ed. Academic Press Inc. 798p.

Hilton, J.W., Harrison, J.W., and Slinger, S.J., 1984. A semi-purified test diet for *Macrobrachium rosenbergii* and the lack of need for supplemental lecithin. *Aquaculture*, 37:209-215.

Holmes, B., 1988. Quality control of raw material and finished production in fullfat soya production. American Soybean Association. MC(P) 8/1/88.

Huner, J.V. and Brown, E.E., 1985. Crustacean and mollusk aquaculture in the United States. AVI publishing company Inc. Westport, Connecticut.

Jayachandran, K.V., and Joseph, N.I., 1989. Food and feeding habitats of slender river prawn, *Macrobrachium idella* (Hilgendorf, 1898) (Decapoda, Palaemonidae) *Mahasagar*. 22:121-129.

Kanazawa, A., Teshima, S., and Sakamoto, M., 1985. Effects of dietary lipids, fatty acids and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. *Aquaculture*, 50:39-49.

Khajarern, J., Sinchermsiri, D., Hanbunchong, A., and Kanto, U., 1987.

Manual of feed microscopy and quality control. In Collaboration with American Soybean Association National Renderers Association and US Feed Grains Council.

Kleinbaum, D.G. and Kupper, L.L., 1978. Applied regression analysis and other multivariable methods. Duxbury Press, 556p.

Kung, L. Jr., and Huber, J.T., 1983. Performance of high producing cows in early lactation fed protein of varying amounts, sources and degradability. *Journal of Dairy Science*, 66:227-234.

Latshaw, J.D., 1974. Soybean processing and its effect on the laying hen. *Poultry Science*, 53:1342-1347.

Latshaw,J.D. and Clayton, P.C., 1976. Raw and heated full fat soybeans in laying diets, *Poultry Science*, 55:1268-1272.

Lee, D.L., 1971. Studies on the protein utilization related to growth in *Penaeus monodon. Aquaculture*, 1:1-13.

Liao, I-chiu and Chao, Nai-Hsien, 1983. Hatchery and grow-out penaeid prawns. *Handbook of Mariculture V.1 Crustacean Aquaculture*. J.P. McVey CRC Press, Inc. Boca Raton, Florida. pp. 155-167.

Lovell, R.T., 1981. Laboratory manual for fish analysis and fish nutrition studies. Auburn University, p65.

Lovell, R.T., 1987. Nutrient requirements of warm water fishes and use of plant proteins in diets of aquatic species. Publication of American Soybean Association, Vol. 1 AQ 1.

Lovett, D.L., and Felder, D.L., 1988. Evaluation of the rotifer *Brachionus plicatilis* as a substitute for *Artemia* in feeding larvae of *Macrobrachium rosenbergii. Aquaculture.* 71:330-338.

Lunchick, C.A., Clawson, J., Armstrong, W.D., and Linnerud, A.C., 1978.

Protein level, lysine level and source interaction in young pigs. *Journal of Animal Science*, 47:177-183.

Malecha, S., 1983. Commercial seed production of the freshwater prawn, *Macrobrachium rosenbergii*, in Hawaii. p.205-230. In *CRC Handbook of Mariculture v.1 Crustacean Aquaculture*. Edited by McVey, J.P. CRC press, Inc. Boca Raton, Florida.

Monari, S., 1991. Full fat soya handbook. Published by American Soybean Association, 40p.

Moran, E.T., Somers, J., and Lomond, E., 1973. Fullfat soybeans for growing and finishing turkeys 1. Live performance and carcass quality. *Poultry Science*, 52:1936-1941.

Murai, T., Daozun, W., and Ogata, H., 1989. Supplementation of methionine to soy flour diets for fingerling carp, *Cyprinus capio. Aquaculture*, 77:373-385.

Najib, H., and Sullivan, T.E., 1982. Influence of protein and SAA levels on egg size and other production criteria during the onset and last phase of lay. *Poultry Science*, 61:1518.

New, M.B.,1976. A review of dietary studies with shrimp and prawns. *Aquaculture*, 9:101-104.

New, M.B. and Singholka, S., 1982. Freshwater prawn farming. A manual for the culture of *Macrobrachium rosenbergii.* Food and Agriculture Organization of the United Nations. Rome. 116p.

National Research Council (NRC), 1983. Nutrient requirements of warm water fishes and shellfishes. Revised edition. National Academy Press. Washington D.C. 102p.

Olson, D.W., Sunde, M.L., and Bird, H.R., 1969. Amino acid supplementation of mandioca meal chick diets. *Poultry Science*, 45(6):1949-1953.

Owen, F.G., Larson, L.I., and Lowry, S.R., 1985. Effects of roasted and grinding of soybeans on lactation performance. *Dairy Science Abatracts*, 68(1):123.

Piedad-Pascual, F. 1986. Effect of supplemental lecithin and lipid sources on the growth and survival of *Penaeus monodon* juveniles *.p 615-618. The First Asian Forum. Proceedings of the First Asian Fisheries Forum*, Manila, Philippines, 26-31 May 1986.

Porter, P.J. and Britten, W.M., 1974. Fatty acid composition of chicks fed fullfat soybeans. *Poultry Science*, 53:1137-1141.

Provenzano, A.J., 1985. The Biology of Crustacea v.10 Economic Aspects: Fisheries and Culture. Academic Press, Inc. 331p.

Reinitz, G.L., Orme, L.E., Lemm, C.A., and Hitzel, F.M., 1978. Full-fat soybean in Rainbow trout diets. *Feedstuffs*, 50:23-24.

Rodwell, V.W., 1983. Biosynthesis of amino acids. p. 265-272. In *Harper's Review of Biochemistry*. 19th ed. Martin, D.W., Mayes, P.A., and Rodwell, V.W., eds. Lange Medical Publications. Los Altos California 94022.

Sandifer, P.A. and Smith, T.I.J., 1985. Freshwater prawns. p.63-65. In *Crustacean and Mollusk Aquaculture in the United States*. Edited by Huner, J.V. and Brown, E.E. AVI Publishing Company, Inc. Westport, Connecticut.

Sandifer, P.A., Smith, T.I.J., Jenkins, W.E., and Stokes, A.D., 1983. Seasonal culture of freshwater prawns in South Carolina. p.189-204. In *CRC Handbook of Mariculture v.1 Crustacean Aquaculture*. Edited by

McVey, J.P. CRC press, Inc. Boca Raton, Florida.

Seerly, R.W., Emberson, J.W., McCampbell, H.C., Burdick, D. and Grimes, L.W., 1974. Cooked soybeans in swine and rat diets. *J.A.S.* 39:1082-1091.

Shen,T.F., Bird, H.R. and Sunde, M.L., 1970. Cooked full-fat soybeans for turkey poults, *Poultry Science*, 49:1738-1740.

Sick, L.V., and Millikin, M.R., 1983. Dietary and nutrient requirements for culture of the Asian prawn, *Macrobrachium rosenbergii*. p.381-389. In *Handbook of Mariculture V.1 Crustacean Aquaculture*. McVey, J.P.,ed. CRC Press, Inc. Boca Raton, Florida.

Smith, N.E., Collar, L.S., Bath, D.L., Dunkley, W.L., and Frank, A.A., 1980. Whole cottonseed and extruded soybean for cows in early lactation. *Journal of Dairy Science*, 63 Supplement 153 Abstract.

Suksri, A., Seripong, S., and Teerapongtanakorn, S., 1990. Effects of organic manures and inoculation on grown and yield of off season soybean cultivars growth on an acid soil. *Thai Agricultural Research Journal*, 8:43-49.

Turner, C.A., Kienholz, C.A., Harper, J.M., and Raghavan, V.C., 1973. Roasted soybeans in growing and finishing diets for large turkeys. *Feedstuffs*, 4:30-31.

Viola,S., Mokady,S and Arieli, Y., 1983. Effects of soybean processing method on the growth of carp (*Cyprinus carpio*). *Aquaculture*, 32:27-38.

Vogt, G., Quinitio, E.T., and Pascual, F.P., 1986. *Leucaena leucocephla* leaves in formulated feed for *Penaeus monodon* : a concrete example of the application of histology in nutrition research. *Aquaculture*, 59:209-234.

Walroup, P.W., and Cotton, T.L., 1974. Maximum usage levels of cooked full-fat soybeans in all mash broiler diets. *Poultry Science*, 53:677-680.

Wee, K.L. and Shu, S., 1989. The nutritive value of boiled full-fat soybean in pelleted feed for Nile Tilapia. *Aquaculture*, 81:303-314.

White,C.L., Greene, D.E., Waldroup, P.W., and Stephenson, E.L., 1967. The use of unextracted soybean for chicks. I. Comparison of infrared cooked, autoclaved, and extruded soybean, *Poultry Science*, 44:1180-1185.

Wickins, J.F., 1982. Opportunities for farming crustaceans in western temperate regions. p.87-178. In *Recent advances in aquaculture*. J.E. Muir and R.J. Roberts eds. Croom helm, London and Canberra.

Wilson, R.P. and Poe, W.E., 1985. Effects of feeding soybean meal with varying trypsin inhibitor activities on growth of fingerling channel catfish. *Aquaculture*, 46:19-25.

Wood,A.S., Summers, J.D., Moran, E.T.Jr., and Pepper, W.F., 1971. The utilization of unextracted, raw and extruded full-fat soybeans by the chick, *Poultry Science*, 50:1392-1399.