ADVANCES IN TROPICAL AQUACULTURE. Tahiti, Feb. 20 - March 4. 1989.
AQUACOP IFREMER. Actes de Colloque 9 pp. 261-270.

27

Protein requirements of Penaeid shrimp

A. KANAZAWA
Faculty of Fisheries, Kagoshima University, 4-50-20. Shimoarata, KAGOSHIMA 890, Japan

Abstract. — *Proteins are indispensable nutrients for growth and maintenance of live of all animals. The optimum protein levels in diets for shrimps are different among the various species. Squid meal is an effective protein source for many penaeids. The effects of dietary protein, lipid, and carbohydrate levels on the growth and survival of larvae of* ***Penaeus japonicus*** *were examined by feeding trials using purified diet with carrageenan as a binder. As a result, the effects of protein levels on growth and survival of* ***P. japonicus*** *larvae varied with dietary carbohydrate levels but not dietary lipid levels.*

Proteins having an essential amino acid profile similar to that of body ar like to have a high nutritive for shrimp. Therefore, the amino acid composition of body protein of shrimp was analyzed, and then diets using various protein sources to simulate the amino acid profile of the body protein of shrimp were chosen. As a result of feeding trials, the shrimp showed good growth and high survival when the amino acid profile of the diet simulated that of the body protein of shrimp.

The superiority of crab protein as a protein source in diets has been demonstrated in the feeding trial of some crustaceans. However, we have found that crab protein as a sole protein source is not good enough to sustain larval growth and survival of ***P.japonicus*** *probably due to both its physical property and unsatisfactory amino acid profile, but it can be used together with other protein sources in diets of larval* ***P. japonicus.***

ESSENTIAL AMINO ACID OF SHRIMP

Proteins are indispensable nutrients for growth and maintenance of life of all animals. As the shrimp and prawn have difficulties of efficiently utilizing free amino acid in diets (Deshimaru and Kuroki, 1974; 1975a,b; Deshimaru, 1982), the incorporation of radioactive acetate into individual amino acid of shrimps was investigated in order to determine requirements of essential amino acid. The shrimp and prawn were shown to require 10 amino acids, arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine and tryptophan (Cowey and Forster, 1971;

Shewbart *et al*, 1972; Miyajima and Broderick, 1977; Coloso and Cruz, 1980; Kanazawa and Teshima, 1981; Pascual and Kanazawa, 1986).

PROTEIN REQUIREMENTS OF PENAEID SHRIMP

Several groups of workers have reported the optimum protein levels in diets for Penaeids : *Penaeus japonicus* (52-57 % : Deshimaru and Yone, 1978), *Penaeus indicus* (43 % : Colvin, 1976), *Penaeus monodon* (46 % : Lee, 1971; 40 % : Aquacop, 1977; 40 % : Khannapa, 1977; 35 % : Bages and Sloane, 1981), *Penaeus aztecus* 23-31 % (Shewbart et *al*, 1973); 40 % : Venkataramiah et *al.*, 1975), *Penaeus setiferus* 28-32 % (Andrews et *al.*, 1972). *Penaeus californiensis* 31 % (Colvin and Brand, 1977), *Penaeus vannamei* (30 % : Colvin and Brand, 1977; 36 % : Smith et *al.*, 1985), *Penaeus stylirostris* (35 % : Colvin and Brand, 1977), *Penaeus merguiensis* (50 % : Aquacop, 1978; 34-42 % : Sedgwick, 1979), *Metapenaeus monoceros* 55 % : Kanazawa et *al.*, 1981), Metapenaeus macleayi (27 % : Maguire and Hume, 1982), and for freswater shrimp : *Macrobrachium resenbergii* (25 % : Clifford and Brick, 1978; 30-40 % : Ashmore et *al.*, 1985).

The optimum protein levels in diets for Penaeid shrimp are different among the various species. I assume that the diversity of optimum protein levels for crustaceans is likely to come from a variety of factors, namely, the discrepancy in food habits, age of specimens, water temperature, protein sources used, and energy level of the diet.

EFFECT OF PROTEIN LIPID, AND CARBOHYDRATE LEVELS ON GROWTH OF PRAWN LARVAE

The effects of dietary protein, lipid, and carbohydrate levels on growth and survival of the prawn larvae were examined by the feeding trials using purified diets with carrageenan as a binder (Teshima and Kanazawa, 1984). In experiment 1, the larvae were fed 12 diets containing various levels of protein (Casein; 25, 35, 45 and 55 %) and lipid (6.5, 11.5 and 16.5 %) at a fixed carbohydrate level of 15 %. In experiment 2, the prawn larvae were given 9 diets containing various levels of protein (35, 45 and 55 %) and carbohydrate (5, 15 and 25 %) at a fixed lipid level of 6.5 %.

The effect of protein levels on growth and survival of the prawn varied with the dietary carbohydrate levels but not with the dietary lipid levels. The elevation of lipid levels from 6.5 % to 16.5 % did not improve growth and survival when the diets contained sufficient levels (15 % or more) of carbohydrate. Contrarily, the elevation of dietary carbohydrate levels from 5 to 25 % improved the survival of the prawn larvae when the diets contained low levels (35-45 %) of protein. On the basis of these data, the optimum protein levels for the prawn larvae were estimated to be around 45 %, 45-55 % and 55 % or more when the diet contained 25 %, 15 % and 5 % levels of carbohydrate, respectively (Fig. 1.).

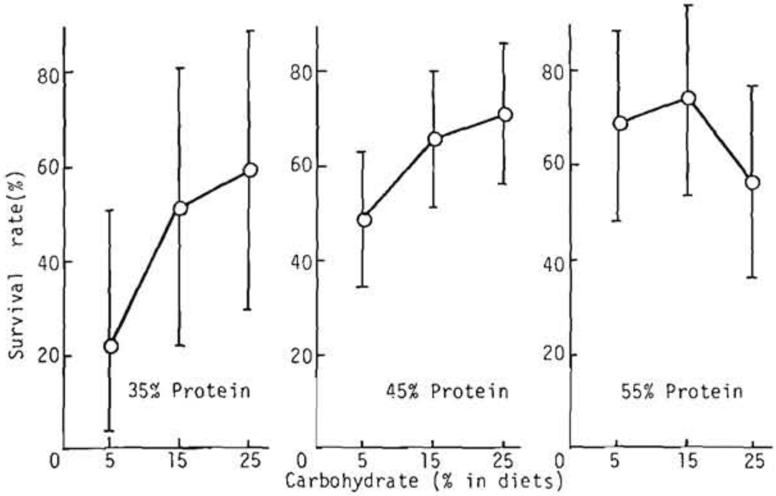


Fig. 1. — Effect of dietary Carbohydrate levels on the survival rates (means ê confidence limits at P=0.95) of *P. Japonicus* larvae.

PROTEIN HAVING ESSENTIAL AMINO ACID PROFILE SIMILAR TO THAT OF SHRIMP BODY

Generally, proteins having an essential amino acid (EAA) profile similar to that of whole body are like to have a high nutritive value for prawn and shrimp (Kanazawa, 1985; Kanazawa, 1986; Kanazawa and Teshima, 1988; Kanazawa, 1988). Therefore, the amino acid composition of whole body proteins of larval prawn *P. japonicus* was analyzed, and then four test diets containing various protein sources to simulate the amino acid profile of larval body protein were formulated using a computer. Four microparticulate diets were formulated with a soybean meal, chicken egg yolk, krill meal, white fish meal, skim milk, squid meal, brown fish meal, yeast powder, gluten meal, short-necked clam and fresh bonito milt (Tables 1 and 2). Feeding experiments were conducted using zoeal larvae of P. *japonicus* obtained from wild egg-bearing females. The zoea larvae were divided into lots of 100 individuals in 1-litre beakers containing sea water (specific gravity, 1.026) filtered a column of cotton and maintained at 29.0 + 1.0°C. Test diets were given to the prawn larvae at a feeding concentration of 0.08 mg/larva twice a day (Teshima and Kanazawa, 1983). The rearing water was renewed every day after the evaluation of growth and survial rates of larvae.

All test diets were based on carrageenan micro-bound diet with particle sizes of <53 μm for zoea 1 - —oea 2, 53-125 μm for zoea 3 — mysis 2 and 125-250 μm for mysis 3 — postlarva 1. Test diets were prepared according to the following process : mixing of powdered ingredients and carrageenan at 85°C, cooling in a refrigerator, freeze-drying, crumbling into particles, and sieving for the desired particle size. Dietary value was evaluated by the survival rate (%) and growth index of larvae,

Tab. 1. — Composition of test diet.

Ingredient (g/100 g)	Diet −1	Diet −2	Diet −3	Diet −4
Soybean meal		5.5	19.3	13.8
Chicken egg yolk powder	20.0	17.4	17.4	17.4
Krill meal	15.0	8.4	8.4	8.4
White fish meal			12.5	12.5
Skim lilk	15.0	3.1		
Squid meal	10.0	11.0	9.2	5.5
Browwn fish meal		19.1		11.4
Yeast powder	10.0	9.4	2.3	2.3
Gluten meal		1.4		
Short-necked clam extract	4.0	4.0	4.0	4.0
Pollack liver oil	7.0	7.0	7.0	7.0
Soybean lecithin	3.0	3.0	3.0	3.0
Cholesterol	0.5	0.5	0.5	0.5
Vitamin mixture	6.0	6.0	6.0	6.0
Mineral mixture	4.0	4.0	4.0	4.0
Cellulose	5.5	0.2	6.4	4.2
TOTAL	100.0	100.0	100.0	100.0
Carrageenan	5.0	5.0	5.0	5.0
Fresh bonito milt	25.0	25.0	25.0	25.0

Tab. 2. — Essential amino acid composition of relative ratio to menthionine.

Amino Acid	P. japonicus	Diet −1	Diet −2	Diet −3	Diet −4
MET	1.00	1.00	1.00	1.00	1.00
THR	1.16	1.22	.41	1.36	1.40
VAL	1.44	1.52	1.66	1.64	1.68
ILE	1.44	1.35	1.48	1.53	1.53
LEU	2.46	2.17	2.51	2.48	2.51
PHE	1.28	1.39	1.56	1.49	1.52
HIS	0.69	0.70	0.89	0.81	0.88
LYS	2.62	2.40	2.76	2.63	2.75
TRP	1.27	0.83	1.14	1.02	1.09
ARG	3.53	2.96	3.23	3.41	3.37

Tab. 3. — Effects of test diets on survival rate and growth index of prawn larvae.

Diet no	Feeding period (day)	Survival rate (%)	Growth index
1 a/b	10	91	7.0
	10	89	7.0
2 a/b	10	94	7.0
	10	90	7.0
3 a/b	10	73	6.8
	10	69	6.9
4 a/b	10	91	7.0
	10	95	7.0

1. Growth index : $zoea_1$, 1; $zoea_2$, 2; $zoea_3$, 3;
: $mysis_1$, 4; $mysis_2$, 5; $post\text{-}larva_1$, 7.

determined daily on 10 samples and on all surviving larvae at the end of the feeding trials when one of the experiment groups reached the stage postlarva 1. Statistical analysis of survival and growth data was performed by analysis of variance. The results of feeding trial are shown in Table 3. The larval prawn fed diets 1, 2 and 4 showed good growth and high survival rates when the amino acid profile of the diet simulated that of the body protein of larval prawn. However, growth and survival of diet 3 containing 19.3 % soybean meal were inferior to diets 1, 2 and 4 (Kanazawa et *al.*, in preparation).

NUTRITIVE VALUE OF METHIONINE ENRICHED SOYBEAN PLASTEIN

Nutritive value of methionine enriched soybean plastein for Tilapia, *Oreochromis niloticus* fry was reported (Teshima and Kanazawa, in press). Feeding trials P. *japonicus* juvenile were conducted by Teshima et *al.* (in preparation) to examine the effects of supplemental methionine as crystalline amino acid or enriched soybean plastein on weight gain, feed conversion efficiency, and protein efficiency ratio. Growth was not enhanced by supplementing diet with methionine enriched soybean plastein gave significant improvements. These results indicate that methionine in soybean plastein is more effectively utilized by the prawn than crystalline methionine. This study proves the possibility of using methionine-enriched plastein to improve the nutritive value of methionine-deficient vegetable protein sources.

NUTRITIONAL EVALUATION OF CRAB PROTEIN FOR PRAWN

The superioritu of crab protein as a protein source in semipurified diets has been demonstrated in the feeding trial of juvenile lobsters, *Homarus americanus* (Boghen et *al.*, 1982) and juveniles of other crustaceans species such as P. *monodon*, P. *vannamei*, P. *stylirostris* and M. *rosenbergii* also grew and survived well with feeding on crab protein based diets (Castell et *al.*, in preparation). However, Koshio et *al.* (in press) have found that crab protein as a sole source is not good enough to sustain larval growth and survival of P. *Japonicus* probably due to both its physical property and unsatisfactory amino acid profile, but it can be used together with other protein sources such as casein in microparticulate diets of larval P. *japonicus* (Table 4 and Fig. 2). While, crab protein was recommended as the sole protein diet formulations for juvenile P. *japonicus*.

EFFECTS AND FREE AMINO ACIDS ON GROWTH OF PRAWN

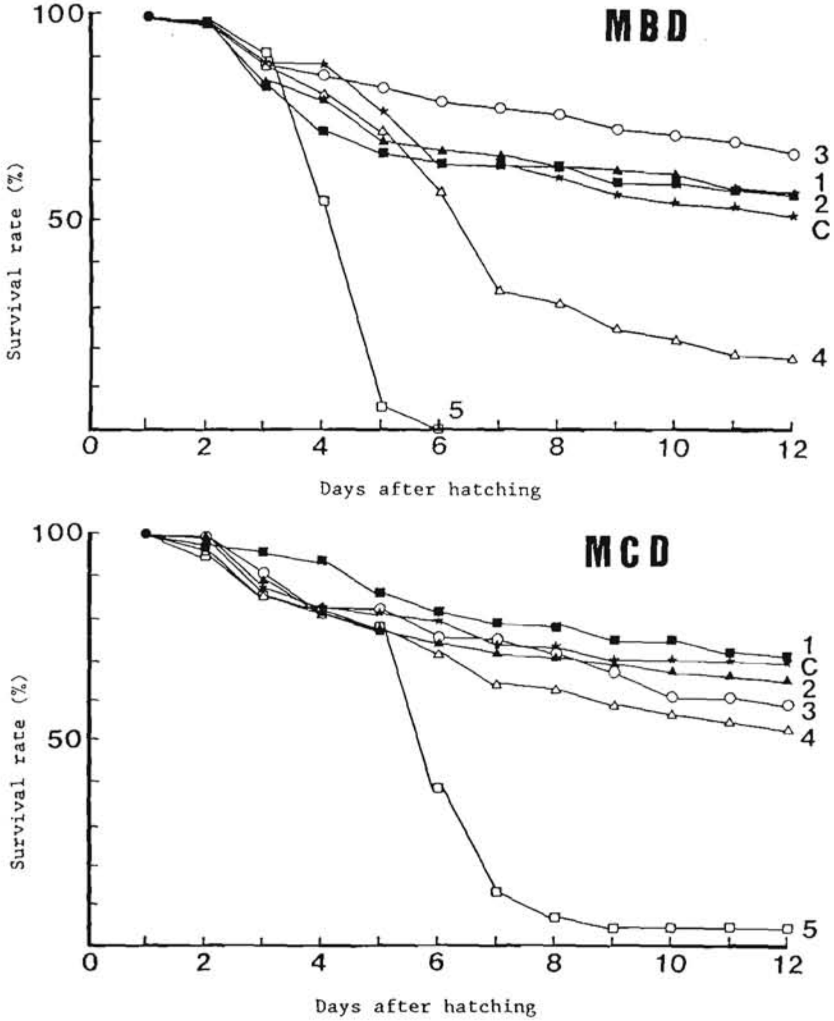
Deshimaru and Kuroki (1974, 1975a, b) and Deshimaru (1982) prepared a diet with a crystalline amino acid mixture instead of protein,

Tab. 4. — Formulation and proximate analysis of test diets.

Ingredient (g/100g)	Diet (MBD)[1]				
	1	2	3	4	5
Crab protein		15.0	35.0	55.0	6
Casein	51.0	39.2	23.4	7.6	
Amino acid mix.[2]	3.0	3.0	3.0	3.0	3
Alpha starch	5.0	5.0	5.0	5.0	5
Dextrin	4.7	4.7	4.7	4.7	4
Feed oil[3]	4.0	4.0	4.0	4.0	4
Corn oil	2.0	2.0	2.0	2.0	2
N^3-HUFA	1.0	1.0	1.0	1.0	1
Cholesterol	0.5	0.5	0.5	0.5	0
Soybean lecithin	3.0	3.0	3.0	3.0	3
Mineral mix.	5.0	5.0	5.0	5.0	5
Vitamin mix.	5.0	5.0	5.0	5.0	5
Glucosamine-HCI	1.0	1.0	1.0	1.0	1
Sodium citrate	0.5	0.5	0.5	0.5	0
Sodium succinate	0.5	0.5	0.5	0.5	0
Alpha cellulose	13.8	10.6	6.4	2.2	
TOTAL	100.0	100.0	100.0	100.0	1
Carrageenan	5.0	5.0	5.0	5.0	5
Crude protein (%)					
MBD	49.9	46.7	47.1	46.3	4
NCD	51.2	47.6	48.2	48.0	4
Crude lipid (%)					
MBD	11.2	7.5	11.2	10.2	8
MCD	2.1	2.8	4.7	4.4	3
Gross energy (Kcal/g)					
MBD	4.29	3.75	4.13	3.99	3
MCD	3.50	3.36	3.57	3.54	3

1 Five MBD and control diet were Cholesterol-lecithin (MCD)
2 L-Phenylalanine 0.24, L-Arginine-HCl 0.54, L-Cystine 0.3, L-Tryptophan 0.2, L-Histidine-HCl, H_2O 0.12, DL-Alanine 0.9, L-Aspartic acid-Na 0.42, L-Lysine-HCl 0.24, L-Valine 0.3, Glycine 0.18.
3 Riken Vitamin Ltd., CO., Japan.

and found that such a diet was unsuitable for sustaining growth and survival of juvenile prawn. The supplemental amino for larval prawn were examined using artificial diets containing carrageenan as a binder (Teshima et *al.*, 1986). The supplementation of a casein diet with crystalline L-arginine HCl improved its nutritive value better than only casein. In the feeding trial, about half the casein in the casein-based diets was replaced with a mixture of crystalline amino acid, either coated or uncoated with a nylon-protein membrane, and balanced to approximate the amino acid protein to that of prawn larval whole body protein. Diet containing crystalline amino acids gave survival rates and growth equal to or higher than the control group receiving live food. These results indicate that prawn larvae are probably able to utilize crystalline amino acid mixture in contrast to juvenile prawns which lack this ability.



Fi. 2. — Survival of larval *P. japonicus* fed MBD and MCD. The diet numbers shown are as follow : diet (CPC casein = 0.51), Diet 2 (15 : 39), Diet 3 (35 : 23), Diet 4 (55 : 8), Diet 5 (65 : 0). 'C' indicates a protein mixture diet (egg yolk powder 15.5, Casein 10.0, Albumin 7.0, Squid meal 15.0, Yeast powder 10.0, Krill powder 15.0, Scallop extract powder 4.0, Amino acid mix, 5.0).

THE DIETARY EFFECTS ON OVARIAN MATURATION OF PRAWN

The technique of eyestalk ablation or removal has been widely used in the operation of seedling production of shrimp due to its effect of induced maturation of gonad and spawning. However, the operation often causes the stress to spawners and therefore high mortality. Mussels, oysters, clams and squids are employed for the broodstock foods and it is suggested that those contain the substances which induce the ovary maturation. The effects of food materials on ovary maturation were investigated in this study.

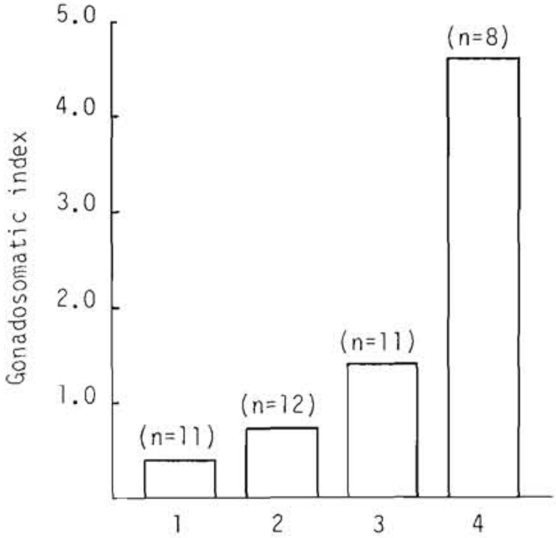


Fig. 3. — Variation of gonadosomatic index by destalking and feeding.
1. Initial (Before treatment)
2. Non-destalking and clam-fed
3. Destalking and unfed
4. Destalking and clam-fed.

Both eyestalks of shrimps (about 20 g) were removed by trying with a surgical thread after the acclimation to the experimental condition. Destalking group was divided into unfed and clam-fed groups, and control group was fed clam. After 10 day rearing period, the tissues were dissected out and gonadosomatic index (GSI) was measured (Fig. 3).

GSI of control groups was 0.69 whereas that of destalking groups was higher (1.42 in unfed and 4.54 in clam-fed groups, respectively). Furthermore, the effect of food after destalking was very important since GSI in clam-fed group was much greater than that in unfed one. It seems that the substances in clam meat, which induce the ovary maturation, are lipid fraction and low molecular nitrogen compounds.

Andrew, J.W., L.V. Sick and G.L. Baptist, 1972. The influence of dietary protein and energy levels on growth and survival of Penaeid shrimp. *Aquaculture*, **1** : 341-347.

Aquacop, 1977. Reproduction in captivity and growth of *Penaeus monodon* Fabricius in Polynesia. *Proc World Maricul. Soc.*, **8** : 927-945.

Aquacop, 1978. Study of nutrition requirements and growth of *Penaeus merguiensis* in tanks by means of purified and artificial diets. *Proc. World Maricu. Soc.*, **9** : 225-234.

Ashmore, S.B., R.W. Stanley, L.B. Moore, S.R. Malecha, 1985. Effect on growth and apparent digestibility of diets varying in grain source and protein level in *Macrobrachium rosenbergii. J. World Maricul. Soc.*, **16** : 205-216.

Bages, M. and L. Sloane, 1981. Effects of dietary protein and starch levels on growth and survival of *Penaeus monodon* (Fabricius) post larvae. *Aquaculture*, **25** : 117-128.

Boghen, A.D., J.D. Castell, D.E. Conklin, 1982. In search of a references protein to replace « vitamin -free casein » in lobster nutrition studies. *Can. J. Zool.*, **60** : 2033-2038.

Clifford, H.C., III and R.W. Brick, 1978. Protein utilization in the freswater shrimp *Macrobrachium rosenbergii. Proc. World Maricul. Soc.*, **9** : 195-208.

Coloso, R.M. and L.J. Cruz, 1980. Preliminary studies in some aspects of amino acid biosynthesis in juveniles of *Penaeus monodon* Fabricius-I. Incorporation of 14C from (U-14 C) acetate into amino acids of precipitable proteins. *Bull. Phil. Biochem. Soc.*, **3** : 12-22.

Colvin, P.M., 1976. Nutritional studies on penaeid prawns : Protein requirements in compounded diets for juvenile *Penaeus indicus* (Milne Edwards). *Aquaculture*, 7 : 315-326.

Colvin, L.B. and C.W. Brand, 1977. The protein requirement of Penaeid shrimp at various life-cycle stages with compounded diets in controlled environment systems. *Proc. World Maricul. Soc.*, **8** : 821-840.

Cowey, C.B. and J.R.M. Forster, 1971. The essential amino acid requirements of prawn, *Palaemon serratus. Mar. Biol.*, **10** : 77-81.

Deshimaru, O., 1982. Protein and amino acid nutrition of the prawn *Penaeus japonicus. In* : G.D. Pruder,C.J. Langdon, and D.E. Conklin (eds.). Proc. Second Intl. Conf. on Aquaculture Nutrition : Biochemical and Phisiological Approaches to Shellfish Nutrition, pp. 106-123.Louisiana State Univ., Baton Rouge, Louisiana.

Deshimaru, O. and K. Kuroki, 1974. Studies on a purified diet for prawn III. A feeding experiment with amino acid test diets. *Nippon Suisan Gakkaishi*, **40** : 1127-1131 (in japanese).

Deshimaru, O. and K. Kuroki, 1975a. Studies on a purified diet for prawn-IV. Evaluation of protein, free amino acid and their mixture as nitrogen source. *Nippon Suisan Gakkaishi*, **41** : 101-103 (in japanese).

Deshimaru, O. and K; Kuroki, 1975b. Studies on a purified diet for prawn-V. Evaluation of casein hydrolyzates as a nitrogen source. *Nippon Suisan Gakkaishi*, **41** : 301-304 (in japanese).

Deshimaru, O. and Y. Yone, 1978. Optimum level of dietary protein for prawn. *Nippon Suisan Gakkaishi*, **44** : 1395-1397.

Kanazawa, A., 1985. Microparticulate diet for larval prawn. *Fish Culture*, **22 (2)** : 44-47 (in japanese).

Kanazawa, A., 1986. New developments in fish nutrition. *In* : J.L.Maclean, L.B. Dizon and L.V. Hosillos (eds), Ist Asian Fish. Forum, Asian Fish Soc., Manila, pp. 9-14.

Kanazawa, A., 1988. Nutritional requirement and artificial feed of starry flounder larvae-I. *Fish Culture*, **25 (9)** : 116-119 (in japanese).

Kanazawa, A. and S. Teshima, 1981. Essential amino acids of the prawn. *Nippon Suisan Gakkaishi*, **47** : 1375-1337.

Kanazawa, A. and S. Teshima, 1988. Microparticulate diets for fish larvae. *In* : A.K. Sparks (ed), New and innovative advances in biology/engineering with potential for use in Aquaculture. NOAA Tech. Rep. NMFS 70, Natl. Mar. Fish. Serv., Seattle, pp. 57-62.

Kanazawa, A., S. Teshima, S. Matsumoto and T. Nomura, 1981. Dietary protein requirement of the shrimp *Metapenaeus monoceros. Nippon Suisan Gakkaishi,* **47** : 1371-1374.

Khannapa, A., 1977. Effect of various protein levels on growth and survival rates of *Penaeus monodon.* Q. Res. Rep., SEAFDEC Aquaculture Dept., 1 : 24-28.

Koshio, S., A. Kanazawa, S. Teshima and J.D. Castell, 1989. Nutritional evaluation of crab protein for larval *Penaeus japonicus* fed microparticulate diets. *Aquaculture* (in press).

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

Maguire, G.B. and I.D. Hume, 1982. A study of the nutritional requirements of school prawns *Metapenaeus macleayi* (Haswell) in some Australian brackish water farming ponds. *Aquaculture,* **29** : 261-278.

Miyajima, L.S. and G.A. Broderick, 1977. Identification of essential amino acids of the freswater shrimp *Macrobrachium ohione. Proc. World Maricul. Soc.,* **8** : 245-250.

Pascual, F.P. and A. Kanazawa, 1986. Specific amino acid-free semi-purified diets for *Penaeus monodon* juveniles. Mem. Kagoshima Univ. Res. Center S; Pac. 7 : 65-72.

Sedgwick, R.W., 1979. Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguiensis* de man. *Aquaculture,* **16** : 7-30.

Shewbart, K.L., W.L. Mies and P.D. Ludwig, 1972. Identification and quantitative analysis of the amino acids present in protein of the brown shrimp *Penaeus aztecus. Mar. Biol.,* **16** : 64-67.

Shewbart, K.L., W.L. Mies and P.D. Ludwig, 1973. Nutritional requirements of the brown shrimp *Penaeus aztecus.* US. Dept. Commer. Res. No. COM-73-11794, pp. 52.

Smith, L.L., P.G. Lee, A.L. Lawrence and K. Strawn; 1985. Growth and digestibility by three sizes of *Penaeus vannamei* Boone : Effects fo dietary protein level and protein sources. *Aquaculture,* **46** : 85-96.

Teshima, S. and A. Kanazawa, 1983. Effects of several factors on growth and survival of the prawn larvae reared with microparticulate diets. *Nippon Suisan Gakkaishi,* **49** : 1893-1896.

Teshima, S. and A. Kanazawa, 1984. Effects of protein, lipid and carbohydrate levels in purified diets on growth and survival rates of the prawn larvae. *Nippon Suisan Gakkaishi,* **50** : 1709-1715.

Teshima, S. and A. Kanazawa, 1989. Nutritive value of methionine-enriched soybean plastein for *Oreochromis niloticus* fry. *In* : Pullin. R. S. V., T. Bhukasawan, K. Tonguthai and J.L. Maclean (eds), 2nd Internal. Symp. on Tilapia in Aquaculture. ICLARM Conf. Proc. (in pres).

Teshima, S., A. Kanazawa and M. Yamashita, 1986. Dietary value of several proteins and supplemental amino acids for larvae of the prawn *Penaeus japonicus. Aquaculture,* **51** : 225-235.

Venkataramiah, A., G.J. Lakshi and G. Gunter, 1975. Effect of protein level and vegetable matter on growth and food conversion efficiency of brown shrimp. *Aquaculture,* **6** : 115-125.