クルマエビの成長と肝膵臓チアミンピロリン酸含量に対する飼料性チアミンおよび炭水化物の相互作用

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Interactive Effects of Dietary Thiamin and Carbohydrate on the Growth and Hepatopancreatic Thiamin Pyrophosphate Content of Kuruma Prawn, *Penaeus japonicus* Juveniles

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Abstract

A 2×2 factorial feeding experiment was conducted using two levels of supplemental thiamin (0 and 20 mg/100 g of diet) and two levels of supplemental carbohydrate (5 and 25 % of diet) for 12 weeks to investigate the interactive effects of these two substances on the growth and thiamin pyrophosphate content of *Penaeus japonicus* juveniles (initial body weight of 0.66 ± 0.04 g). Analysis of variance showed that there was significant interaction ($p < 0.05$) of thiamin and carbohydrate levels on weight gain, whole body glycogen content, muscle pyruvate content and hepatopancreatic TPP, but none on survival, feed intake, FCE, and contents of glycogen and pyruvate in the hepatopancreas. Weight gain of the prawns fed the thiamin supplemented diet was higher for each level of carbohydrate. These groups showed also higher survival, feed intake and feed conversion efficiency (FCE) than the thiamin-free groups. Whole body glycogen content and muscle pyruvate content of prawn fed the 25 % carbohydrate-supplemented diet was higher than in other groups, especially in the thiamin-free groups. Hepatopancreatic thiamin pyrophosphate (TPP) was lower in prawns fed the 25 % carbohydrate-supplemented diet independent of thiamin content of the diet.

Requirement levels of a particular nutrient may be affected by the level of another nutrient in either the diet or metabolically in the animal, hence the study of nutrient interactions and interrelationships[1]. Since thiamin and its derivatives are vital nutrient for all animals, including aquatic crustaceans[2-3], the thiamin requirements of some penaeid shrimp have been investigated. The requirement of *Penaeus japonicus* was estimated to be 6 mg and 12 mg per 100 g diet, based on the growth and the thiamin content of the body of the prawn, respectively[3]. On the contrary, the requirement of *Penaeus monodon* was reported only to be 1.3 mg, 1.4 mg and 5.8 mg per 100 g diet, based on growth, hemolymph thiamin content, and hemolymph thiamin pyrophosphate (TPP) content, respectively[4]. These figures are much lower than that for *P. japonicus*. The reasons for the apparent disparity in thiamin requirement among shrimp species are still not clear. Some possibilities such as diet composition, carbohydrate and lipid contents[1], size of shrimp and leaching

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of diet have been considered.

Carbohydrates are considered the least expensive form of dietary energy for animals, but their utilization and metabolism by shrimp are limited. Carbohydrates may also serve as precursors for various metabolic intermediates for growth, i. e., non-essential amino acids, nucleic acids and chitin[5-6]. Sucrose and glycogen are reportedly good sources of carbohydrate for *P. japonicus*[7]. Generally, disaccharides and polysaccharides are better carbohydrate sources for penaeid shrimp compared to monosaccharide[8-10].

Since thiamin serves as a coenzyme in carbohydrate metabolism in the oxidative decarboxylation of pyruvate and the transketolase of the phentose phosphate shunt, the presence of dietary thiamin is essential in the utilization of dietary carbohydrate to provide metabolic energy. Interaction of dietary thiamin and carbohydrate for rainbow trout has also been studied[11]. This research reported no significant effects of dietary carbohydrate on the thiamin requirement of fish, but differences in the transketolase activity of the kidney and liver, and pyruvate and lactate levels of the plasma were detected.

However, there is no available information that establishes the relationship between dietary thiamin and carbohydrate on shrimp. The present study was designed to investigate interactive effects between levels of dietary thiamin and carbohydrate on the growth performance of *P. japonicus* juveniles, and the effects on the hepatopancreatic TPP content as a parameter of the thiamin nutritional status of shrimp.

Materials and methods

Test diets

Four test diets were prepared containing either one of 2 levels of thiamin and carbohydrate. Test diets

Table 1. Composition of experimental diets (g/100 g diet)

Ingredients	Diet no.			
	1	2	3	4
Casein (vitamin free)	44.00	44.00	44.00	44.00
Squid meal	5.00	5.00	5.00	5.00
Arginine	1.52	1.52	1.52	1.52
Carbohydrate*1	5.00	5.00	25.00	25.00
Thiamin-HCl	-	0.02	-	0.02
Glucosamine-HCl	0.80	0.80	0.80	0.80
Na-succinate	0.30	0.30	0.30	0.30
Na-citrate	0.30	0.30	0.30	0.30
Pollack liver oil	2.00	2.00	2.00	2.00
Squid liver oil	3.00	3.00	3.00	3.00
Oleic acid ethyl ester	8.50	8.50	-	-
Soybean lecithin	3.00	3.00	3.00	3.00
Cholesterol	0.50	0.50	0.50	0.50
Attractant*2	2.20	2.20	2.20	2.20
Mineral mix*3	5.37	5.37	5.37	5.37
Vitamin mix (thiamin free)*4	1.99	1.99	1.99	1.99
α-Cellulose	11.52	11.50	0.02	-
Agar	5.00	5.00	5.00	5.00
Total	100	100	100	100

*1 Carbohydrate: mixture of sucrose, dextrin, and α-starch (2 : 1 : 2).

*2 Attractant (g per 100 g diet): betaine, 0.5; taurine, 0.5; proline, 0.3; alanine, 0.3; IMP, 0.1; glutathione, 0.1 and Na-glutamate, 0.4.

*3 Mineral (g per 100 g diet): K_2HPO_4, 1.203; $Ca_3(PO_4)_2$, 1.709; $MgSO_4$ $7H_2O$, 1.904 and NaH_2PO_4 $2H_2O$, 0.554.

*4 Vitamin (mg per 100 g diet): ρ-amino benzoic acid, 15.80; biotin, 0.63; inositol, 632.00; niacin, 63.20; Ca-pantothenate, 94.80; pyridoxine-HCl, 18.96; vitamin C (phospitan), 136.07; riboflavin, 12.64; menadione, 6.34; vitamin A-palmitate, 30.34; α-tocoferol, 31.60; calciferol, 1.88; cyanocobalamine, 0.13; folic acid, 1.26 and choline chloride, 948.00.

were isonitrogenous and isocaloric. Oleic acid was used to adjust the caloric content of the diet. Total proportions were adjusted to 100 % by the addition of α-cellulose (Table 1). The diets were prepared in the following manner. Agar, as binder, was dissolved in 160 m*l* of boiling water and was cooked gently for about 5 min. The diet premix was then poured into it and mixed thoroughly for 10 min. During the mixing, 1 N NaOH was added carefully to adjust the pH of the diet to 6. The diet was then steamed without pressure for 10 min, stuffed in Kurehalon plastic tubes, and again steamed for another 5 min. After cooling in air, diets were stored in the refrigerator (4 ℃) until used.

Feeding trial

Juveniles of the kuruma prawn, obtained from a commercial farm, were kept in a round holding tank and fed commercial feeds until they were used in the experiment. A total of 240 prawns (0.66 ± 0.04 g average body weight) was randomly selected for the experiment. Twenty prawns per tank with three replicate tanks per treatment were randomly housed in 54-*l* rectangular tanks ($60 \times 30 \times 30$ cm) with sand bottoms (2 cm thickness). Seawater, 33 ppt, was continuously supplied at a flow rate of 10 *l*/h. The seawater was recirculated through a sand bottom filter. Water temperature range during the feeding experiment was 23.6-28.5 ℃.

The prawns were fed once a day at 6 % (dry matter basis) body weight based on average initial weight. Feeding level was adjusted thereafter according to the feeding response of the prawn. Uneaten feed was collected and exuviae were removed each morning. All prawns were weighed and counted every two week. At the same time, all tanks and sand bottoms were cleaned and washed.

Thiamin and its phosphate ester analyses

Thiamin contents of the test diets, thiamin and its phosphate ester content in the hepatopancreas of prawns were determined by modified thiochrome methods using high performance liquid chromatography (HPLC) analysis[12-13]. The HPLC instrument was a Shimadzu LC-3A equipped with a Shimadzu fluorescence detector RF-535 (excitation wavelength at 375 nm and emission wavelength at 435 nm) and a chromatopac C-R1B integrator (Shimadzu). The analytical column was Cosmosil 5C18-AR, 10×250 mm, which was connected with Cosmosil 5C18-AR, 4.6×10 mm as guard column (from Nacalai Tesque, Inc.).

The mobile phase was 0.02 M sodium phosphate buffer (pH 4.3): methanol, (99 : 1, v/v) at a flow rate of 1.0 m*l*/min. The post-column derivatization reagent, 0.01 % potassium ferricyanide in 15 % NaOH (freshly prepared), was pumped into the eluent leaving the column through a T-junction at a flow rate of 0.3 m*l*/min.

The diet or hepatopancreas was mixed with 10 % cool trichloroacetic acid (TCA) and homogenized using a glass homogenizer in an ice bath. After centrifugation (4000 rpm, 25 min), the supernatant was extracted 4 times with three volumes of water-saturated diethyl ether. The collected sample was kept uncapped for 20-30 min to remove excess ether. The sample was then filtered through a 0.45 μm cellulose nitrate membrane filter (Advantec, Toyo Roshi Kaisha, Japan) and 20 μl of filtrate was introduced into the HPLC injection port. Thiamin-HCL, thiamin pyrophosphate chloride (Wako Pure Chemical Industries, Ltd., Japan) and thiamin monophosphate chloride (Sigma) were used as standards.

Glycogen and pyruvate analyses

Glycogen contents in the hepatopancreas and whole body of prawns were analyzed according to Carrol *et al.*[14] using anthrone reagent. The freeze-dried sample was homogenized in 5 % TCA, centrifuged at 3000 rpm for 5 min and the supernatant was decanted upon an acid-washed filter paper placed in a funnel draining into a volumetric flask. The residue was homogenized again in 5 % TCA and centrifuged, and the supernatant was poured through the same filter. Two more extractions were made in the same manner and the combined supernatant was made up to the desired volume with TCA 5 %.

To 1 m*l* supernatant in the test tube, 5 m*l* of 95 % ethanol was added carefully. The test tube was capped and allowed to stand overnight at room temperature. The mixture was then centrifuged at 3000 rpm for 15

min. The clear liquid was decanted, and the residue (glycogen) was allowed to dry at room temperature. The glycogen was dissolved in 2 m*l* water, and then 10 m*l* anthrone reagent was added. The tube was capped, shaken well, and then immersed in a boiling water bath for 15 min. The tube was allowed to cool to room temperature and the optical density of the glycogen solution was read at 620 nm. The standard was prepared by pipetting 2 m*l* of benzoic acid solution containing 0.1 mg glucose into a similar tube. The glucose was processed in the same way as described for the sample. Glycogen content was calculated as: mg glycogen / g tissue = (DS / DG) × (volume of extract / g of tissue) × 0.1 × 0.9, where DS: optical density of the sample; DG: optical density of the glucose standard; 0.1: mg of glucose in 2 m*l* standard solution; and 0.9: the factor for converting glucose value to glycogen value.

For pyruvate analysis, fresh tissue (hepatopancreas or muscle) was immediately homogenized in cool 8 % (w/v) perchloric acid and centrifuged at 5000 rpm, 4 ℃ for 15 min. Collected supernatant was recentrifuged to obtain a clear supernatant for analysis. Samples were stored at 4 ℃ before further analysis. Pyruvate was analyzed with a commercial kit (Sigma Chemical Co. Tech. Bull. Nos. 726-UV), which employs the lactate dehydrogenase. The reduced nicotinamide adenine dinucleotide (NADH) is then measured spectrophotometrically. Calibration curves were prepared by using pyruvic acid standard solution (Sigma Chemical Co.).

Statistical analysis

Statistical test for the measured parameters was performed using analysis of variance (ANOVA) (Super ANOVA, Abacus Concepts, Inc., 1991). All references to statistical significance were at the 5 % level.

Results

Growth, survival, feed intake and FCE

Changes in the weight gain of prawns fed experimental diets during the 12 week feeding trial are presented in Fig. 1. Prawns fed diets 3 and 4, which both contained 25 % carbohydrate, consistently resulted in higher weight gains compare to the other groups. Factorial analysis of variance indicated that different levels of carbohydrate and thiamin in the diet had significant effects on weight gain, survival, feed intake and feed conversion efficiency (FCE). Effects of carbohydrate on weight gain were significant after 4 weeks, while effects of thiamin were significant after 6 weeks. Among these above mentioned parameters, only weight gain was significantly different when the interactive effects of these two factors was considered (Table 2). This significance was first observed at the 10^{th} week of feeding experiment.

Glycogen and pyruvate

Glycogen content in the hepatopancreas and body, and pyruvate content in the hepatopancreas and

Table 2. Performance of the prawn fed experimental diets for 12 weeks[*1]

Dietary factors		Diet no.	Final weight (g)	Weight gain (%)	Survival (%)	Feed intake[*2]	FCE[*3]
Carbohydrate	Thiamin						
5	0	1	2.17±0.04	229±6	82	5.77±0.17	0.36±0.01
5	20	2	2.50±0.02	279±4	88	5.92±0.12	0.43±0.01
25	0	3	2.64±0.04	301±6	72	6.04±0.10	0.44±0.01
25	20	4	3.21±0.06	387±9	78	6.47±0.14	0.53±0.02
Carbohydrate				0.0001[*4]	0.0003	0.0008	0.0001
Thiamin				0.0001	0.0039	0.0058	0.0001
Carbohydrate × thiamin				0.0012	NS[*5]	NS	NS

[*1] Initial weight is 0.66 ± 0.04 g. Each value is mean ± s. d. of data for three-plicate tanks.
[*2] Feed intake: sum of daily feed intake (g dry weight) / 0.5 (number of prawn at start + number of prawn at the end).
[*3] Feed conversion efficiency: weight gain (g) / feed intake (g).
[*4] Probability significance.
[*5] Non-significant ($p > 0.05$).

muscle of the prawn are shown in Table 3. Factorial analysis of variance indicated that different levels of carbohydrate in the diet had significant effects on glycogen content in the hepatopancreas and body, and pyruvate content in the hepatopancreas and muscle of the prawn. Higher contents of glycogen and pyruvate were found in prawns fed diets containing 25 % carbohydrate. Different levels of thiamin in the diet had significant effects on glycogen contents of both hepatopancreas and body, and pyruvate content in both hepatopancreas and muscle. Interactive effect of these two factors was significant only for glycogen and pyruvate contents in the body and muscle, respectively.

Vitamin B-1 content in the hepatopancreas

Thiamin pyrophosphate (TPP) and thiamin monophosphate (TMP) contents in the hepatopancreas are shown in Table 4. Factorial analysis of variance indicated that different levels of carbohydrate and thiamin in the diet had significant effects on the TPP content of the hepatopancreas. However, only effect of thiamin was significant on the TMP content. Interactive effect of these two factors was significant only for the TPP content. Prawns fed diets high in carbohydrate had lower contents of TPP. The lowest TPP content was observed in prawns fed the thiamin-free diet with high carbohydrate content (Fig. 2).

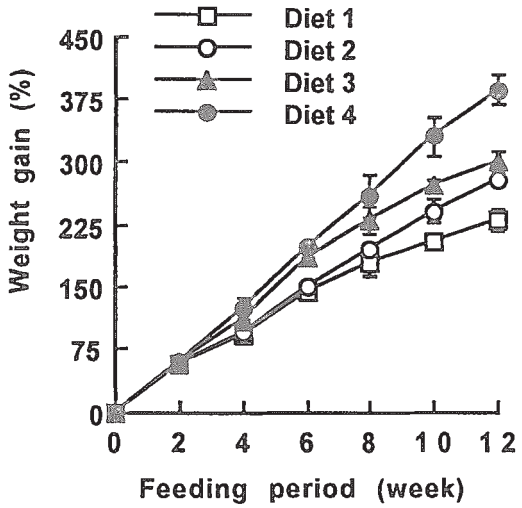

Fig. 1. Weight gain of prawn fed experimental diets for 12 weeks.

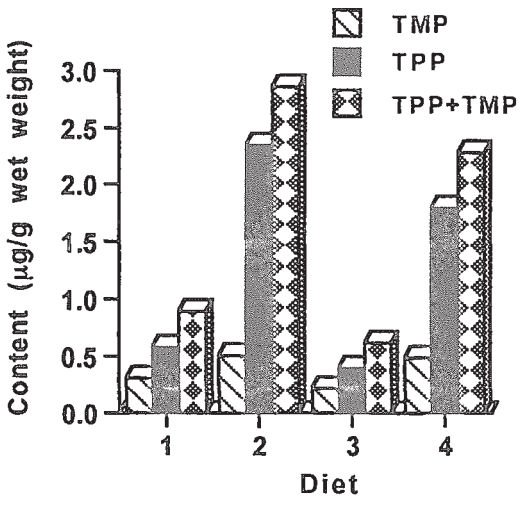

Fig. 2. Thiamin pyrophosphate (TPP) and thiamin monophosphate (TMP) contents in the hepatopancreas of prawn fed experimental diets for 12 weeks.

Table 3. Glycogen and pyruvate contents in the tissues of prawn fed experimental diets for 12 weeks[*1]

Dietary factors		Diet	Glycogen (mg/g dry weight)		Pyruvate (μg/g wet weight)	
Carbohydrate	Thiamin	no.	Hepatopancreas	Whole body	Hepatopancreas	Muscle
5	0	1	17.40±1.52	5.79±0.51	17.73±0.45	10.13±0.82
5	20	2	16.43±1.90	5.60±0.39	15.34±1.05	9.76±0.38
25	0	3	24.87±1.85	10.78±0.54	24.37±1.39	13.43±1.14
25	20	4	21.21±1.77	6.93±0.60	21.80±1.21	11.45±0.63
Carbohydrate			0.0008[*2]	0.0001	0.0010	0.0112
Thiamin			0.0489	0.0002	0.0317	0.0066
Carbohydrate × thiamin			NS[*3]	0.0004	NS	0.0372

[*1] Values is mean ± s. d. of data for three-plicate tanks.
[*2] Probability significance.
[*3] Non-significant ($p > 0.05$).

Table 4. Thiamin pyrophosphate (TPP) and thiamin monophosphate (TMP) contents in the hepatopancreas of prawn fed experimental diets for 12 weeks*1

Dietary factors		Diet	TPP	TMP
Carbohydrate	Thiamin	no.	(μg/g wet weight)	(μg/g wet weight)
5	0	1	0.58±0.03	0.31±0.06
5	20	2	2.35±0.10	0.51±0.12
25	0	3	0.40±0.03	0.22±0.01
25	20	4	1.79±0.10	0.49±0.07
Carbohydrate			0.0001*2	NS*3
Thiamin			0.0001	0.0007
Carbohydrate × thiamin			0.0022	NS

*1 Values is mean ± s. d. of data for three-plicate tanks.
*2 Probability significance.
*3 Non-significant ($p > 0.05$).

Discussion

The present study showed that the growth of prawn was significantly improved by thiamin supplementation. Growth of prawns fed thiamin-free diets retarded after 6 weeks, both for prawns fed the low and high carbohydrate diets. However, growth of prawns fed the diet high in carbohydrate was better than that of prawns fed low carbohydrate, both for the thiamin-free and thiamin supplemented diet. Since all diets were isonitrogenous and isocaloric, the results indicated that dietary carbohydrate could be utilized more efficiently as an energy source than dietary lipid (oleic acid), when endogenous thiamin was available to support carbohydrate metabolism to produce metabolic energy. In our previous study, it was found that the TPP content in the hepatopancreas of prawns fed thiamin-free diets reduced after 4 weeks, but that its growth was significantly retarded only by the 8th week of feeding (unpublished data). This agreed with the present study that growth of prawns fed thiamin-free-high-carbohydrate diets reduced after 6 weeks of feeding, but prawns fed thiamin-supplemented-high-carbohydrate diets still grew well until the end of the experiment.

Optimum levels of dietary carbohydrate for penaeid shrimp differ according to the carbohydrate source and shrimp species. Disaccharide, sucrose and polysaccharides such as dextrin and starch have been reported to have high nutritive value for *P. japonicus*[7,15]. On the other hand the monosaccharide glucose has been shown to inhibit the growth of *P. japonicus*[15], *P. aztecus*[9], and *P. duorarum*[10]. *Penaeus japonicus* juveniles have been reported to contain glucose, acetyl glucosamine and trehalose as the major carbohydrates in the body tissues[16]. Quantitative requirements of carbohydrate are usually discussed in term of protein-sparing effects. Inclusion of carbohydrate levels of 25 %, 15 % and 5 % in diets containing 45 %, 45-55 % and 55 % protein, respectively, was optimum for *P. japonicus* larvae[17]. Dietary sugar levels of 20 % in the diet containing 45 % protein and 10 % lipid resulted in the best growth for *P. monodon*[18], whereas 30 % dietary sugar level resulted in significantly lower growth.

Generally, glycogen is the major storage form of carbohydrate in animals. While glycogen was stored, *Metapenaeus sp.* preferred to sustain the intake of carbohydrate from food for both oxidative metabolism and chitin synthesis[19]. During starvation, hepatopancreatic glycogen of *P. japonicus* decreased rapidly[8]. Higher glycogen contents in prawns fed the high carbohydrate diets in the present study indicate that some dietary carbohydrate was converted and stored as glycogen. The same results have been reported for rainbow trout fed isocaloric and isonitrogenous diet containing different levels of carbohydrate. Its liver glycogen content significantly increased with increasing dietary carbohydrate levels[20]. The highest glycogen content was found in prawns fed thiamin-free-high-carbohydrate diet, both in the hepatopancreas and whole body. These results indicate that thiamin had a significant effect on the utilization of carbohydrate to produce metabolic energy. General pathway of carbohydrate

metabolism including glycolysis, penthose phosphate shunt, and the citric acid cycle has been reported presence in the hepatopancreas of lobster[21], and the glycolysis is the principal catabolic route of glucose in crustancean muscle[22]. The lack of thiamin could inhibit carbohydrate metabolism, resulting in more carbohydrate stored as glycogen.

Under aerobic conditions, the pyruvate formed in glycolysis is further oxidized in the citric acid cycle. The decarboxylation of pyruvate is accompanied by oxidation to give acetyl coenzyme A, which then enters the citric acid cycle where the acetate can be further oxidized to CO_2 and H_2O. This process is accomplished by pyruvate oxidase enzyme, which require thiamin pyrophosphate (TPP) as a coenzyme[23-24]. Pyruvate oxidase was more drastically inhibited than α-ketoglutarate dehydrogenase in the liver of mice fed a thiamin deficient diet[25]. In the present study, although the interactive effect of dietary carbohydrate and thiamin did not significant on pyruvate content of the hepatopancreas, higher levels of pyruvate was found in the hepatopancreas and muscle of prawns fed thiamin free diets, both in diets with high and low carbohydrate content. The mobilization of pyruvate into the citric acid cycle could also be reduced by stress resulting in higher pyruvate levels in the muscle[26-27]. Since the present results are obtained under the same conditions, the varying levels of pyruvate among the dietary groups could be the effects of treatment, It was reported that blood pyruvate level increased significantly in thiamin-deficient animal and human[23], and could be considered as a good measure of thiamin status.

Significantly lower TPP content was found in hepatopancreas of prawn fed high carbohydrate diets compared to those on the low one, and the lowest content was found in prawns fed the thiamin-free-high-carbohydrate diet (diet 3). Thiamin pyrophosphate (TPP) has been reported as a sensitive indicator of thiamin status in rainbow trout[28] and in mice[25]. In our previous study it was also found that TPP content in the hepatopancreas of *P. japonicus* fed thiamin free diets reduced drastically after 4 weeks of feeding experiment (unpublished data). The presence of significant interactive effect of dietary thiamin and carbohydrate on TPP content, indicated that dietary carbohydrate content affected thiamin metabolism in the prawn, and higher dietary thiamin was required in the diet containing high levels of carbohydrate. Thiamin requirement of *P. japonicus* was estimated to be about 6-12 mg per 100 g diet[3]. Compared to the requirement of *P. monodon*, which was only 1.3-1.4 mg thiamin per 100 g diet[4], the requirement of *P. japonicus* is markedly higher. Chen[29] also reported the requirement of *P. monodon* for riboflavin and niacin to be much lower than the requirement of *P. japonicus*. One factor that may be involved in the apparent differences in the dietary vitamin requirements between shrimp species is the more omnivorous nature of *P. monodon* compared to *P. japonicus*.

References

1) Hilton, J. W. (1989): The interaction of vitamins, minerals and diet composition in the diet of fish. *Aquaculture*, 79(1-4), 223-244.

2) Catacutan, M. R. and M. De-La Cruz (1989): Growth and mid-gut cell profile of *Penaeus monodon* juveniles fed water-soluble vitamin-deficient diets. *Aquaculture*, 81(2), 137-144.

3) Deshimaru, O. and K. Kuroki (1979): Requirement of prawns for dietary thiamin, pyridoxine, and choline chloride. *Bulletin of the Japanese Society of Scientific Fisheries*, 45(3), 363-367.

4) Chen, H. Y., F. C. Wu, and S. Y. Tang (1991): Thiamin requirement of juvenile shrimp (*Penaeus monodon*). *J. Nutr.*, 121(12), 1984-1989.

5) Akiyama, D. M., W. G. Dominy, and A. L. Lawrence (1991): Penaeid shrimp nutrition for the commercial feed industry: revised, in "Proceeding of the Aquaculture Feed Processing and Nutrition Workshop" September 17-25, 1991, Thailand and Indonesia (ed. by D. M. Akiyama nad R. K. H. Tan), American Soybean Association, Singapore, pp. 80-98.

6) Freedland, R. A. and S. Briggs (1977): A Biochemical Approach to Nutrition, Chapman and Hall, London, pp. 63.

7) Deshimaru, O. and Y. Yone (1978): Effects of dietary carbohydrate source on the growth and feed efficiency of prawn. *Bulletin of the Japanese Society*

of Scientific Fisheries, 44(10), 1161-1164.

8) Abdel-Rahman, S. H. (1979): Studies on carbohydrate metabolism in the prawn *Penaeus japonicus* (Bate). Master's thesis, Kagoshima University, Kagoshima, pp. 112.

9) Andrews, J. W., L. V. Sick, ana G. J. Baptist (1972): The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. *Aquaculture*, 1(4), 341-347.

10) Sick, L. V. and J. W. Andrews (1973): The effects of selected dietary lipids, carbohydrates and proteins on the growth, survival and body composition of *Penaeus duorarum*. *Proc. World Mariculture Society*, 4, 263-276.

11) Morito, C. L. H., D. H. Conrad, and J. W. Hilton (1986): The thiamin deficiency signs and requirement of rainbow trout (*Salmo gairdneri*, Richardson). *Fish Physiology and Biochemistry*, 1(2), 93-104.

12) Kimura, M., B. Panijpan, and Y. Itokawa (1982): Separation and determination of thiamin and its phosphate esters by reversed-phase high-performance liquid chromatography. *Journal of Chromatography*, 245, 141-143.

13) Kawasaki, T.(1986): Determination of thiamin and its phosphate esters by high-performance liquid chromatography, in "Methods in Enzymology" Vol.122 (ed. by F. Chytil and D. McCormick), Academic Press, New York, pp. 15-19.

14) Carrol, N. V., R. W. Longley, and J. H. Roe (1956): The determination of glycogen in liver and muscle by use of anthrone reagent. *J. Biol. Chem.*, 220(2), 583-593.

15) Abdel-Rahman, S. H., A. Kanazawa, and S. Teshima (1979): Effects of dietary carbohydrate on growth and the levels of the hepatopancreatic glycogen and serum glucose of prawn. *Bulletin of the Japanese Society of Scientific Fisheries*, 45 (12), 1491-1494.

16) Kanazawa, A. (1983): Penaeid nutrition, in "Proceeding of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition" (ed. by G. D. Pruder, C. J. Langdon, and D. E. Conklin), Lousiana State University, Delaware, pp. 87-105.

17) Kanazawa, A. (1989): Protein requirements of penaeid shrimp, in "Advances in Tropical Aquaculture" (ed. by J. Barret, j. Calvas, G. Cuzon, J. Fuchs, and M. Weppe), IFREMER, Tahiti, pp. 261-270.

18) Alava, V. R. and F. P. Pascual (1987): Carbohydrate requirements of *Penaeus monodon* (Fabricius) juveniles. *Aquaculture*, 61(3/4), 211-217.

19) Dall, W. (1965): Studies on the physiology of a shrimp, *Metapenaeus sp.* (crustacea: decapoda: penaeidae), IV: carbohydrate metabolism. *Aust. J. Mar. Freshw. Res.*, 16(2), 163-180.

20) Hilton, J. W. and J. L. Atkinson (1982): Response of rainbow trout (*Salmo gairdneri*) to increased levels of available carbohydrate in practical trout diets. *Br. J. Nutr.*, 47(3), 579-607.

21) Hochachka, P. W., J. M. Teal, and M. Telford (1962): Pathways of carbohydrate metabolism in lobster hepatopancreas. *Can. J. Biochem. Physiol.*, 40(8), 1043-1050.

22) Chang, E. S. and J. D. O'Connor (1983): Metabolism and transport of carbohydrates and lipids, in "The Biology of Crustacea" Vol. V (ed. by L. H. Mantel), Academic Press, New York, pp. 263-289.

23) Gubler, C. J. (1991): Thiamin, in "Handbook of Vitamin" Second edition (ed. by L. J. Machlin), Marcel Dekker, Inc., New York, pp. 233-282.

24) Malinovskaya, M. V. (1988): Pathways of carbohydrate metabolism in fishes in relation to temperature adaptation (a review). *Hydrobiological Journal*, 24(6), 30-40.

25) Trebukhina, R. V., Y. M. Ostrovsky, V. G. Petushok, M. G. Velichko, and V. N. Tumanov (1981): Effect of thiamin deprivation on thiamin metabolism in mice. *J. Nutr.*, 111(3), 505-513.

26) Reddy, M. S. and K. V. R. Rao (1988): Modulation of carbohydrate metabolism in the selected tissues of marine prawn, *Penaeus indicus* (H. Milne Edwards), under phosphanidon-induced stress. *Ecotoxicology and Environmental Safety*, 15(2), 212-220.

27) Schwalme, K. and W. C. Mackay (1991): Mechanisms that elevate the glucose concentration of muscle and liver in yellow perch (*Perca flavescens* Mitchill) after exercise-handling stress. *Can. J.*

Zool., 69(2), 456-461.

28) Masumoto, T., R. W. Hardy, and Z. Casillas (1987): Comparison of transketolase activity and thiamin pyrophosphate levels in erythrocytes and liver of rainbow trout (*Salmo gairdneri*) as indicator of thiamin status. *J. Nutr.*, 117(8), 1422-1426.

29) Chen, H. Y. (1993): Recent advances in nutrition of *Penaeus monodon*. *Journal of the World Aquaculture Society*, 24(2), 231-240.

クルマエビの成長と肝膵臓チアミンピロリン酸含量に対する飼料性チアミンおよび炭水化物の相互作用

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飼料性チアミンと炭水化物が，成長と体チアミンピロリン酸（TPP）含量におよぼす相互作用をしらべるために，クルマエビ稚エビ（平均体重0.66±0.04 g）を用いて，二段階のチアミン添加量（飼料100 g中0および20 mg）と二段階の炭水化物添加量（5および25％）の2×2因子飼育実験を12週間行なった。すべてのパラメーターの分散分析より，増重，エビ体のグリコーゲン含量，筋肉中のピルビン酸含量および肝膵臓中のTPP含量に有意な相互作用（$p < 0.05$）が検出されたが，生残，FCEおよび肝膵臓のグリコーゲンおよびピルビン酸含量にはみられなかった。炭水化物添加量が同じ場合，チアミン添加区が無添加区に比べ高い増重を示した。また，チアミン添加区は無添加区に比べ，増重，生残，摂餌量，飼料転換効率（FCE）で高い値を示した。肝膵臓のグリコーゲンおよびピルビン酸含量は，炭水化物25％添加区が高い値を示し，その中でチアミン無添加区が最も高い値を示した。肝膵臓中のTPP含量は，チアミン添加量に関係なく，炭水化物25％添加区が低い値を示した。