EFFECTS OF DIETARY PYRIDOXINE LEVELS ON GROWTH AND VITAMIN B_6 PROFILE OF JUVENILE KURUMA PRAWN, *Penaeus japonicus*

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ABSTRACT

The effects of dietary pyridoxine levels (0, 5, 15, and 25mg/100g diet) on growth and vitamin B_6 profile of juvenile *Penaeus japonicus* (0.69 ± 0.03g; mean ± sd) were investigated during 8 weeks of feeding experiments. Dietary pyridoxine (PN) significantly ($p < 0.05$) improved weight gain and survival of prawns. The highest weight gain and survival were observed in prawns fed diet with 5mg of supplemental PN/100g dry diet.

Pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP) were the dominant forms of vitamin B_6, both in the hepatopancreas and muscle of prawns, accounting for 74-87% and 87-100% of the total respectively. PLP and PMP contents in the hepatopancreas and muscle of prawns fed diet without supplemental PN were markedly reduced and were lower than in other groups. Increasing levels of dietary pyridoxine supplement from 5 to 25mg/100g diet slightly increased PMP content in the hepatopancreas, but had no effect on the PLP and PMP contents of muscle nor in the PLP contents of the hepatopancreas.

The present study showed that juvenile *P. japonicus* required 4.3mg PN/100g diet for maximum weight gain. The level of 5mg PN/100g diet was required for maximum PLP contents in the hepatopancreas and muscle.

KEYWORDS: *Penaeus japonicus*, pyridoxine, growth, vitamin B_6 profile.

INTRODUCTION

Dietary requirements for vitamin B_6 have been established for several species of fish by giving them diets containing different levels of pyridoxine (PN). The criteria used for measuring vitamin B_6 requirements included growth, mortality, food conversion, concentration of vitamin B_6 in selected tissues, activity of various aminotransferases in body tissues, and pathological changes in body tissues or organs (Adron *et al.*, 1978; Kissil *et al.*, 1981; Halver, 1972; Smith *et al.*, 1974 and Albrektsen *et al.*, 1993). Although a qualitative requirement of *P. japonicus* for water-soluble vitamins has been established (Guary *et al.*, 1976; Deshimaru and Kuroki, 1976; Deshimaru and Kuroki, 1979; Kanazawa *et al.*, 1976; Kanazawa, 1983 and 1990), very few studies have been done concerning vitamin B_6 nutrition in prawns. Deshimaru and Kuroki (1979) reported that in a casein based diet, juvenile *P. japonicus* required 12mg PN/100g diet for maximum growth and PN content in the body. Kanazawa (1990) reported that larvae of *P. japonicus* fed a carrageenan microbound diet without PN-supplementation could not reach the postlarval stage (died during the mysid stage) and suggested supplementation of 12mg PN/100g

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diet was required. Coburn (1994) considered that a level of 12mg PN/100g diet for juvenile prawns was too high.

Vitamin B_6 acts as a precursor to the coenzyme pyridoxal phosphate which is required in protein and amino acid metabolism. Due to the multiple roles of this vitamin in various metabolic pathways, there are a number of signs that can be identified in vitamin B_6 deficient animals. Poor growth, high mortality, anorexia, anemia, dark coloration and loss of equilibrium have been observed in fish deficient in PN (Baker and Davies, 1995; Albrektsen *et al.*, 1993; Wanakowat *et al.*, 1989; Herman, 1985; Kissil *et al.*, 1981 and Smith *et al.*, 1974). Low aspartate aminotransferase (ASAT) activity was also reported in the muscle of Atlantic salmon, *Salmo salar* (Albrektsen *et al.*, 1993; 1995) and in the liver of rainbow trout, *Salmo gairdneri* (Hardy *et al.*, 1987) fed PN-deficient diets. However, only high mortality and growth depression were reported from prawns fed PN-deficient diets (Deshimaru and Kuroki, 1979; Kanazawa, 1990).

The present study aims to investigate the effects of dietary PN levels on growth and vitamin B_6 contents in the hepatopancreas and muscle of juvenile *P. japonicus*. It also aims to determine possible PN deficiency symptoms.

MATERIAL AND METHODS

Test diets

Four test diets (PN-0, PN-5, PN-15, and PN-25) were prepared to contain 0, 5, 15, and 25mg of supplemental PN/100g diet as shown in Table 1. Diets were isonitrogenous and isocaloric. Total proportions were adjusted to 100% by the addition of α-cellulose. The diets were prepared according to the following procedure. Agar, as binder, was dissolved in 160mL of boiling water and was cooked gently in the water bath for about 5 minutes. The diet premix was then added and mixed thoroughly for 10 minutes. During mixing, 1N NaOH was added carefully to adjust the pH to 6. The diet was then steamed at 100°C without pressure for 10 minutes, pressed into Kurehalon plastic tubes, and steamed again for another 5 minutes. After cooling at room temperature, diets were stored at 4°C until used.

Feeding trial

Post larvae of kuruma prawn, obtained from a commercial hatchery, were reared in 500L round holding tanks and fed a commercial diet (Higashimaru Co., Japan) until they were used in the experiment. A total of 288 prawns (weighing 0.69 ± 0.03g) were selected for the experiment. Twenty-four prawns per tank with three replicate tanks per treatment were randomly housed in 54L rectangular tanks (60 x 30 x 30cm) with sand bottoms (2cm thickness). Sea water, 33ppt, was continuously supplied at a flow rate of 2L/h, and 50% of the water in the tank was changed every morning. The sea water was recirculated through a sand bottom filter. Water temperature during the 8 weeks of feeding experiment was maintained between 22-25°C using an electric heater.

The prawns were fed once a day with 10% (dry matter basis) of their body weight based on average initial weight. The feeding level was adjusted every 2 weeks according to the feeding response and body weight of the prawns. Uneaten feed was collected and exuviae were removed each morning. All prawns were weighed and counted every 2 weeks. All tanks and sand bottoms were cleaned and washed at the same time.

Every 2 weeks, hepatopancreas and muscle of three prawns from each replicate tank were collected, pooled for each treatment, frozen in dry ice-methanol and stored at -84°C until used for the vitamin B_6 and glutamate oxaloacetate transaminase (GOT) analyses.

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

Ingredients	Diets			
	PN-0	PN-5	PN-15	PN-25
Pyridoxine	0.000	0.005	0.015	0.025
α-cellulose	0.590	0.585	0.575	0.565
Casein (vitamin free)	49.00	49.00	49.00	49.00
Squid meal[*1]	5.00	5.00	5.00	5.00
Sucrose	5.00	5.00	5.00	5.00
Dextrin	4.37	4.37	4.37	4.37
α- starch	5.00	5.00	5.00	5.00
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	4.00	4.00	4.00	4.00
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]	6.00	6.00	6.00	6.00
Vitamin mix (PN-free)[*4]	1.99	1.99	1.99	1.99
Lysine	1.20	1.20	1.20	1.20
Arginine	2.75	2.75	2.75	2.75
Agar	5.00	5.00	5.00	5.00
Total	100	100	100	100
Analysis				
PN (mg/100g)	nd[*5]	4.72	14.09	23.68
Crude protein (%)	54.9	54.9	54.5	55.0
Gross energy (kcal/g)[*6]	4.3	4.3	4.1	4.2

[*1] Squid meal was prepared using fresh squid muscle. After removing the insides, head, and skin, the muscle was freeze-dried, powdered, and sieved (< 250μm). The powder was then extracted 3 times with chloroform : methanol (1 : 1), dried, and again sieved (< 250μm).

[*2] Attractant (g per 100g) : Betaine, 0.5; taurine, 0.5; proline, 0.3; alanine, 0.3; I M P, 0.1; glutathione, 0.1 and Na-glutamate, 0.4.

[*3] Mineral (g per 100g): K_2HPO_4, 1.403; $Ca_3(PO_4)_2$, 1.909; $MgSO_4.7H_2O$, 2.134 and $NaH_2PO_4.2H_2O$, 0.554.

[*4] Vitamin mix (mg/100g) :ρ-amino benzoic acid, 15.80; biotin, 0.63; inositol, 632.00; niacin, 63.20; Ca-pantothenate, 94.80; thiamin-HCl, 15.00; Vit. C (phospitan), 136.07; riboflavin, 12.64; folic acid, 1.26; cyanocobalamine, 0.13; choline chloride, 948.00; menadione, 6.34; Vit. A palmitate30.34; α-tocoferol, 31.60; calciferol, 1.88.

[*5] Undetected

[*6] Gross energy calculated using caloric equivalents of: protein, 5.65kcal g^{-1}; lipid, 9.45kcal g^{-1}; and carbohydrate, 4.10kcal g^{-1}.

Analysis of vitamin B_6

The vitamin B_6 content of the test diets, and its content in the form of a phosphate ester in the hepatopancreas and muscle of prawns were determined by high performance liquid chromatography (HPLC) based on the methods of Sampson and O'Connor (1989) and van de Kamp *et al.* (1995). The HPLC instrument was a LC-6A equipped with SCL-6B System Controller, RF-535 Fluorescence HPLC Monitor (excitation wavelength at 328nm and emission wavelength at 393nm), and C-R7Ae plus Chromatopac (all from Shimadzu, Japan). The analytical column was LiChrosorb RP18-5, 4.6 x 250mm (GL Sciences Inc., Japan).

The mobile phase A was 0.033M phosphoric acid and 0.008M 1-octanesulfonic acid, adjusted to pH 2.2 with 6N KOH. Mobile phase B was 0.033M phosphoric acid and 10% (v/v) 2-propanol, adjusted to pH 2.2 with 6 N KOH (Sampsom and O'Connor, 1989). The post-column reagent described by Coburn and Mahuren (1983) was used at a flow rate of 0.2mL $minute^{-1}$ to enhance fluorescence of pyridoxal phosphate (PLP). The reagent was freshly prepared and contained 1g of sodium bisulfite per 1000mL of 1.0M potassium phosphate buffer adjusted to pH 7.5 with 6 N KOH. B_6 vitamers were separated using the following binary gradient program: sample injection followed by 100% solvent A for 5 minutes, a linear gradient to 90% solvent B in 25 minutes, 90% solvent B for 6 minutes, a linear gradient to 100% solvent A in 2 minutes, column equilibration for 7 minutes with 100% solvent A. The flow rate was 1.2mL $minute^{-1}$ throughout the gradient.

The diets, hepatopancreas, and muscle were homogenized separately in 5% cold metaphosphoric acid in an ice bath using a glass homogenizer and 4-deoxypyridoxine was added as an internal standard (Gregory and Feldstein, 1985). After centrifugation (13,900 x g at 4°C for 25 minutes), the supernatant was washed 4 times with two volumes of methylene chloride. The collected sample was then filtered through a 0.45µm membrane filter (Advantec, Toyo Roshi Kaisha, Japan) and 20µL of the filtrate was injected into the HPLC injection port. Pyridoxine-HCl, pyridoxal-HCl, pyridoxal phosphate monohydrate (Wako Pure Chemical Industries Ltd., Japan), 4-deoxypyridoxine, pyridoxamine phosphate-HCl (Nacalai Tesque Inc., Japan) and pyridoxamine dihydrochloride (Sigma Chemical Co.) were used as standards.

Determination of GOT activity

Prawn muscle (100mg) was homogenized in 6ml of an extraction buffer (0.1M sodium phosphate, pH 7.2; 20% glycerol; 0.02% triton X-100; and 1.5mM dithiothreitol) using a glass homogenizer (Casillas *et al.*, 1982). The homogenate was then centrifuged at 13,900 x g at 4°C for 20 minutes. The collected supernatant was again centrifuged for 20 minutes to obtain a clearer product, stored at 4°C for further analysis. GOT activity in muscle, expressed as IU g^{-1} wet weight, was analyzed using a reagent kit (Kanto Chemical, Japan) and measured using a RaBA-Hi super System (Chugai, Japan).

Statistical analysis

Statistical tests for weight gain, survival, feed intake, and feed conversion efficiency (FCE) were performed using one-way analysis of variance (ANOVA). The differences between individual treatments were tested using Duncan's new multiple range test (Steel and Torrie, 1960). All references to statistical significance were at 5% level. The dietary PN requirement for maximum weight gain of kuruma prawns was estimated according to Zeitoun *et al.* (1976).

RESULTS

Growth and survival

Although all groups showed high survival (78% or more), the survival of prawns fed diet

without supplemental PN was significantly lower ($p < 0.05$) than PN-supplemental groups (Table 2). The highest survival was observed on prawns fed diet with 5mg of supplemental PN/100g diet.

Weight gain of the prawns increased with time over the 8 weeks of the feeding experiment. After 8 weeks, weight gain was significantly ($p < 0.05$) lower on prawns fed diet without supplemental PN than diets with supplemental PN. The highest weight gain was observed on prawns fed diet with 5mg of supplemental PN/100g diet (Table 2). Increasing levels of supplemental PN in diets from 5 to 25mg/100g diet resulted in a slightly lower weight gain, though this was not significantly different ($p > 0.05$).

Prawns fed diet without supplemental PN showed significantly ($p < 0.05$) lower FCE and feed intake than those of PN-supplemented groups. There were no significant differences of FCE and feed intake among prawns fed PN-supplemented diets (Table 2 and Table 5).

Vitamin B_6 contents

Vitamin B_6 profiles in the hepatopancreas and muscle of prawns are shown in Table 3 and Table 4 respectively. In the hepato-pancreas, PLP and PMP are the main forms of vitamin B_6 accounting for 74-87% of total, followed by PM (10-17%). Only small amounts of PL and PN are present. The concentration of PLP was lower than that of PMP for almost all groups of prawns. After the 8 weeks experimental period PLP contents of prawns fed diet with 5, 15, and 25mg of supplemetal PN/100g diet were almost the same, but twice as high as those given PN-unsupplemented. Meanwhile, PMP contents increased slightly over time, with increasing levels of PN-supplementation, and became 7 to 8 times higher than that of the PN-unsupplemented diet at the end of the feeding period. In the hepatopancreas of prawns fed diet without supplemental PN, about 42% and 32% of vitamin B_6 was present in the PLP and PMP forms, respectively. The proportions of PLP and PMP changed to 22% and 61% respectively in prawns fed PN-supplemented diets.

Table 2. Weight gain, survival and FCE of prawns fed experimental diets for 8 weeks[*1]

Diets	Final weight (g)	Weight gain [*2] (%)	survival (%)	FCE [*3]
PN-0	1.81 ± 0.12	161 ± 19[a]	78[a]	0.27 ± 0.04[a]
PN-5	2.53 ± 0.03	265 ± 4[b]	99[b]	0.37 ± 0.01[b]
PN-15	2.43 ± 0.07	251 ± 10b	89[c]	0.39 ± 0.02[b]
PN-25	2.33 ± 0.13	237 ± 17[b]	94[bc]	0.35 ± 0.03[b]

[*1] Initial weight = 0.69 ± 0.03 g. Values are means ± SE. Values in the column with the same superscript are not significantly different ($P > 0.05$).
[*2] Weight gain = (final weight - initial weight) x 100/initial weight.
[*3] Feed conversion efficiency = weight gain (g)/feed intake (g).

Table 3. Vitamin B_6 contents in the hepatopancreas of prawns fed experimental diets[*1]

B6 vitamer (μg/g wet weight)	Diets	Feeding period (week)			
		2	4	6	8
PLP	PN-0	1.058	0.826	0.654	0.594
	PN-5	1.101	1.186	1.209	1.274
	PN-15	1.151	1.174	1.225	1.255
	PN-25	1.205	1.280	1.272	1.185
PMP	PN-0	0.974	0.931	0.498	0.452
	PN-5	2.238	2.947	2.759	3.092
	PN-15	2.104	3.261	3.582	3.505
	PN-25	2.351	3.439	3.293	3.554
PL	PN-0	0.100	0.066	0.023	0.023
	PN-5	0.141	0.071	0.054	0.046
	PN-15	0.190	0.075	0.047	0.074
	PN-25	0.223	0.116	0.065	0.071
PN	PN-0	0.150	0.164	0.050	0.110
	PN-5	0.135	0.132	0.062	0.100
	PN-15	0.148	0.135	0.101	0.175
	PN-25	0.166	0.138	0.125	0.151
PM	PN-0	0.470	0.312	0.215	0.232
	PN-5	0.499	0.662	0.620	0.628
	PN-15	0.675	0.616	0.563	0.762
	PN-25	00.659	0.655	0.750	0.793

* Initial contents of PLP, PMP, PL, PN, and PM were 0.979, 1.563, 0.054, 0.091, and 0.057μg/g wet weight, respectively.

Only PLP, PMP, and PN were detected in the muscle of prawns (Table 4). PLP and PMP were also the predominant forms and accounted for 87-100% of the total vitamin B_6 in the muscle. PLP content in the muscle was much higher (69-80%) than that of PMP (17-22%) (Fig. 1).

Muscle GOT activity

Muscle GOT activity was markedly reduced in the prawns fed diet without supplemental PN compared with PN-supplemented groups (Table 5). Increasing level of dietary PN (from 5 to 25mg/100g diet) did not increase the GOT activity. Muscle GOT activity of prawns fed diet without supplemental PN was markedly reduced after the second week of feeding (Fig. 2). In terms of percentage stimulation, inclusion of PLP in the samples before analysis markedly increased the muscle GOT activity of prawns fed diet without supplemental PN and resulted in higher percentage stimulation. Inclusion of PLP did not elicit increased GOT activity in prawns fed PN-supplemented diet (Fig. 2). Since the GOT reaction requires PLP or PMP, the relationships between PLP and PMP in muscle and muscle GOT activity were calculated. A high positive correlation ($r = 0.99$; $n = 4$) was found between PLP content and muscle GOT activity . Also a positive correlation ($r = 0.94$; $n = 4$) was found between PMP and muscle GOT activity.

Table 4. Vitamin B_6 contents in the muscle of prawns fed experimental diets[*1]

B6 vitamer (µg/g wet weight)	Diets	Feeding periods (weeks)			
		2	4	6	8
PLP	PN-0	0.824	0.655	0.521	0.499
	PN-5	0.983	1.008	0.983	0.956
	PN-15	0.955	1.007	0.858	0.840
	PN-25	0.989	0.927	1.006	0.939
PMP	PN-0	0.195	0.166	0.136	0.128
	PN-5	0.244	0.250	0.256	0.239
	PN-15	0.232	0.264	0.261	0.243
	PN-25	0.225	0.251	0.241	0.221
PN	PN-0	0.151	0.110	0.036	0.089
	PN-5	0.110	0.120	0.100	nd[*2]
	PN-15	0.156	0.151	0.133	nd
	PN-25	0.107	0.134	0.100	0.098

[*1] Initial contents of PLP, PMP, and PN were 1.063, 0.244, and 0.112 µg/g wet weight, respectively.
[*2] Undetected.

Table 5. Muscle glutamate oxaloacetate transaminase (GOT) activity and feed intake of prawns fed experimental diets[*1].

B6 vitamer (µg/g wet weight)	Diets	Feeding periods (weeks)			
		2	4	6	8
PLP	PN-0	9.18	7.13	6.77	5.24
	PN-5	11.64	10.84	11.42	10.85
	PN-15	11.51	11.35	11.76	10.04
	PN-25	11.70	12.35	10.74	11.33
PMP	PN-0	48.60	50.79	59.84	79.82
	PN-5	11.97	11.31	11.00	13.76
	PN-15	11.88	11.40	15.46	22.16
	PN-25	11.89	11.40	12.50	15.32
PN	PN-0	0.65	0.89	1.23[a]	1.45[a]
	PN-5	0.66	0.88	1.40[b]	1.94[b]
	PN-15	0.67	0.86	1.35[b]	1.89[b]
	PN-25	0.67	0.85	1.35[b]	1.92[b]

[*1] Initial : GOT activity : 11.572 IU/g wet weight and % stimulation : 8.9
[*2] Percentage stimulation = (GOT activity with PLP - GOT activity without PLP) x 100/GOT activity without PLP.
[*3] Feed intake = sum of daily feed intake (g dry weight)/0.5 (number of prawn at start + number of prawn at the end). Daily feed intake was measured by subtracting the amount of uneaten feed from that of feed offered.

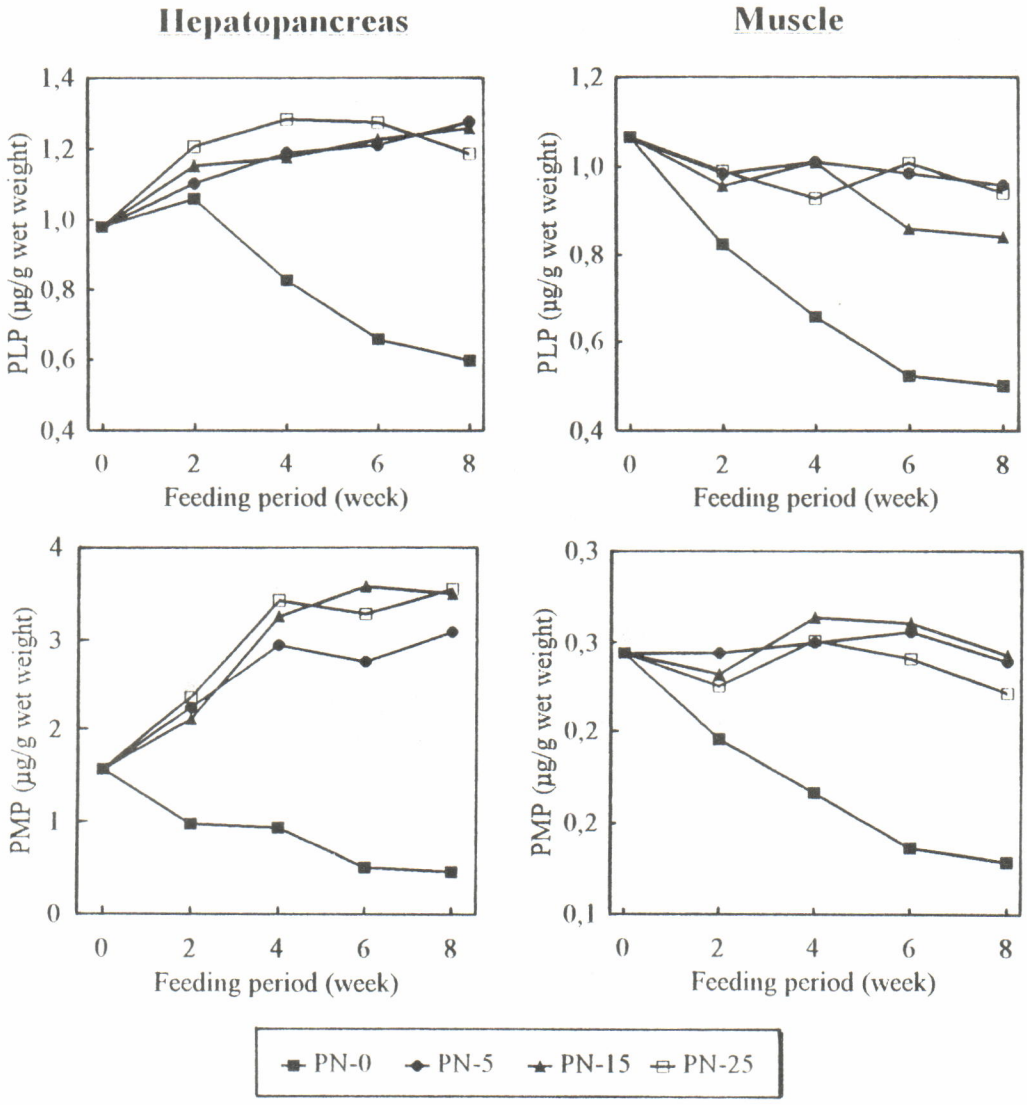

Fig. 1. Change in PLP and PMP contents in the hepatopancreas and muscle of prawns fed test diets for 8 weeks.

Pyridoxine requirement

Based on a broken-line analysis for weight gain of prawns at the end of the feeding experiment and dietary pyridoxine levels, the PN requirement for maximum weight gain of juvenile *P. japonicus* was found to be 4.3mg/100 g dry diet. PN supplementation of 5mg/100g diet resulted in the highest PLP contents both in the hepatopancreas and muscle of prawns.

DISCUSSION

The present study showed that PN-supplementation improved weight gain and survival of juvenile *P. japonicus*. The prawns fed diet with 5, 15, and 25mg of supplemental PN/100g diet showed weight gains of 265%, 251%, and 237%, respectively. The prawns fed diet without supplemental PN grew well only for the first 4 weeks, after which growth was significantly

retarded. This result agrees with the finding of Deshimaru and Kuroki (1979) who reported that growth of juvenile *P. japonicus* fed PN-deficient diet was retarded after 4 weeks of feeding. In their study, dietary PN levels of 0, 6, 12, and 24mg were tested on prawns (0.5g average body weight). At the end of the feeding period (week 12), they found that the growth of prawns fed a diet with 24mg of supplemental PN/100g diet was slightly higher than that of PN-deficient prawns. However, during the first 10 weeks of feeding, the growth of the prawns receiving PN at 24mg/100g diet was consistently lower than PN-deficient prawns. In the present study, the growth of prawns fed high levels of PN (25mg of supplemental PN/100g diet) was slightly lower than those fed with 5mg and 15mg levels, but they were not significantly different. Feed intake and FCE were also not different for groups fed PN-supplemented diets.

Vitamin B_6 has been reported to exist, in at least six biologically active derivatives in biological materials (Toukairin-Oda *et al.*, 1989). Among these six, only PLP and PMP showed coenzyme activity of vitamin B_6 requiring enzymes. Aminotransferases can use either PLP or PMP, while all other vitamin B_6 dependent enzymes require PLP for reconstitution of an active holoenzyme. Five vitamers (PLP, PMP, PL, PN, and PM) in the hepatopancreas and three (PLP, PMP, and PN) in the muscle of prawns were identified in the present study. The dominant forms were PLP and PMP, which accountied for 74-87% and 87-100% respectively in the hepatopancreas and muscle.

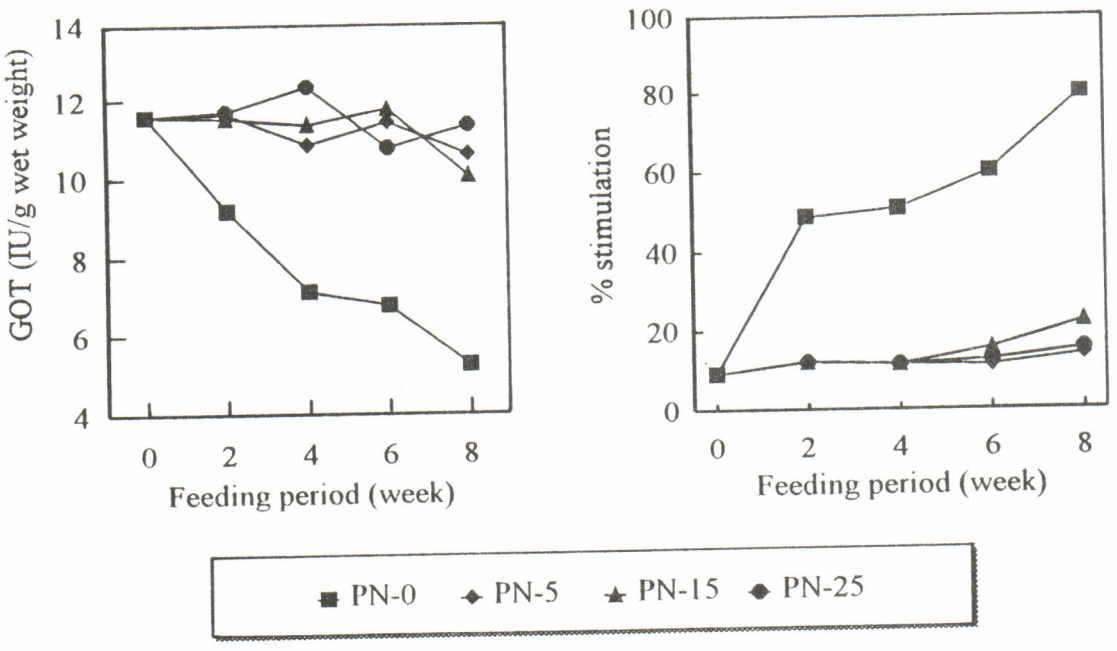

Fig. 2. Muscle GOT activity and its percentage stimulation of prawns fed test diets during 8 weeks of feeding.

PLP and PMP contents in the hepatopancreas of prawns fed diet without supplemental PN were reduced with time during the feeding experiment. At the end of experiment, PLP and PMP contents were 60% and 30% respectively of initial contents. Increasing levels of PN supplementation from 5mg to 25mg/100g diet resulted in the increase in the PMP content, but not for the PLP content. This indicates that PLP in the hepatopancreas reached saturation level with inclusion of 5mg or more PN/100g diet. PMP content was also higher than PLP content in the initial sample. This indicates that excess vitamin B_6 in the hepatopancreas might have been stored as PMP.

In the muscle of prawns fed diet without PN supplementation, PLP and PMP contents declined by 50% during the feeding experiment. Contrary to the situation in the hepatopancreas, PLP content in muscle is always higher than that of PMP content (4 : 1). Their contents were almost the same for all groups fed PN-supplemented diets and this remained un-changed during the experimental period. The results showed that the PLP: PMP ratio in muscle might be strictly controlled and that excess vitamin B_6 was not stored in the muscle as PLP or PMP. Furth-Walker *et al.* (1989) reported that there was a tightly controlled mechanism regulating the ratio of two active forms of vitamin B_6, PLP : PMP (1 : 1) in the liver of mouse. Recently the ratio of 2 : 1 was reported in the liver of mice by van de Kamp *et al.* (1995). Discrepancies among these reported values might be affected either by the species, by vitamin B_6 intake, or by vitamin B_6 status in the body of the animals during the experiment. Tryfiates and Saus (1976) reported the equilibrium ratio of PLP : PMP to be 1 : 2 in the liver of vitamin B_6 deficient rats reached after 5 days of injection with PN. Ratios of less than 1 for low intake (30µg/day) and 1.5 for high intake (75µg/day or more) of vitamin B_6 were reported in the livers of rats fed different levels of PN (van den Berg *et al.*, 1982). It was not clear whether the PMP form could be converted to PLP when the PLP requirement increased. In rat liver, interconversion of PLP and PMP through enzymatic transamination and pyridoxamine-5-phosphate oxidase reactions was suggested. The enzymatic transamination reaction is affected by the ratio of amino acids and keto acid as a substrate (van den Berg *et al.*, 1982). In human liver PMP is subsequently oxidized to PLP by pyridoxamine-5-phosphate oxidase (Merrill *et al.*, 1984). There is no information available concerning the vitamin B_6 metabolism in prawns.

Since aminotransferases require PLP as a coenzyme, assays of this enzyme in various tissues and organs are often used as the basis for measuring vitamin B_6 status in animals. Aspartate aminotransferase (ASAT or GOT, EC 2.6.1.1) and alanine aminotransferase (ALAT or GPT, EC 2.6.1.2) have been used for evaluating vitamin B_6 status in several species of fishes such as rainbow trout (d'Apollonia and Anderson, 1980), turbot (Adron *et al.*, 1978), and gilthead sea bream (Kissil *et al.*, 1981). In the present study, it was observed that muscle GOT activity of prawns fed PN-supplemented diets was unchanged, while it was reduced to 50% of normal in prawns fed diets without supplemental PN. Meanwhile, addition of PLP to the sample increased the percentage stimulation of GOT in prawns fed diet without supplemental PN, but had no effect on prawns fed PN-supplemented diets. These results indicate that the amount of apoenzyme present was not affected by the dietary PN levels, and not all the apoenzyme present in the muscle of prawns fed diet without supplemental PN was saturated with the coenzyme pyridoxal phosphate.

Values of muscle GOT activity showed similar patterns to PLP and PMP contents of the muscle. High positive correlations were found between muscle GOT activity and PLP content in muscle ($r = 0.99$), and between muscle GOT activity and PMP content ($r =$

0.94) in the present study. These results agree with the findings of Albrektsen *et al.* (1993) and Albrektsen *et al.* (1995) who reported that GOT activity in muscle of Atlantic salmon was significantly correlated with muscle vitamin B_6 content. The present results suggest that muscle GOT activity, PLP and PMP contents in muscle might be good indicators to evaluate the nutritional status of vitamin B_6 in the prawns, *P. japonicus* in addition to growth.

The present study showed that the minimum dietary PN requirement for juvenile *P. japonicus* was less than 5mg/100g diet for maximum weight gain. Supplementation of 5mg/100g diet was required for maximum PLP contents in the hepatopancreas and muscle of prawns. These values were much lower than those reported by Deshimaru and Kuroki (1979), who suggested 12mg PN/100g diet based on both weight gain and PN content of juvenile *P. japonicus*. In their study, however, the higher weight gain of prawns fed diet with 12mg of supplemental PN/100g diet was observed only at the end of the experiment, while weight gain during the first 10 weeks of feeding was almost the same as for prawns fed diet with 6mg of supplemental PN/100g diet. The authors also did not indicate whether the improvement over 6mg PN/100g diet group was significant, nor was there any replication performed in their study to enable statistical analysis. Based on PN content in the body of prawns reported by Deshimaru and Kuroki (1979); Coburn (1994) estimated that the vitamin B_6 requirement of *P. japonicus* was about 6.2mg/100g diet (10mg vitamin B_6/day/prawn).

No specific deficiency signs caused by lack of vitamin B_6 were observed in the present study, although for poor growth, poor survival, low FCE, and low feed intake were noted. Since kuruma prawns are bottom-living and live buried in the sand bottom, observations of other deficiency signs become more difficult compared to free-swimming animals. Low muscle GOT, and PLP and PMP contents in the hepatopancreas and muscle of prawns fed without supplemental PN in the present study might be considered as sensitive indicators for the early detection of vitamin B_6 deficiency in *P. japonicus*.

CONCLUSION

1. Vitamin B_6 is essential for normal growth and prevention of its deficiency signs of *P. japonicus*.
2. Deficiency signs of vitamin B_6 were poor growth and survival, low PLP and PMP levels in both hepatopancreas and muscle, and low muscle GOT activity.
3. Dietary pyridoxine requirement for maximum weight gain of juvenile *P. japonicus* was 4.3 mg/100 g diet. Dietary level of 5 mg/100 g diet was required for maximum PLP content in the hepatopancreas and muscle of prawns.
4. Predominant forms of vitamin B_6 were PLP and PMP, both in the hepatopancreas and muscle of prawns, and their levels were influenced by dietary PN. PLP level in the hepatopancreas was lower than PMP level. However, PLP level in the muscle was much higher than PMP level.

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