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PYRIDOXINE REQUIREMENT OF JUVENILE ABALONE, *HALIOTIS DISCUS HANNAI* INO

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ABSTRACT Juvenile abalone, *Haliotis discus hannai* Ino (initial mean body weight: 0.56 ± 0.01 g, shell length: 15.74 ± 0.13 mm) were fed six diets with graded levels of pyridoxine (PN) (0.40, 12.5, 22.7, 43.5, 87.8, and 172.2 mg/kg diet). Each diet was fed to three replicate groups of juvenile abalone for 16 wk. Dietary PN significantly ($P < 0.05$) affected abalone growth, tissue concentrations of PN and pyridoxal 5′-phosphate (PLP), and the activities of asparatate aminotransferase (AST) and alanine aminotransferase (ALT). The responses of all these parameters to dietary pyridoxine levels fitted the broken-line model. Broken-line regression analyses showed that the breakpoints were 23 on the basis of growth data, 32–38 on tissue concentrations of PN and PLP, 20–21 on the activities of visceral AST and ALT, and 39–40 on the activities of muscle AST and ALT. Hence, the requirement of *H. discus hannai* for dietary pyridoxine should be 23 mg/kg diet for maximum growth, and about 40 mg/kg diet for the saturation of tissue PN and PLP, or tissue aminotransferase activities.

KEY WORDS: abalone, *Haliotis discus hannai*, pyridoxine, requirement, aminotransferases, feeding and nutrition

INTRODUCTION

Vitamin B_6, including pyridoxine (PN) and pyridoxamine (PM), is an essential nutrient of animals, including aqueous animals such as fishes and crustaceans. Its major active forms in animals are pyridoxal 5′-phosphate (PLP) and pyridoxamine 5′-phosphate (PMP). Vitamin B_6 deficiency can result in anorexia, anemia, dark coloration, loss of balance, poor growth, and high mortality in fish (Smith et al. 1974, Kissil et al. 1981, Herman 1985, Albrektsen et al. 1993). In crustaceans, only high mortality and growth depression were reported for prawns fed a PN-deficient diet (Deshimaru & Kuroki 1979). There is no information available so far on the requirement of pyridoxine and its avitaminosis in any mollusc species.

In addition to growth, survival and tissue concentration of vitamin B_6 vitamers, aminotransferases in various tissues and organs are often used as the basis for evaluating vitamin B_6 status in animals, because these enzymes require PLP as a coenzyme. Aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) have been measured in several species of aquatic animals, such as gilthead seabream (Kissil et al. 1981) and in shrimp *Penaeus japonicus* Bate (Giri et al. 1997).

Haliotis discus hannai is one of the most commercially important gastropods in aquaculture. Studies on the nutrition of water-soluble vitamins for this abalone have previously been reported by our laboratory (Mai 1998, Mai et al. 2001, Wu et al. 2002, Zhu et al. 2002, Chen et al. 2005). The purpose of this study is to investigate the effect of dietary pyridoxine on survival, growth, tissue pyridoxine concentration, and aminotransferases activity of juvenile abalone, *H. discus hannai*.

MATERIALS AND METHODS

Experimental Diet and Animal Rearing

Formulations of the experimental diets and their proximate composition are shown in Table 1. Crude protein level of the experimental diets was 28.2%, which is considered to be sufficient to maintain optimum growth for *H. discus hannai* (Mai et al. 1995a). Soybean oil and menhaden fish oil (1:1) was used as the basal lipid source. Dietary lipid level was 3.75%, which was sufficient to support optimum growth and provide enough essential fatty acids for the abalone (Mai et al. 1995b). The vitamin (pyridoxine free) and mineral mixture were slightly modified from those used by Uki et al. (1985). Six experimental diets were formulated from the purified ingredients to provide graded levels of pyridoxine (0.40; 12.5; 22.7; 43.5; 87.8; 172.2 mg/kg diet, determined by high performance liquid chromatography (HPLC).

PN-HCl was encapsulated with sodium alginate by emulsion coacervation process before being supplemented in the experimental diets. The method of encapsulation was derived from Bodmeier & Wang (1993) and similar to that used in Zhu et al. (2002).

Procedures for food preparation were from the method of Mai et al. (1995a). All the ingredients (passed through a mesh with 125-μm pore size) were mixed thoroughly and were made to a paste by gradually adding water (about 120%, volume/weight). The paste was shaped into 0.5 mm thick sheets, which were cut into 1-cm^2 flakes. The flakes were dipped in an aqueous solution of $CaCl_2$ (5%, w/v) for about one minute, and then the surplus solution was drained naturally. The diet flakes were sealed in sample bag and stored at –20°C until use.

Juvenile abalone (*H. discus hannai*, approximately 0.56 ± 0.01 g body weight and 15.74 ± 0.13 mm shell length), used in this experiment were obtained from a spawning at Mashan Fisheries Co., Shandong province of China. There were six treatments with three replicates per treatment. The abalone were assigned to 18 plastic cages (20 × 20 × 15 cm), and each cage stocked with 30 animals was used as a replicate. A corrugated plastic plate in each cage was used as a shelter for the abalone. All 18 cages were distributed randomly in a rectangle pool (6 × 2 × 1.5 m) that was continuously supplied with fresh seawater, flowing through 30-μm primary sand filters, then through 10-μm secondary composite sand filters. The flow rate was about 0.5 L per min per cage. Cages were kept in dim light by screening a

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TABLE 1.

Ingredient composition and proximate of experimental diets (% on dry weight basis).

Ingredients	Content (g/100 g diet)
Casein, vitamin free (Sigma Chemical, St. Louis, MO)	25
Gelatin (Sigma)	6
Dextrin (Shanghai Chemical Co., Shanghai, China)	34
CM-cellulose (Shanghai Chemical Co)	5
Sodium alginate (Shanghai Chemical Co.)	20
Vitamin mix.[a], pyridoxine free	2
Mineral mix.[b]	4
Choline chloride (Shanghai Chemical Co.)	0.5
SO/MFO[c]	3.5
Proximate analysis (means of triplicates)	
Protein (%)	28.2
Lipid (%)	3.75
Ash (%)	8.34

[a] Vitamin mix: each 1,000 g of diet contained thiamin HCl, 120 g; riboflavin, 100 mg; folic acid 30 mg; PABA, 400 g; niacin, 200 mg; Ca pantothenate, 200 mg; inositol, 4,000 mg; biotin, 12 mg; vitamin E, 450 mg, menadione, 890 mg; VB_{12}, 0.18 mg; ascorbic acid, 4,000 mg; retinol acetate, 100,000 IU; cholecalciferol, 2,000 IU; ethoxyquin, 400 mg.
[b] Mineral mix: each 1,000 g of diet contained NaCl, 0.4 g; $MgSO_4 \cdot 7H_2O$, 6.0 g; $NaH_2PO_4 \cdot 2H_2O$, 10 g; KH_2PO_4, 12. 8 g; $Ca(H_2PO_4) \cdot H_2O$, 8 g; Fecitrate, 1.0 g; Ca-lactate, 1.4 g; $ZnSO_4 \cdot 7H_2O$, 141.4 mg; $MnSO_4 \cdot 4H_2O$, 64 mg; $CuSO_4 \cdot 5H_2O$, 12.4 mg; $CoCl_2 \cdot 6H_2O$, 0.4 mg; KIO_3, 1.2 mg.
[c] Soybean oil and menhaden fish oil (1:1) with 0.001% ethoxyquin.

large drape above. Before the experiment, the abalone underwent a 1-wk conditioning period. They were fed appropriate diets once daily at 1700 hours at a satiation level. The plastic cages were cleaned at 0800 hours every morning to remove the uneaten feed and feces. During the experimental period, the water temperature was 15–25°C, salinity 30–34, and pH 7.6–7.9. Dissolved oxygen was not less than 7 mg/L, and there were negligible levels of free ammonia and nitrite. The pyridoxine in the seawater was below measurable levels. The whole feeding experiment was conducted for 16 wk.

Sample Collection and Analysis

At the termination of the experiment, all abalone were fasted for 3 days, then removed from the cages, counted, weighed, and shell length measured, then frozen at –70°C for subsequent chemical analysis. Growth was expressed as specific growth rate (SGR, %/day) and daily increment in shell length (DISL, μm/day), the calculation formula is as follows:

$$\mathrm{SGR}\,(\%/\mathrm{day}) = [(\ln W_t - \ln Wi)/t] \times 100$$

$$\mathrm{DISL}\,(\mu\mathrm{m}/\mathrm{day}) = [(SLt - SLi)/t] \times 1,000$$

where, W_t, Wi are final and initial mean weight (g), SLt, SLi are final and initial mean length (mm), respectively; t is the feeding trial period (day).

The samples of abalone from each replicate were slightly thawed, and the soft-body were separated from the shells, and then divided into visceral tissues and muscular tissues. Both tissues from each replicate were pooled, respectively, fine cut and prehomogenized in ice baths. Appropriate aliquots were taken from the homogenate for the determinations of proximate composition. Proximate analysis to determine protein, lipid, and moisture was conducted using standard methods (AOAC 1995).

PN and PLP concentrations in tissues were analyzed by HPLC using the method modified from Giri et al. (1997). About 0.5 g prehomogenized sample or diet was homogenized with 3 mL of 1.0 M perchloride acid. The mixture was centrifuged at × 6000 g for 10 min at 4°C. The pH of the supernatant was adjusted to 3–4, and centrifuged again at × 6000 g for 10 min at 4°C. When the concentration of PN or PLP in a sample was too low, the sample was concentrated with nitrogen blow at 60°C. The supernatant was filtered through 0.45-μm filter and then analyzed with a HPLC (Hewlett Parkard 1100). The injection volume was 10 μL. An ODS Hypersil (4 × 250 mm) column was used for separation. The mobile phase A was 0.033 M phosphoric acid, adjusted pH to 2.2 with 6 N KOH. Mobile phase B was 0.033 M phosphoric acid and 6.33% 2-propanol, adjusted pH to 2.2 with 6 N KOH, A:B = 50:50. The flow rate was 1.0 mL/min. PN and PLP were detected at 280 nm with a diode array detector (DAD). PN-HCl and PLP (Sigma Co.) was solved in 0.01 M HCl as standards.

AST and ALT activities were measured by the method modified from Giri et al. (1997). About 0.5 g prehomogenized viscera or muscle of test abalone was homogenized again in 4 mL of extraction buffer (0.1 M sodium phosphate, pH 7.2; 20% glycerol; 0.02% Triton X-100 and 1.5 mM dithiothreitol). The homogenate was then centrifuged at × 10,000*g* at 4°C for 20 min. The collected supernatant was again centrifuged for 20 min to obtain a clearer supernatant. The supernatant was analyzed using Beckman autobiochemistric analyzer (CX-7, USA) with a test kit (Bekman-Doulter). The unit of the enzyme activity was expressed as micromole products of 1g wet tissues in 1 min.

Water Stability of Experimental Diets

The leaching of dietary pyridoxine was measured with the method used in Zhu et al. (2002). Test feeds were put onto 100-μm mesh screens and allowed to settle to the bottom of experimental cages without abalone. Temperature and flow rate were 20 ± 0.8°C and about 0.5 L per min per cage, respectively, which match those of the experiment. At the end of the allotted time (0, 1, 2, 6, and 12 h, respectively), the remaining feed were removed from the cages and lyophilized for 24 h. Then the PN–HCl concentrations were measured by HPLC (HP1100).

Statistical Analysis

All percentage data were square-root arcsine transformed prior to analysis. Data from each treatment were subjected to one-way ANOVA. When overall differences were significant at less than 5%, Tukey HSD test was used to compare the mean values between individual treatments. Pyridoxine requirements of the juvenile abalone were estimated on the basis of the specific growth rate (SGR,%/day), daily increment in shell length (DISL), concentrations of PN and PLP, activities of AST and ALT in abalone viscera and muscle using broken-line

analysis (Robbins et al. 1979). Statistical analyses were performed using the STATISTICA package (Version 5.1 for Windows).

RESULT

Leaching

The result of the leaching test with experimented diets is shown in Table 2. The dietary PN-HCl content declined during the text period. The retentions of the microencapsulated PN-HCl in diets were significantly higher than that of crystal form ($P < 0.05$) at any allotted time. In the first 2 h, the retentions of microencapsulated PN-HCl ranged from 83.9% to 86.5%, whereas that of crystal PN-HCl was only 57.4%.

Deficiency Signs

The 16-wk feeding trial showed that pyridoxine deficiency resulted in poor growth and anorexia of abalone, *H. discus hannai*. No other overt deficiency sign was observed.

Survival, Specific Growth Rate, and Daily Increment In Shell Length

There were no significant differences in survival of the abalone among the dietary treatments (Table 3). The SGR and DISL, however, were significantly affected by dietary PN over the 16-wk rearing period. The lowest SGR and DISL were observed in the abalone fed the pyridoxine-deficient diet, and SGR and DISL increased as the dietary PN increased from 0.4–22.7 mg/kg, and then leveled off when dietary PN levels got higher. Both SGR and DISL responded to increasing dietary PN in a broken-line model. The equations describing the relationship between SGR or DISL and dietary pyridoxine level (X) are $Y = 0.789 - 0.0025\ (23 - X)$, and for DISL, $Y = 63.273 - 1.486\ (23 - X)$, respectively. The breakpoints in the regression lines, which are considered to be the minimum dietary requirements for optimum responses, were both 23, indicating that the minimum dietary requirement of dietary pyridoxine for optimal growth in term of SGR and DISL is 23 mg/kg diet.

Tissue Contents of PN and PLP

The PN and PLP levels in abalone viscera and muscle were sensitive to dietary PN level (Table 4). Increasing levels of dietary pyridoxine yielded positive effects on tissue PN and PLP ($P < 0.01$), which reached the maximum levels in the abalone fed the diet containing 43.5 mg PN/kg diet and leveled off when the dietary PN increased further. Hence, they responded to dietary pyridoxine also in a broken-line manner. The breakpoints of broken-line regression analyses on the basis of visceral PN, visceral PLP, muscle PN, and muscle PLP were 32, 38, 37, 38 mg/kg diet, respectively.

Aminotransferase Activities in Viscera and Muscle

The AST and ALT activities in the viscera and muscle of abalone fed with different levels of PN are shown in Table 5. The AST and ALT activities were all significantly affected by dietary PN level. The activities of these two enzymes in the viscera increased when the dietary PN increase from 0.4–22.7 mg/kg diet, and then leveled off. The broken-line model can also describe the relationship between enzyme activities and dietary pyridoxine level. The breakpoints of the regression analyses were 20 and 21 respectively. The activities of the two aminotransferases in abalone muscle, however, were much higher than those in the viscera and increased with increasing dietary PN, and leveled off when the dietary PN was more than 43.5 mg/kg. The corresponding breakpoints were 40 and 39 respectively.

DISCUSSION

To prevent the dietary pyridoxine from leaching during the period before the diets were consumed by abalone, the PN-HCl was microencapsulated with sodium alginate by emulsion coacervation process before supplementation. Sodium alginate, the wall material of microencapsulation, is easily digested by abalone because they have abundant alginate lyase in their digestive system (Nakagawa & Nagayama 1988, Takami et al. 1998). As a result, the leaching of the dietary pyridoxine was greatly reduced (Table 2). In the first 2 h, the values of leaching of the microencapsulated dietary PN-HCl were only 13.8% to 15.6%, significantly lower than that with crystalline pyridoxine

TABLE 2.

Retention of PN-HCl (%) in the test diets with graded levels of PN-HCl*.

Dietary Pyridoxine (mg/kg)	Immersion Time				
	0	1	2	6	12
0.40	—	—	—	—	—
12.5	100	91.5 ± 2.3[a]	86.5 ± 2.2[a]	66.9 ± 2.6[a]	47.2 ± 2.5[a]
22.7	100	91.0 ± 3.4[a]	85.3 ± 4.1[a]	67.0 ± 1.6[a]	46.9 ± 2.3[a]
43.5	100	90.7 ± 2.7[a]	84.2 ± 3.6[a]	66.2 ± 3.1[a]	45.4 ± 2.7[a]
87.8	100	90.2 ± 3.5[a]	84.3 ± 3.2[a]	65.7 ± 3.5[a]	45.5 ± 2.4[a]
172.2	100	89.3 ± 1.9[a]	83.9 ± 2.4[a]	65.4 ± 2.8[a]	44.6 ± 3.4[a]
40 (crystal form)	100	69.5 ± 2.6[b]	57.4 ± 3.1[b]	37.7 ± 2.5[b]	28.2 ± 2.4[b]
ANOVA					
F value		17.312	26.487	24.154	32.528
P value		0.003	0.000	0.000	0.000

* Mean ± SE for duplicates.

Means in each column not sharing a common superscript are significantly different according to Tukey test ($P < 0.05$).

TABLE 3.

Growth and survival of abalone fed diets containing graded levels of pyridoxine for 16 wk[1].

Dietary Pyridoxine (mg/kg diet)	Initial Weight (mg)	Initial Shell Length (mm)	Final Weight (mg)	Final Shell Length (mm)	SGR[2] (%/d)	DISL[3] (um/day)	Survival (%)
0.4	550 ± 8.9	15.69 ± 0.18	1104.6 ± 17.63[a]	19.10 ± 0.82[a]	0.62 ± 0.02[a]	31.0 ± 2.26[a]	96.7 ± 2.74
12.5	547.8 ± 16.6	15.58 ± 0.31	1217.4 ± 33.19[ab]	20.51 ± 0.50[ab]	0.71 ± 0.02[ab]	44.8 ± 2.29[ab]	97.8 ± 3.16
22.7	566.7 ± 17.9	15.88 ± 0.21	1445.6 ± 94.72[b]	22.48 ± 0.89[b]	0.83 ± 0.04[b]	64.3 ± 3.52[b]	90.0 ± 0.00
43.5	561.1 ± 15.0	15.78 ± 0.07	1375.1 ± 64.82[b]	22.14 ± 0.52[b]	0.80 ± 0.05[b]	63.2 ± 3.81[b]	95.5 ± 5.63
87.8	546.6 ± 40.4	15.63 ± 0.76	1392.6 ± 94.01[b]	22.09 ± 1.30[b]	0.83 ± 0.04[b]	62.7 ± 4.06[b]	97.8 ± 3.16
172.2	566.7 ± 32.7	15.87 ± 0.63	1451.4 ± 102.1[b]	22.58 ± 1.62[b]	0.84 ± 0.04[b]	64.0 ± 4.61[b]	97.8 ± 3.16
ANOVA							
F value	0.443	0.239	3.457	19.154	5.275	31.162	1.062
P value	0.810	0.937	0.036	0.000	0.009	0.000	0.429

Means in each column not sharing a common superscript are significantly different according to Tukey test ($P < 0.05$).
[1] Mean ± SE, for triplicates.
[2] Specific growth rate.
[3] Daily increment of shell length.

(44.1%). Results of a previous study indicated that the digestive tracts of most abalone were full of food within the first 2 h of feeding (Mai et al. 1998). Additionally, the selected criteria relating to abalone growth, the tissue concentrations of PN and PLP, and the aminotransferase activity responded to increasing dietary PN in a broken-line manner. Hence, it is reliable to evaluate the effects of dietary pyridoxine on abalone using these feeds.

Based on growth data, SGR and DISL, broken-line regression analysis showed that the minimum dietary requirement of dietary pyridoxine for optimal growth of abalone is 23-mg/kg diet. Based on the activities of the two aminotransferases (AST and ALT) in abalone viscera, the breakpoints of broken-line regression analysis is 20–21. Based on the activities of AST and ALT in abalone muscle, however, the breakpoints are 39–40. Using the data of tissue concentrations of PN and PLP for regression analysis, the estimated requirement of *H. discus hannai* for dietary pyridoxine is 32–38 mg/kg. Hence, it can be concluded that the requirement of *H. discus hannai* for dietary pyridoxine is 20–40 mg/kg diet. These values are higher than those of fishes (1–20 mg/kg diet; NRC 1993), but lower than those of shrimp (60–89 mg/kg diet; Deshimaru & Kuroki 1979; Shiau & Wu 2003).

Generally, the pyridoxine requirements of fishes based on maximum growth are lower than those based on the maximum storage of this vitamin (NRC 1993), which was also observed in the present study. Considering the high water solubility of dietary pyridoxine and the differences in feeding behavior among fish, shrimp, and abalone, leaching of this vitamin before consumption is probably one of the main factors resulting in the wide variation in estimated requirements of dietary pyridoxine. Most fish can swallow feed pellets soon as the feed is provided. In contrast it takes much longer for shrimp to ingest feeds and for abalone to locate and rasp feed pellets. Such slow feeding behavior allows soluble nutrients to leach out from feed pellets. Even though the microencapsulation process reduced leaching, the leaching may have been higher in the feeding trial than in the leaching test because of the scraping nature of feeding by abalone. Hence, a higher supplementation level may be necessary to compensate for leaching. The requirements of shrimp for dietary pyridoxine are as high as 60–89 mg/kg diet, which were estimated using uncoated or unencapsulated pyridoxine

TABLE 4.

Pyridoxine (PN) and pyridoxal 5-phosphate (PLP) in viscera and muscle of the abalone fed graded levels of dietary PN-HCl for 16 wk (μg/mg tissue)[1].

Dietary Pyridoxine (mg/kg)	Viscera		Muscle	
	PN	PLP	PN	PLP
0.4	0.65 ± 0.05[a]	1.41 ± 0.11[a]	0.63 ± 0.05[a]	1.22 ± 0.13[a]
12.5	0.93 ± 0.10[ab]	1.62 ± 0.12[ab]	0.85 ± 0.17[ab]	1.37 ± 0.13[ab]
22.7	1.17 ± 0.11[bc]	2.07 ± 0.13[bc]	1.13 ± 0.14[bc]	1.83 ± 0.16[bc]
43.5	1.40 ± 0.10[c]	2.34 ± 0.15[c]	1.39 ± 0.12[c]	2.12 ± 0.18[c]
87.8	1.35 ± 0.13[c]	2.56 ± 0.28[c]	1.47 ± 0.12[c]	2.10 ± 0.18[c]
172.2	1.42 ± 0.12[c]	2.51 ± 0.22[c]	1.45 ± 0.14[c]	2.34 ± 0.16[c]
ANOVA				
F value	17.132	13.941	14.644	16.134
P value	0.000	0.000	0.000	0.000

[1] Mean ± SE for triplicates.
Means in each column not sharing a common superscript are significantly different according to Tukey test ($P < 0.05$).

TABLE 5.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the viscera and muscle in the abalone fed diets containing graded levels of pyridoxine for 16 wk (Unit[1]/g tissue)[2].

Dietary Pyridoxine (mg/kg)	Viscera		Muscle	
	AST	ALT	AST	ALT
0.4	5.97 ± 0.43[a]	2.22 ± 0.17[a]	7.43 ± 0.45[a]	2.96 ± 0.28[a]
12.5	7.99 ± 0.45[b]	2.63 ± 0.25[b]	12.82 ± 1.26[ab]	4.37 ± 0.23[ab]
22.7	9.26 ± 0.32[b]	2.90 ± 0.17[b]	16.23 ± 1.46[b]	5.49 ± 0.45[b]
43.5	9.24 ± 0.44[b]	2.95 ± 0.21[b]	23.46 ± 2.08[c]	7.28 ± 0.43[c]
87.8	9.30 ± 0.61[b]	2.87 ± 0.25[b]	23.70 ± 2.26[c]	7.31 ± 0.46[c]
172.2	9.27 ± 0.68[b]	2.91 ± 0.32[b]	22.50 ± 2.24[c]	7.32 ± 0.85[c]
ANOVA				
F value	14.201	12.133	29.239	28.064
P value	0.000	0.000	0.000	0.000

[1] The Unit was number of micromoles of products in 1 min of 1g wet tissue.
[2] Mean ± SE for triplicates.
Means in each column not sharing a common superscript are significantly different according to Tukey test ($P < 0.05$).

(Deshimaru & Kuroki 1979, Shiau & Wu 2003). These values are about 10 times higher than those of fishes (NRC 1993). It is likely that such high requirement estimates are caused by a very high leaching loss of unencapsulated vitamins. Therefore, reducing soluble nutrient leaching can further reduce the supplemental levels in the diets for slow feeders in water.

The present study found that the activities of both AST and ALT were sensitive to the pyridoxine status in abalone, indicating that in abalone pyridoxine functions similarly to fish and other higher animals. In abalone viscera the activities of both AST and ALT were lower and leveled off at the lower dietary PN level than those in abalone muscle. Abalone, like other marine molluscs, have high tissue concentrations of free amino acids, especially taurine, alanine, glycine, and arginine (Mai et al. 1994). These amino acids have been revealed to play an important role in the energy metabolism by sustaining glycolysis through the formations of opines under hypoxic conditions in abalone (Gäde & Grieshaber 1986, Sato et al. 1991). Higher activities of AST and ALT in abalone muscle than in the viscera probably indicate more vigorous metabolism of amino acids in the muscle to provide energy for motion or firm adhesion on substrates by the foot muscle.

Some PN deficient signs have been reported in aquatic animals. However, some bottom-living fishes and shrimp that spend much time motionless on the bottom are less likely to develop the typical deficiency signs as observed in free swimming fish (Cowey et al. 1975, Chen et al. 1991, Deshimaru & Kuroki 1979). In the present study, only poor growth was observed after in the 16-wk feeding experiment. This may be partly because of the fact that abalone are fairly sedentary. Additionally, abalone grow more slowly compared with most culture fishes. With a longer trial period, some deficiency signs may eventually be observed.

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