Chitin but Not Chitosan Supplementation Enhances Growth of Grass Shrimp, *Penaeus monodon*[1,2]

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ABSTRACT The effect of chitin, poly-β-(1 → 4)-*N*-acetyl-glucosamine, and chitosan, a polymer of glucosamine obtained by the deacetylation of chitin, on growth and nutrient digestibility was studied in grass shrimp, *Penaeus monodon*. Shrimp were fed for 8 wk diets containing no supplement (control) or 2, 5 or 10 g/100 g chitin or chitosan. Each diet was fed to triplicate groups of shrimp with a mean initial body weight of 0.45 ± 0.05 g. Significantly higher body weight gains were observed in shrimp fed the 5% chitin diet than in those fed the 10% chitin or the control diet. The weight gain of shrimp decreased as dietary chitosan supplementation level increased ($r = 0.87$, $P < 0.05$). Feed efficiencies (FE) and protein efficiency ratios (PER) followed the same pattern. Lower protein and lipid digestibilities and lower body protein and lipid contents were observed in shrimp fed all chitosan-containing diets than in controls ($P < 0.05$). Carbohydrate digestibility was lower in shrimp fed the 10% chitosan diet than in those fed the control diet. Lower protein and lipid digestibilities, body lipid content and blood cholesterol concentration were observed in shrimp fed the 10% chitin diet compared with controls ($P < 0.05$). Higher weight gains, body lipid contents and blood cholesterol concentrations were observed in shrimp fed the 2 and 5% chitin diets than in those fed the chitosan diets. Shrimp fed the 5% chitin diet had higher protein and lipid digestibilities and higher body protein content than those fed the 5% chitosan diet ($P < 0.05$). These data suggest that dietary chitin, supplemented at 5%, enhances *P. monodon* growth, whereas chitosan depresses shrimp growth, regardless of the supplementation level. J. Nutr. 128: 908–912, 1998.

KEY WORDS: • *grass shrimp* • *Penaeus monodon* • *chitin* • *chitosan* • *fiber*

Dietary fiber has received much attention lately as a topic of research activity in human and animal nutrition (Edwards 1995, Mackeown-Eyssen and Bright-See 1984, Schneeman and Tinker 1995). The binding abilities of fiber have caused some concern about the effects of a higher fiber diet on nutrient availability. The inclusion of certain types of fiber in the diet have been reported to produce lower apparent availability of minerals (Oku et al. 1982, Ward and Reichert 1986) and to decrease the utilization of protein (Shah et al. 1982, Ward and Reichert 1986, Shiau and Liang 1994). However, knowledge of the responses of crustaceans to increased consumption of dietary fiber appears to be almost nonexistent.

Chitin is a polymer of glucosamine that is found in shells or walls of invertebrates, fungi and yeasts. It is the main component of crustacean exoskeletons and is made up of calcium oxide and protein units. Chitin forms 50–80% of organic compounds in crustaceans shells (Muzzarelli 1977). Chitosan, an aminopolysaccharide, is prepared from shellfish chitin by treatment with alkali. Both chitin and chitosan are non-starch polysaccharides and have the potential to be regarded as components of dietary fiber. The effects of dietary chitin and chitosan on crustacea are not well studied. Kitabayashi et al. (1971) demonstrated that the addition of 0.52% glucosamine to diets improved growth of *Penaeus japonicus*, but growth was retarded if chitin was added to the diet. Deshimaru and Kuroki (1974) stated that a dietary source of glucosamine is unnecessary for *P. japonicus*. Fox (1993) reported that chitin was not directly utilized by *Penaeus monodon*. However, Akiyama et al. (1992) recommended a minimum dietary level of 0.5% chitin in shrimp feed.

The purpose of this study was to elucidate the effects of chitin and chitosan on growth and nutrient digestibility of juvenile grass shrimp, *P. monodon*.

MATERIALS AND METHODS

Diet preparation. The formulation and proximate composition (AOAC 1995) of the seven experimental diets are presented in **Tables 1** and **2**. The basal diet contained 50.4% fishmeal (Norsemink, Norwegian Herring Oil and Meal Industries, Bergen, Norway) and 25.2% cornstarch (Sigma Chemical, St. Louis, MO) as protein and carbohydrate sources, respectively. Either chitin or chitosan (both Sigma Chemical) was added to the basal diet at 2, 5 and 10 g/100 g. A control group without fiber supplementation was also included for a total of seven dietary groups. The diets were prepared by thoroughly mixing the dry ingredients with oil and then adding cold water until a stiff dough resulted. This was passed through a mincer with die and the resulting "spaghetti-like" strings were dried using both air-conditioners and electrical fans. After drying, the diets were broken up, sieved through 2-mm mesh and stored at −20°C.

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TABLE 1

Composition of the basal diet

Ingredient	g/100 g dry weight
Fish meal	50.4
Cornstarch	25.2
Corn oil	5.0
Amino acid mix[1]	3.0
Carboxymethylcellulose	2.5
Sodium citrate	0.3
Sodium succinate	0.3
Sodium hexametaphosphate	1.0
Cholesterol	0.5
Vitamin mix[2]	2.7
Mineral mix[3]	8.6
Cr_2O_3	0.5

[1] Amino acid mix: L-alanine, 0.6 g/100 g; glycine, 0.6 g/100 g; L-glutamic acid, 0.6 g/100 g; betaine, 1.2 g/100 g.

[2] Vitamin mixture (mg/g mixture): *para*-aminobenzoic acid, 10 mg; inositol 100 mg; pyridoxine-HCl, 1.5 mg; riboflavin, 0.8333 mg; nicotinic acid, 0.2667 mg; thiamine-HCl, 0.5185 mg; β-carotene, 1 mg; menadione, 1.4815 mg; all-*rac*-α-tocopherol, 10 mg; vitamin B-12, 0.0074 mg; calciferol, 0.0037 mg; folic acid, 0.2963 mg; choline HCl, 200 mg; ascorbic acid (L-ascorbyl-2-monophoshate), 1.4815 mg; calcium pantothenate, 60 mg; biotin, 0.15 mg. All ingredients were diluted with α-cellulose to 1 g.

[3] Mineral mixture (mg/g mixture): K_2HPO_4, 100 mg; $NaHPO_4$, 215 mg; $Ca(H_2PO_4)\cdot H_2O$, 265 mg; $CaCO_3$, 105 mg; Ca-lactate, 165 mg; KCl, 28 mg; $MgSO_4\cdot 7H_2O$, 100 mg; Fe-citrate, 10 mg; $CuCl_2$, 0.15 mg; $AlCl_2\cdot 6H_2O$, 0.24 mg; $ZnSO_4\cdot 7H_2O$, 4.76 mg; $MnSO_4\cdot 6H_2O$, 1.07 mg; $CoCl_2\cdot 6H_2O$, 1.4 mg; α-cellulose, 2.15 mg.

Experimental procedure. Juvenile *P. monodon* were supplied by the Tungkang Marine Laboratory (Tungkang, Pingtung, Taiwan). Upon arrival, they were acclimated to laboratory conditions for 2 wk in a plastic tank [74 cm (w) × 95 cm (l) × 45 cm (h)] and fed a commercial diet (grass shrimp no. 2 feed, Yung-Hsien, Taipei, Taiwan). The proximate composition (g/100 g) of the commercial diet was as follows: moisture, 9.36; crude protein (N × 6.25), 37.30; lipid, 5.03; and ash, 12.53. At the beginning of the experiment, 21 aquaria ($60 \times 60 \times 45$ cm^3) were each stocked with 16 shrimp with an average wet weight of 0.45 ± 0.05 g. Each experimental diet was fed to three groups of shrimp. Each aquarium received continuous aeration. In each aquarium, impurities in the water were removed every day and 75% of the water was exchanged at 2, 4 and 6 wk to maintain water quality. Dissolved oxygen concentration was monitored weekly and maintained at 7.5 mg O_2/L throughout the experimental period. Water temperature ranged from 25 to 29°C, pH from 6.3 to 6.5 and salinity from 19 to 21 g/kg. A photoperiod of 12 h light, 12 h dark (0800–2000 h) was used. Group of shrimp were fed their respective diets at a rate of 8 g/(100 g body weight · d). This daily ration was subdivided into two equal feedings at 0900 and 1700 h. Shrimp were weighed biweekly and the daily ration was adjusted accordingly. The duration of the study was 8 wk.

At the end of the feeding trial, the shrimp were weighed. Growth (as measured by the percentage of body weight gain), feed efficiency (FE) and protein efficiency ratio (PER) were calculated as described previously (Shiau and Chou 1991, Shiau and Liu 1994). After the final weighing, three shrimp were randomly removed from each aquarium, blood samples were collected from the ostium of each shrimp and pooled for blood cholesterol concentration estimation (Abell et al. 1952); three other shrimp were then taken randomly from each aquarium and pooled for body composition analysis (AOAC 1995).

The apparent digestibilities of protein, lipid and carbohydrate were determined by using diets containing 0.5% chromic oxide as an indicator. After feeding, fecal material was collected by siphoning into a plastic sieve; similarly, any leftover food was siphoned off after each feeding. Care was taken to prevent breaking up fecal strands while siphoning. Fecal collections were made in the morning (1 h after the first feeding; voided between 1000 and 1200 h), and in the afternoon (1 h after the 2nd feeding; voided between 1800 and 2000 h). Fecal collections were conducted throughout the experiment. After each collection, the samples were pooled for shrimp fed each diet, and frozen at −20°C and stored for subsequent analysis. Samples of test diets and freeze-dried feces were analyzed for chromium concentration by atomic absorption spectrophotometry (Arthur 1970). Apparent digestibility of protein, lipid and carbohydrate was calculated as follows:

$$\%\text{Digestibility} = 100 - \left(\frac{\%\ \text{Cr in feed}}{\%\ \text{Cr in feces}}\right) \times \left(\frac{\%\ \text{ingredient in feces}}{\%\ \text{ingredient in feed}}\right) \times 100$$

Statistical analysis. A two-way ANOVA was used to test the effects of fiber sources and supplementation levels and their interactions by using the SAS/PC statistical software (SAS Institute, Cary, NC); significance was set at $P < 0.05$. When a significant main effect was found without the interaction effect, individual mean differences were determined by Duncan's new multiple range test. If a significant interaction ($P < 0.05$) was presented, simple main effects were tested by Student's t test for independent means; to reduce the risk of Type I error, the level of significance was set at $P < 0.01$ rather than $P < 0.05$.

TABLE 2

Formulations and proximate composition of each diet

	Chitin				Chitosan			
	0	2	5	10	0	2	5	10
	g/100 g							
Basal diet	100	98	95	90	100	98	95	90
Chitin	—	2	5	10	—	—	—	—
Chitosan	—	—	—	—	—	2	5	10
Moisture	10.54	10.57	9.40	9.42	10.54	11.41	9.73	9.76
Protein	34.99	34.66	34.54	34.29	34.99	34.30	34.46	33.70
Ether extract	9.70	9.69	9.26	8.61	9.70	9.20	8.91	8.17
Ash	12.13	11.81	11.14	11.31	12.13	11.88	11.75	11.12
Crude fiber	1.35	3.34	5.14	7.87	1.35	3.34	5.67	8.07
N-free extract[1]	31.29	29.93	30.52	28.50	21.29	29.87	29.48	29.18

[1] N-free extract = 100 − (moisture + protein + ether extract + ash + crude fiber).

TABLE 3

Weight gain, feed efficiency (FE), protein efficiency ratio (PER) and survival of P. monodon *fed diets containing various levels of chitin or chitosan for 8 wk*[1]

	Fiber supplementation level (g/100 g)				Pooled SED[2]	ANOVA		
	0	2	5	10		Fiber level (A)	Fiber source (B)	A × B
Weight gain, *g/100 g*								
Chitin	291.84^{ab}	346.32^{bcy}	380.73^{cy}	230.68^{a}	31.23	<0.05	<0.05	NS
Chitosan	291.84^{b}	234.20^{abx}	221.56^{ax}	216.28^{a}	32.62			
FE, *g gain/100 g feed*								
Chitin	52.08^{b}	54.35^{by}	60.24^{cy}	38.17^{a}	1.20	<0.05	<0.05	NS
Chitosan	52.08^{c}	39.37^{bx}	36.50^{ax}	34.48^{a}	1.06			
PER								
Chitin	1.48^{bc}	1.34^{ab}	1.57^{cy}	1.15^{a}	0.12	<0.05	<0.05	NS
Chitosan	1.48^{c}	1.15^{b}	0.90^{ax}	0.85^{a}	0.15			
Survival, %								
Chitin	76.62	83.64	74.17	82.22	5.21	NS	NS	NS
Chitosan	76.62	67.83	63.48	65.45	6.66			

[1] Values are means of three groups of shrimp with 16 shrimp per group. Results were analyzed by two-way ANOVA to determine the effect of fiber source and the effect of fiber supplementation level and their interaction. ^{abc} Significant ($P < 0.05$) differences between fiber supplementation levels within fiber source; ^{xy} differences between fiber sources within fiber supplementation level ($P < 0.05$). NS, not significant ($P > 0.05$).
[2] SED, standard error of the differences.

RESULTS

In the chitin fed groups, higher relative weight gains were observed in shrimp fed the 5% chitin diet than in those fed (in descending order) the 2% diet, the unsupplemented control diet and the 10% chitin diet (**Table 3**). The differences between the 5% group and the control and 10% groups, and between the 2 and 10% groups were significant ($P < 0.05$). The weight gain of shrimp fed chitosan generally decreased as the dietary chitosan supplementation level increased ($y = -2.12x + 236.02$, $r = -0.87$). Significant differences were observed between shrimp fed 10 or 5% chitosan and the control group. Shrimp fed 2 and 5% chitin diets had higher weight gains than shrimp fed comparable chitosan diets. Patterns of feed efficiencies (FE) and protein efficiency ratios (PER) were similar to those of the weight gain. Survival of shrimp fed chitin and chitosan diets ranged from 74 to 84% and 64 to 77%, respectively.

Shrimp fed the 10% chitin diet had lower protein and lipid digestibilities than shrimp fed the control diet (**Table 4**). Higher protein and lipid digestibilities were found in shrimp fed the 5% chitin diet than in those fed the 5% chitosan diet. Lower protein and lipid digestibilities were observed in shrimp fed chitosan diets than in those fed the control diet, regardless of the chitosan supplementation level. Lower carbohydrate digestibility was found in shrimp fed the 10% chitosan diet than in those fed the control diet (Table 4).

Shrimp fed the 10% chitin diet had lower body protein content than those fed the 5% chitin or control diet and had lower body lipid content than shrimp fed 5 or 2% chitin or the control diet (**Table 5**). Body protein and lipid contents were lower in shrimp fed chitosan-containing diets than in controls. Moisture content in shrimp generally inversely reflected their lipid content. Ash content in shrimp was not affected by the diets. Shrimp fed the 10% chitin diet had lower blood cholesterol concentration than shrimp fed 5 or 2% chitin or the control diet. Blood cholesterol concentration in shrimp generally decreased as the dietary chitosan supplementation level increased ($y = -3.22x + 319.94$, $r = -0.94$). Significant differences were observed between chitosan-supplemented groups and the control group and between the 5 and 10% chitosan groups and the 2% chitosan group. Shrimp fed the 5% chitin diet had higher body protein content than those fed the 5% chitosan diet, whereas shrimp fed the 2 and 5% chitin diets had higher body lipid contents and higher blood cholesterol concentrations than shrimp fed the 2 and 5% chitosan diets.

DISCUSSION

The results of this investigation made clear that dietary chitin but not chitosan supplementation enhanced the growth of *P. monodon*. Both chitin and chitosan are acetylated aminopolysaccharides; the difference between them is the extent of acetylation present (Muzzarelli 1977). Chitin, poly-β-(1 → 4)-*N*-acetyl-D-glucosamine, is a cellulose-like biopolymer. It is highly acetylated (15–21% acetyl content) and is insoluble in common solvents. Chitosan, in contrast, is only 3–5% acetylated and is prepared from chitin by deacetylation with alkali (Fillar and Wirick 1978). This deacetylation results in a polysaccharide that behaves as a weak anion exchange resin and that has viscosity properties similar to those of certain water-soluble dietary fibers such as guar gum and pectin (Furda 1983).

Nutrient absorption depends on the rate at which nutrients are in contact with the absorptive epithelium. The influence of dietary fiber on the movement of nutrients along the gastrointestinal tract likely affects nutrient absorption. Water-soluble fibers such as guar gum and pectin have been reported to delay stomach emptying (Schwartz et al. 1982, Tadesse 1982). The delay has been attributed to the increased viscosity of the test diet (Ehrlein and Prove 1982). The rate of nutrient absorption depends on the rate at which nutrients come into contact with the absorptive epithelium. Accordingly, the relative influence of dietary fiber on the movement of nutrients along the gastrointestinal tract will be likely to affect nutrient absorption. Thus the delay in stomach emptying caused by soluble dietary fiber might influence the absorption rate of nutrients. Chitosan has been reported to interfere with fat digestion and absorption in the intestinal tract of rats, and to facilitate the excretion of dietary fat into the feces (Deuchi et al. 1994). This may explain the lower protein and lipid digestibilities of *P. monodon* fed diets supplemented with chito-

TABLE 4

Protein, lipid and carbohydrate digestibility of P. monodon *fed diets containing various levels of chitin or chitosan for 8 wk*[1]

	Fiber supplementation level (g/100 g)				Pooled SED[2]	ANOVA		
	0	2	5	10		Fiber level (A)	Fiber source (B)	A × B
			%					
Protein digestibility								
Chitin	88.80^{b}	87.30^{ab}	87.88^{aby}	86.06^{a}	0.62	<0.05	<0.05	NS
Chitosan	88.80^{b}	86.10^{a}	85.87^{ax}	85.61^{a}	0.83			
Lipid digestibility								
Chitan	92.91^{b}	92.20^{b}	92.33^{by}	89.94^{a}	0.60	<0.05	<0.05	NS
Chitosan	92.91^{b}	89.89^{a}	88.38^{ax}	87.80^{a}	0.92			
Carbohydrate digestibility								
Chitin	91.69	88.17	88.08	87.60	1.21	<0.05	NS	NS
Chitosan	91.69^{b}	88.06^{ab}	87.28^{ab}	85.15^{a}	1.42			

[1] Values are means of three groups of shrimp with 16 shrimp per group. Results were analyzed by two-way ANOVA to determine the effect of fiber source and the effect of fiber supplementation level and their interaction. ^{ab} Significant ($P < 0.05$) differences between fiber supplementation levels within fiber source; ^{xy} differences between fiber sources within fiber supplementation level ($P < 0.05$). NS, not significant ($P > 0.05$).
[2] SED, standard error of the differences.

san (Table 4), which in turn resulted in growth depression (Table 3).

Sterols are considered essential nutrients for crustaceans because of the important role of cholesterol as a cell constituent and as a metabolic precursor of steroid hormones and molting hormones (Teshima 1972). Although various aspects of sterol metabolism, including biosynthesis, side-chain modification, absorption and transport, are not fully understood, it is generally believed that most crustaceans require a dietary source of cholesterol (Teshima 1997). It has been reported that *P. monodon* require 0.5 % dietary cholesterol for maximal growth (Chen 1993). In this study, blood cholesterol concentrations were lower than those of controls in shrimp fed diets containing chitosan ($r = 0.94$, Table 5). In rabbits, ingested chitosan forms micelles with cholesterol and dietary cholesterol in the alkaline fluids in the upper part of the intestine, resulting in the depression of the absorption of dietary cholesterol and the circulation of cholic acid to the liver. Cholic acid is synthesized from blood cholesterol in the liver, resulting in a decrease of blood cholesterol concentration. The micelles are digested by chitinases secreted by intestinal microorganisms in the large intestine, and bile acids and sterols are ex-

TABLE 5

Body composition and blood cholesterol concentration of P. monodon *fed diets containing various levels of chitin or chitosan for 8 wk*[1]

	Fiber supplementation level (g/100 g)				Pooled SED[2]	ANOVA		
	0	2	5	10		Fiber level (A)	Fiber source (B)	A × B
			g/100 g					
Moisture								
Chitin	76.43^{a}	78.38^{ab}	76.92^{a}	79.32^{b}	0.81	<0.05	NS	NS
Chitosan	76.43^{a}	77.61^{ab}	77.67^{ab}	79.16^{b}	0.92			
Crude protein								
Chitin	18.05^{b}	16.84^{ab}	17.81^{by}	16.04^{a}	0.45	<0.05	<0.05	NS
Chitosan	18.05^{b}	16.43^{a}	15.99^{ax}	15.55^{a}	0.40			
Crude lipid								
Chitin	2.59^{b}	2.28^{by}	2.08^{by}	1.38^{a}	0.22	<0.05	<0.05	NS
Chitosan	2.59^{b}	1.68^{ax}	1.42^{ax}	1.26^{a}	0.23			
Ash								
Chitin	4.33	3.57	3.50	3.32	0.43	NS	NS	NS
Chitosan	4.33	3.55	4.17	3.60	0.46			
Blood cholesterol concentration								
Chitin	7.27^{b}	6.99^{by}	6.83^{by}	5.30^{a}	0.38	<0.05	<0.05	NS
Chitosan	7.27^{c}	5.84^{bx}	5.02^{ax}	4.69^{a}	0.36			

[1] Values are means of three groups of shrimp with 16 shrimp per group. Results were analyzed by two-way ANOVA to determine the effect of fiber source and the effect of fiber supplementation level and their interaction. ^{abc} Significant ($P < 0.05$) differences between fiber supplementation levels within fiber source; ^{xy} differences between fiber sources within fiber supplementation level ($P < 0.05$). NS, not significant ($P > 0.05$).
[2] SED, standard error of the differences.

creted as free forms into feces without absorption (Hirano and Akiyama 1995). This mechanism has not yet been shown in crustacea, but our results clearly demonstrated that chitosan supplementation markedly decreased blood cholesterol concentration in shrimp, whereas chitin exhibited such an effect only at the highest supplementation level (i.e., 10%). Another possibility is that because chitosan is an aminopolysaccharide, it is therefore likely to bind acidic compounds such as bile acids. Future study is required to examine this possibility in crustacea.

In this study, we found that chitin supplementation enhances *P. monodon* growth in contrast with the findings of Fox (1993). In his study, *P. monodon* were fed diets containing 0, 4, 8, 12 and 16% chitin, and no growth improvement was observed. The author concluded that the commercial chitin had been prepared with the use of diluted mineral acids, which would have removed a proportion of the test materials, i.e., chitin (Fox 1993). It was then suggested that native chitin, such as that found in shrimp head meal, may be of greater nutritional value to shrimp (Fox 1993). The chitin used in this study was from the same commercial source (Sigma) as that used in the Fox's study. The reason for the different results obtained in the two studies is not known. Fiber can be added to a diet either by substituting an equivalent amount of one of the dietary components or by adding fiber to the total diet. Unlike our study in which fiber was added to the basal diet, in Fox's study, polyethylene was used to compensate for the amount of chitin added to the experimental diet. Polyethylene is also a biopolymer (fiber). The extent to which polyethylene influences the growth of *P. monodon* is not known. Furthermore, the interaction of polyethylene and chitin with respect to the chitin effects on the growth of *P. monodon* certainly requires more investigation. The low chitinoclastic bacteria numbers and decreased chitase levels with increased dietary chitin observed in Fox's study led to a conclusion that shrimp could not utilize dietary chitin directly. It was suggested that the rate of production of endogenous chitinases was too slow to allow digestion of a continual supply of dietary chitin (Fox 1993). Bacteriological and enzyme assays were not performed in the present study. This may also explanain the difference in results between the two studies. The importance of the gut microflora in crustacean nutrition requires further study, especially considering the widescale use of antibiotics in intensive shrimp aquaculture. Despite this unresolved issue, this study clearly shows the growth enhancement of chitin supplementation in *P. monodon*. However, the decrease in growth at the higher (10%) supplementation level suggests that dietary chitin levels should not exceed 5%.

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