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Cholesterol requirement of juvenile tiger shrimp (*Penaeus monodon*)

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Abstract

The effect of dietary cholesterol on the growth and survival of juvenile tiger shrimp (*Penaeus monodon*) was tested using semi-purified diets. Twenty-four shrimp per treatment, with an initial mean weight of 268.2 mg, were individually housed in black nylon mesh pots in 40-liter aquaria containing 30 ppt seawater. Diets containing 0, 0.2, 0.4, 0.5, 0.8, and 1.0% supplemental cholesterol were evaluated. The duration of the experiment was 63 days. Shrimp fed diets containing 0% and 1% cholesterol had significantly lower weight gain and poorer survival than those fed the other diets. There was no significant difference in weight gain of *P. monodon* fed diets containing 0.2–0.8% cholesterol. A diet containing 1% cholesterol had an adverse or toxic effect on *P. monodon* growth.

1. Introduction

Cholesterol is an important animal sterol which occurs free or in combination with fatty acids in all cells and in blood. It serves as a precursor of a number of compounds, such as sex hormones, adrenal corticoids, bile acids and vitamin D. Most animals can synthesize sterol from acetate, but crustaceans have been shown to be incapable of *de novo* sterol synthesis from acetate (Teshima and Kanazawa, 1971). Therefore, dietary cholesterol is considered to be essential for good growth and survival of crustaceans. Kanazawa et al. (1971) reported that *Penaeus japonicus* fed a sterol-free diet had poor growth and survival. Other workers have also demonstrated the necessity of dietary cholesterol for good growth of *P. japonicus* (Deshimaru and Kuroki, 1974), crayfish, *Pacifastacus leniusculus*

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(D'Abramo et al., 1985b) and American lobster, *Homarus americanus* (Castell et al., 1975; D'Abramo et al., 1984).

Some lack of agreement exists in the reported requirement values of cholesterol for *P. japonicus*. Kanazawa et al. (1971) reported a value of 0.5% dietary cholesterol for good growth, whereas other researchers have obtained the best growth of *P. japonicus* with diets containing 0.2% (Shudo et al., 1971) and 2.1% (Deshimaru and Kuroki, 1974) cholesterol. These contradictory results with *P. japonicus* are considered to be due to differences in the composition of the test diets used. Castell et al. (1975) reported that the optimum level of cholesterol for *H. americanus* was 0.5%. However, D'Abramo et al. (1984) found that a diet containing as low as 0.12% cholesterol was satisfactory for normal growth of *H. americanus* and no significant difference was observed in the growth of animals fed diets containing 0.19–0.59% cholesterol. Recently, Kean et al. (1985) re-evaluated the cholesterol requirement for *H. americanus* and reported that 0.25–0.5% cholesterol was optimal. D'Abramo et al. (1985b) studied the sterol requirement of *P. leniusculus* and found that 0.4% dietary sterol was associated with the best growth of juvenile crayfish.

The interaction between cholesterol and phosphatidylcholine on the weight gain of *P. penicillatus* and *P. monodon* has been investigated by Chen and Jenn (1991) and Chen (1993), respectively. These studies used three levels of dietary cholesterol (0, 0.5, and 1%) and the results indicated that 0.5% dietary cholesterol was required. These workers concluded that 0.5% dietary cholesterol was required for good growth of penaeid shrimp. Therefore, the purpose of this study was to investigate the growth-promoting effects of dietary cholesterol and determine the optimum level for juvenile *P. monodon*.

2. Material and methods

Juvenile *P. monodon* were obtained from outdoor ponds of a local commercial shrimp hatchery farm. Animals were held in 58×28×31 cm aquaria and separated individually by placing them in 12×12×26 cm cages made from black screen net. Each aquarium contained 8 animals. The aquaria were fitted with under-gravel filters consisted of crushed coral sand. Salinity was maintained at 30 ppt and water temperature ranged between 30 and 33°C.

There were 6 experimental diets containing 0, 0.2, 0.4, 0.5, 0.8 and 1% cholesterol (Table 1). Diets contained 40% fish meal containing 62% crude protein as the sole protein source. Lipid was extracted from the fish meal with hot 95% ethanol (1:1, w/v) in 4 successive treatments to minimize the contribution of dietary cholesterol. Levels of corn starch and cellulose were changed accordingly to maintain isoenergetic diets. The wheat flour served to bind the diets.

Dry ingredients of the experimental diets were mechanically mixed to insure homogeneity. Distilled water was added and the mixture was blended thoroughly by hand until consistent extrusion was achieved. Diets were cold-extruded through a Nippon Career chopper (2.5 mm die diameter). All experimental diets were dried in a vacuum freeze-dryer (Dura-Dry MP freeze-dryer, model FD-6-55 CMPO, FTS systems, Inc.) for 8 h until the moisture was reduced to less than 10%. All diets were stored frozen (−20°C) in air-tight sealed plastic bags until fed to the animals. The experimental diets were analyzed for

Table 1
Ingredient composition (dry weight %), proximate analysis and amount of cholesterol in the experimental diets

Ingredient	Diets					
	A	B	C	D	E	F
Cholesterol	0.0	0.2	0.4	0.5	0.8	1.0
Corn starch	21.1	20.7	20.2	20.0	19.3	18.85
Cellulose	15.5	15.7	16.0	16.1	16.5	16.75
Basal ingredients[a]	63.4	63.4	63.4	63.4	63.4	63.4
Analyzed composition (as fed)						
Moisture	7.9	4.7	12.0	3.2	10.8	5.2
Crude protein[b]	34.1	34.3	33.7	36.0	33.9	33.7
Ash[b]	11.7	12.0	11.9	11.8	11.9	12.4
Crude fat[b]	6.4	5.9	5.9	6.5	6.7	6.6
Cholesterol[b]	0.02	0.19	0.43	0.48	0.81	0.95

[a]Lipid-extracted fish meal 40%, wheat gluten 5%, dextrin 5%, vitamin mix[1] 4%, mineral mix[2] 4%, vitamin E (500 IU/g) 0.2%, vitamin A (500,000 IU/g) 0.1%, vitamin D (500,000 IU/g) 0.1%, choline chloride 1%, and lipid mix (2:1 cod liver oil and corn oil) 4%.
[b]Expressed as percent dry weight.
[1]Thiamine (HCl) 0.5%, riboflavin 0.8%, niacinamide 2.6%, *d*-biotin 0.1%, Ca-pantothenate 1.5%, pyridoxine HCl 0.3%, folic acid 0.5%, cyanocobalamin 0.1%, inositol 18.1%, ascorbic acid 12.1%, BHT 0.1%, *p*-aminobenzoic acid 3.0%, and cellufil 60.3%.
[2]Bernhart-Tomarelli modified (Bernhart and Tomarelli, 1966).

proximate composition based on AOAC (1984) methods. Crude protein was determined with a Kjeltec semi-autoanalyzer model 1007 (Tecator, Sweden). Crude lipid was determined by the ether extraction method (Folch et al., 1957). Ash and moisture were determined by the conventional method using a muffle furnace and a 200°C oven.

The cholesterol level in the diet and whole body tissue excluding exoskeleton was determined by lipid extraction, saponification and gas-liquid chromatography (GLC). The extracted lipid samples were saponified by refluxing in 50% ethanolic KOH solution for 40 min. Upon cooling, the samples were diluted with 40 ml distilled water and the unsaponifiable lipids extracted 3 times with 20, 30, and 40 ml of ether. The ether phase was washed several times with distilled water and then evaporated to dryness. The cholesterol was analyzed using GLC (Hitachi 163 instrument, Hitachi Ltd, Japan) equipped with a flame ionization detector. The cholesterol was then separated on a glass column (3.0 m × 2 mm) packed with 3% SE-30. The column, injector and detector temperatures were maintained at 275, 285 and 285°C, respectively. Nitrogen was used as the carrier gas. Cholesterol was identified by comparison with the retention time of authentic cholesterol. The magnitude of each peak on the chromatogram was quantified by a Hitachi Chromato-integrator D-2000.

There were 6 dietary treatments consisting of 6 different levels of cholesterol. Each diet was fed to triplicate groups of 8 animals for 63 days. The shrimp were fed to excess twice daily at 10.00 and 17.00. Uneaten feed and other waste material were removed from each cage prior to each feeding period. Individual wet weights were determined every 3 weeks, and recorded to the nearest 0.1 mg. Proximate analyses of pooled whole body tissue excluding exoskeleton were conducted at the end of the study.

Mean wet weights and survival associated with the dietary treatments were compared by a one-way ANOVA (SAS, 1985). If significant differences were indicated at or less than the 0.05 level, Duncan's multiple range test was used to identify significant differences among treatment means (Steel and Torrie, 1980).

3. Results

The crude protein and crude lipid content in the experimental diets ranged from 33.7 to 36% and 5.9 to 6.7%, respectively (dry weight basis) (Table 1). The analysis of cholesterol indicated that the diets formulated to contain 0, 0.2, 0.4, 0.5, 0.8 and 1% cholesterol actually contained 0.02, 0.19, 0.43, 0.48, 0.81 and 0.95%, respectively.

Growth performance and survival of juvenile *P. monodon* fed the experimental diets for 63 days are shown in Table 2. At the end of the feeding trial, the mean percent weight gains of juvenile *P. monodon* fed diets containing 0% and 1% cholesterol were significantly lower ($P<0.05$) than those fed the other treatment diets. There was no significant difference ($P>0.05$) in percent weight gains of *P. monodon* fed diets containing 0.2, 0.4, 0.5, and

Table 2
Mean ± s.d. (mg) wet weights of initial and final, percent weight gain of juvenile *Penaeus monodon* and survival rates of the feeding trial

Cholesterol level	Initial weight	Final weight	Weight gain (%)	Survival rates (%)
0.02	258 ± 65	396 ± 80	101[a]	13.3[a]
0.19	286 ± 68	1180 ± 420	333[b]	83.3[b]
0.43	261 ± 59	1082 ± 390	336[b]	86.7[b]
0.48	270 ± 52	1188 ± 339	348[b]	93.3[b]
0.81	274 ± 62	1218 ± 304	348[b]	93.3[b]
0.95	295 ± 69	931 ± 325	196[a]	33.3[a]

Weight gain percentages and survival rates with different superscripts are significantly different ($P<0.05$).

Table 3
Results of proximate analyses and cholesterol level (mg/g of body weight) of whole body tissue excluding exoskeleton of juvenile *Penaeus monodon* fed test diets

Cholesterol level	Whole body tissue composition (% dry weight)			
	Crude protein	Crude fat	Ash	Cholesterol
Initial	76.85	10.61	12.54	0.49
0.02	–[a]	12.83	–[a]	0.44
0.19	85.41	9.12	5.47	1.09
0.43	87.33	6.71	5.96	1.03
0.48	85.70	5.57	5.99	0.59
0.81	86.88	7.11	6.00	0.53
0.95	83.53	11.30	5.16	0.61

[a]Missing data.

0.8% cholesterol. Survival rates of *P. monodon* fed diets containing 0% and 1% cholesterol were 13.3 and 33.3%, respectively, whereas survival rates of the remaining groups were in the range of 83.3–93.3%.

Body composition (dry weight basis) and cholesterol content of the whole body tissue of juvenile *P. monodon* fed the different diets are shown in Table 3. *P. monodon* fed diets containing 0% and 1% cholesterol had unexpectedly higher whole body lipid (12.8 and 11.3%, respectively) than those fed the other dietary treatments. The cholesterol content in the whole body tissue increased from 0.44 to 1.09% and then decreased to 0.61% with increasing dietary cholesterol levels. *P. monodon* fed the diet containing 1% cholesterol had lower cholesterol content in their whole body tissue than those fed diets containing 0.2 and 0.4% cholesterol.

4. Discussion

The present study quite clearly indicates that *P. mondon* require a dietary supplement of cholesterol to achieve maximum weight gain and survival. A dietary cholesterol level as low as 0.2% (dry weight) was found to be satisfactory for good growth and survival of *P. monodon* and feeding a diet without supplemental cholesterol caused poor growth and low survival. D'Abramo et al. (1984) also reported that a dietary cholesterol level as low as 0.12% (dry weight) in a purified diet and 0.2% in an unrefined diet was satisfactory for good growth and survival of *Homarus* sp. In contrast, Castell et al. (1975) reported that a 0.1% dietary cholesterol level produced inferior growth and survival in *H. americanus*. Kanazawa *et al.* (1971) reported that 0.5% dietary cholesterol was optimum for good growth and survival of *P. japonicus*. The optimum level of cholesterol for *P. monodon* (Chen, 1993) and *P. penicillatus* (Chen and Jenn, 1991) was also found to be 0.5%. The lack of agreement between these results may be due in part to the design of the experimental diets. The supplemental cholesterol levels used in previous experiments on *P. japonicus* (Kanazawa et al., 1971), *H. americanus* (Castell et al., 1975), *P. penicillatus* (Chen and Jenn, 1993) and *P. monodon* (Chen, 1993) used only two or three levels (0, 0.5 and 1% or higher) of cholesterol. The exact cholesterol requirement cannot be determined from these experimental designs. Therefore, based on the results of this study, it would appear that the requirement for crustaceans may be less than the recommended level of 0.5% cholesterol.

Studies by D'Abramo et al. (1985a) and Teshima and Kanazawa (1983) have shown that digestion and assimilation of dietary cholesterol is affected by dietary lipids and phospholipid. Cholesterol added to a lipid-free diet is almost completely lost in the feces. Adding dietary phospholipids enhances digestibility of sterols. Thus the effective dietary level of cholesterol is very much a function of other dietary factors. With no supplemental lecithin, the 2.1% dietary cholesterol required for *P. japonicus* reported by Deshimaru and Kuroki (1974) was very likely the level required for the dietary formulation they were using. With no supplemental lecithin and using casein-based diets, Castell et al. (1975) found that 0.1 or 0.2% was inferior to 0.5% dietary cholesterol. With varying levels of lecithin supplements, Chen (1993) and Chen and Jenn (1991) found that 0.5% or higher dietary cholesterol was required for *P. monodon* or *P. penicillatus*. However, with varying levels of lecithin sup-

plements, Kean et al. (1985) found 0.25% cholesterol to be sufficient. With no supplemental lecithin, 0.2% dietary cholesterol is a minimum requirement for *P. monodon*. Therefore, the physiological effect of phospholipid on the cholesterol requirement of crustaceans needs further investigation.

The results of this study clearly indicate that *P. monodon* require a dietary source of cholesterol. The growth and survival data indicated that 0.02% dietary cholesterol led to high mortality and poor growth and 0.2% dietary cholesterol produced growth and survival significantly better than the deficient control diet, but not significantly different from 0.4, 0.6 or 0.8% dietary cholesterol. Therefore, the definitive dietary cholesterol requirement must be between 0.2 and 0.8%. However, feeding a level of 1% dietary cholesterol resulted in an adverse effect on growth in this study. Excessive protein levels may have a detrimental effect on fish and crustaceans (Lim et al., 1979; Sedgwick, 1979) and high lipid levels may also have a detrimental effect on crustaceans (Andrews et al., 1972; Forster and Beard, 1973; Sick and Andrews, 1973; Sheen and D'Abramo, 1991). Mercer (1982) indicated that physiological responses to nutrients are, as a rule, graded. Therefore, for most essential nutrients, there is a characteristic nutrient–response curve which increases to a point and then tend to level off. The high level of dietary cholesterol which caused the negative growth response may be a nutrient–response characteristic rather than toxicity in some instances.

It has been reported that *H. americanus* (Castell et al., 1975) and *P. japonicus* (Kanazawa et al., 1971) fed diets containing high levels of dietary steroids showed inferior growth. D'Abramo *et al.* (1984) indicated that a dietary cholesterol level greater than 0.5% for *Homarus* sp. was unnecessary, because 0.3–0.5% cholesterol was found in natural prey of juvenile lobsters, such as rock crab and mussels (Teshima and Kanazawa, 1972). In the present study, inferior growth and low survival of *P. monodon* occurred when they were fed the diet containing 1% cholesterol. Thus, the diets containing excessive levels of cholesterol may cause adverse effects on growth and survival of various prawns. However, the mechanism of this apparent toxic effect is still unknown and further investigation on its metabolism is needed.

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