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Dietary cholesterol requirement of juvenile mud crab *Scylla serrata*

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Abstract

The effect of dietary cholesterol on growth, molting frequency and survival of juvenile mud crab, *Scylla serrata* was investigated using semi-purified diets. Six isoenergetic and isonitrogenous diets containing 0.04%, 0.21%, 0.50%, 0.79%, 1.12% and 1.44% supplemental cholesterol were evaluated. Fifteen mud crabs per treatment, with an initial mean weight of 84.4 ± 30.9 mg, were individually housed in 3 l containers. After termination of the experiment, crabs fed the diets containing 0.5 and 0.79% cholesterol had significantly higher weight gain than those fed the other diets. Also, crabs fed the diet without cholesterol addition had the lowest survival and molting frequency among the groups. Survival of crabs fed cholesterol supplemented diets ranged from 73% to 93%. Diets containing more than 1.12% cholesterol had an adverse effect on mud crab growth. Based on percent weight gain data using broken-line analysis, the optimal dietary cholesterol requirement of juvenile mud crab was found to be approximately 0.51%.

Keywords: Mud crab; *Scylla serrata*; Nutrition; Cholesterol

1. Introduction

Cholesterol is an important animal sterol that occurs free or chemically bound to fatty acids in all cells and blood. It serves as a precursor of numerous physiological compounds, such as sex hormones, molting hormones, adrenal corticoids, bile acids and vitamin D. Most animals can synthesize sterol from acetate, but crustaceans are

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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. For example, *Penaeus japonicus* (Kanazawa et al., 1971), larval *P. japonicus* (Teshima and Kanazawa, 1983) and *P. monodon* (Sheen et al., 1994) fed a sterol-free diet had poor growth and survival.

Several investigations have demonstrated the necessity of dietary cholesterol and a wide range of estimates have been reported for the cholesterol requirement of crustaceans. Kanazawa et al. (1971) observed that when juvenile *P. japonicus* were fed a diet containing 0.5% cholesterol, better growth was achieved. However, other researchers have obtained the best growth of this species with diets containing 0.2% (Shudo et al., 1971) and 2.1% (Deshimaru and Kuroki, 1974) cholesterol. D'Abramo et al. (1984) found that growth of juvenile *Homarus americanus* fed diets containing 0.19–0.59% cholesterol was satisfactory. Kean et al. (1985) re-evaluated the cholesterol requirement for *H. americanus* and reported that 0.25–0.5% dietary cholesterol was optimal. D'Abramo et al. (1985b) also studied the sterol requirement of juvenile crayfish, *Pacifastacus leniusculus* and indicated that 0.4% dietary cholesterol was associated with the best growth of this species. Chen (1993) estimated that 0.5% dietary cholesterol was optimal for growth of juvenile *P. monodon*. Sheen et al. (1994) further indicated that 0.2–0.8% dietary cholesterol is required for good growth of *P. monodon*. White shrimp, *P. vannamei* fed diets containing 0.23–0.42% cholesterol had better growth than those fed other treatment diets (Duerr and Walsh, 1996). Emery (1987) also reported that postlarval *P. vanammei* required 0.5% dietary cholesterol. The range of values reported for crustacean species is considered to be due to differences in the composition of the experimental diets.

Mud crab, *Scylla serrata*, (Keenan et al., 1998) is an important crustacean species farmed in Taiwan. They are polycultured with milkfish or shrimp. Limited knowledge is available concerning nutritional requirements of this crustacean species. Optimal diet for *Carcinus maenas* was first proposed by Adelung and Ponat (1977). Then, Ponat and Adelung (1980) indicated that casein and cod liver oil provided good protein and lipid sources for *C. maenas*. Ponat and Adelung (1983) further reported that *C. maenas* require 1.5% cholesterol in the diet. Recently, Sheen and Wu (1999) pointed out that a dietary level of 5.3 to 13.8% lipid is required for *S. serrata*.

The objective of this study was to determine the cholesterol level required for juvenile crab, *S. serrata*. Six different levels of cholesterol were added to a basal diet. Survival, weight gain and molting frequency of each crab were determined.

2. Materials and methods

S. serrata megalopa were collected from the southern coast of Taiwan and transported by air, in tightly sealed bags, one quarter filled with seawater and inflated with oxygen, enclosed in an insulated container. Upon arrival, megalopae were acclimated to laboratory conditions in 500-l FRP tank and fed the basal diet containing 0.04% cholesterol until they metamorphosed into postlarvae. After megalopa metamorphosed into postlarvae, crabs were randomly selected. Animals were held individually in 3-l

containers, each having its own individual supply of air. Each container was filled with 30‰ filtered seawater and entire water was exchanged daily to remove uneaten food and maintain good water quality.

There were six dietary treatments consisting of six different levels of cholesterol. Ingredient compositions of the six isoenergetic and isonitrogenous purified diets that contained 0%, 0.2%, 0.5%, 0.8%, 1.1% and 1.4% cholesterol are provided in Table 1. Before preparation of diets, lipid was extracted from casein using hot ethanol (1:1, w/v) in four successive treatments to minimize the contribution of dietary lipid. Levels of dietary corn starch and cellulose were changed accordingly to maintain isoenergetic diets. Agar–agar served to bind the diets. The treatment diets were prepared by adding mixed ingredients to 500 ml of an 11.486% agar–agar aqueous solution cooled to 35°C. The mixture was stirred to a homogenous paste, poured into Petri dishes, where it quickly set to a jelly. The jelly was then cut into approximate $1 \times 1 \times 0.3$ cm cubes that were stored frozen (−40°C) in air-tight sealed plastic bags until fed to the animals. The cubes remained intact for more than 24 h in seawater.

The proximate composition of each of the experimental diets and pooled whole crabs from each dietary treatment were analyzed for 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 chloroform–methanol (2:1, v/v) extraction method (Folch et al., 1957). Ash and moisture were determined by conventional methods using a muffle furnace and a 200°C oven, respectively. The crude protein and crude lipid content in the experimental diets ranged from 46.1% to 47.1% and 8.0% to 9.5%, respectively (dry weight).

Table 1
Composition (% dry weight) of test diets fed mud crab for experiment

Ingredients	Dietary cholesterol levels (% dry weight)					
	0	0.2	0.5	0.8	1.1	1.4
Basal mix[a]	77.896	77.896	77.896	77.896	77.896	77.896
Cholesterol	0.0	0.2	0.5	0.8	1.1	1.4
Corn starch	16.75	16.30	15.625	14.95	14.275	13.60
α-Cellulose	5.354	5.604	5.979	6.354	6.729	7.104
Analyzed composition (as fed)						
Moisture[b]	78.3	78.7	78.2	78.1	77.7	78.2
Crude protein[b]	46.1	46.9	46.6	46.9	47.0	46.7
Crude lipid[b]	8.0	8.3	8.6	8.9	9.2	9.5
Crude fiber[b]	5.4	5.5	5.8	6.2	6.5	6.9
Ash[b]	7.6	7.6	7.6	7.7	7.6	7.6
Total cholesterol[b]	0.04	0.21	0.50	0.79	1.12	1.44

[a] Lipid-free casein 50%, lipid mix (2:1 cod liver oil and corn oil) 6%, agar–agar 11.486%, vitamin mix, Thiamin 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%, inositol 18.1%, ascorbic acid 12.1%, BHA 0.1%, *p*-amino-benzoic acid 3.0%, cyanocobalamine(1%) 0.1% and cellufil 60.3%. 4%, mineral mix, Bernart–Tomarelli modified (Bernart and Tomarelli, 1966). 4%, astaxanthin 0.01%, taurine 1%, choline chloride 1%, vitamin A (500,000 IU/g) 0.1%, vitamin D_3 0.1%, and vitamin E 0.2%.

[b] Expressed as percent dry weight.

The actual cholesterol level in the diets and whole crab body tissue were measured using a commercial assay kit (Boehringer Mannheim, Germany). The analytical principle is a stoichiometric relationship between cholesterol and lutidine-dye, which is produced after enzymatic reactions catalysed by cholesterol oxidase and catalase. The dye was measured by colorimetry at 405 nm using a Hitachi U-2000 spectrophotometer. The analyses of cholesterol contents indicated that the diets formulated to contain 0%, 0.2%, 0.5%, 0.8%, 1.1,% and 1.4% cholesterol actually contained 0.04%, 0.21%, 0.50%, 0.79%, 1.12% and 1.45%, respectively (Table 1).

Each treatment was randomly assigned to containers. There were 15 crabs per treatment, one crab per container. Each crab was fed to excess once daily at 0900 h. The experiment was conducted in darkness except for feeding and checking for exuviae, approximately 90 min/day. Feed residue and feces were removed by siphon before each feeding. Water temperature was maintained at 25 ± 2°C and recorded daily. Wet weights of all surviving individuals for each treatment were determined every 3 weeks. Weighted molting frequency was defined as the summation of the total number of molts of all the mud crabs for each treatment and then divided by 15 (the initial number for each treatment). The duration of the experiment was 84 days.

Mean % weight gain of crabs representing different treatments were subjected to Statistical Analysis System (SAS-PC) (Joyner, 1985) using the General Linear Model procedure for a one-way ANOVA. If significant differences among treatments were indicated at or less than the 0.05 level, the Duncan's multiple range test was used to identify significant differences between treatment means (Steel and Torrie, 1980). Broken-line regression model was used to determine the break-points in the growth curve, which estimated the optimal dietary cholesterol requirement of juvenile crabs (Robbins et al., 1979). This model assumes that when the cholesterol requirement is met, the % weight gain plateaus abruptly.

3. Results

Growth performance and survival of juvenile crab fed the experimental diets at the termination of the experiment are shown in Table 2. The mean % weight gains of

Table 2
Percent weight gain and survival of juvenile crab *S. serrata* fed the purified diets for 84 days
Within weight gain column, means with different letters are significantly different ($P < 0.05$).

Cholesterol levels (%)	Initial weight (mg)	Final weight (mg)	Weight gain (%)	Survival (%)
0.04	88.3 ± 31.1	201.0 ± 58.0	*	*
0.21	86.4 ± 46.9	589.1 ± 237.2	622[b]	93
0.50	83.4 ± 28.5	827.6 ± 370.8	948[a]	73
0.79	87.2 ± 37.9	823.4 ± 381.4	932[a]	87
1.12	86.4 ± 25.3	629.2 ± 177.3	657[b]	87
1.44	74.8 ± 15.6	524.5 ± 103.0	605[b]	87

* No survival.

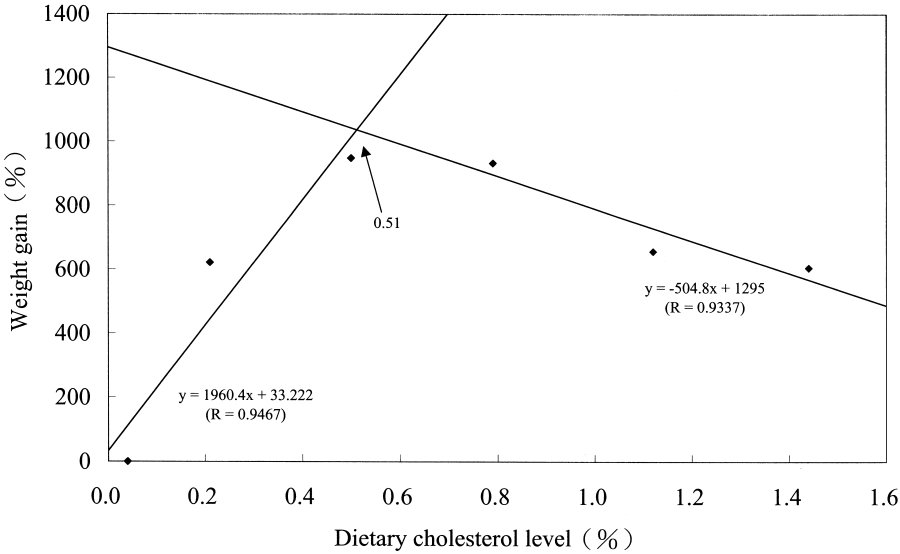

Fig. 1. Effect of dietary cholesterol level on the growth of *S. serrata*. Each point represents the means of surviving mud crabs. The dietary cholesterol requirement for *S. serrata* occurs at 0.51% when analyzed by a broken-line model.

juvenile crab fed diets containing 0.21%, 1.12% and 1.44% cholesterol were significantly lower ($P < 0.05$) than those of crabs fed diets containing 0.50% and 0.79% cholesterol. There was no significant difference ($P > 0.05$) between the % weight gains of crabs fed diets containing either 0.50% or 0.79% cholesterol. When broken-line regression analysis was used to estimate the optimal cholesterol level for mud crabs, as shown in Fig. 1, the regression equations were $Y = 1960.4X + 33.222$ and $Y = -504.8X + 1295$. The breakpoint occurred at 0.51%, which was estimated to be the amount of dietary cholesterol to provide maximal growth of *S. serrata*. No crab fed the diet without supplemented cholesterol survived. Survival of juvenile crabs fed diets containing 0.21–1.44% cholesterol ranged from 73% to 93%.

Table 3
Proximate analysis of whole body tissue of juvenile crab *S. serrata* fed test diets containing graded levels of cholesterol for 84 days

Cholesterol levels (%)	Whole body composition (% dry weight)				
	Moisture	Crude lipid	Crude protein	Ash	Total cholesterol
0.04	*	*	*	*	*
0.21	66.1	6.2	27.9	29.0	0.17
0.50	67.7	9.7	35.4	29.0	0.19
0.79	66.1	10.4	39.9	26.3	0.19
1.12	66.2	7.2	33.8	27.3	0.16
1.44	67.5	8.0	38.8	29.9	0.25

* No survival.

Table 4
Weighted molting frequency of juvenile crab *S. serrata* fed test diets containing graded levels of cholesterol for 84 days

Molting frequency	Dietary cholesterol levels (%)					
	0.04	0.21	0.50	0.79	1.12	1.44
0	6	0	0	0	0	0
1	7	0	0	0	0	0
2	2	2	0	1	2	2
3	0	10	7	8	9	8
4	0	2	4	4	2	3
Mean	0.73	2.80	2.47	2.80	2.60	2.67

Body composition (dry weight basis) and cholesterol content of the whole body tissue of juvenile crab fed the different diets are shown in Table 3. The crude lipid level in the whole body tissue increased from 6.15% to 10.42% and then decreased to 8.02% with increasing dietary cholesterol levels. The cholesterol content in the whole body tissue also increased from 0.169% to 0.193% cholesterol as dietary cholesterol increased from 0.21% to 0.79%.

Weighted molting frequency of juvenile crabs fed treatment diets is shown in Table 4. In general, crabs fed diet containing graded level of cholesterol had three to four molts during experimental period. Crabs fed diet without supplemented cholesterol had the lowest mean weighted molting frequency (0.73). However, crabs fed diets supplemented with cholesterol had 2.47–2.80 weighted molting frequencies.

4. Discussion

This trial clearly indicates that *S. serrata* requires dietary cholesterol to achieve maximum weight gain and survival. A dietary cholesterol level of 0.51% was found to be an optimum requirement for maximum growth of mud crab. The optimal level of cholesterol for *P. japonicus* (Kanazawa et al., 1971), *P. monodon* (Chen, 1993) and *P. penicillatus* (Chen and Jenn, 1991) was also reported to be 0.5%. Teshima et al.(1997) further indicated that the optimal dietary cholesterol requirement for juvenile *P. japonicus* ranged from 0.26% to 0.6% at feeding levels of 3–7%. However, Thongrod and Boonyaratpalin (1998) indicated that banana shrimp, *P. merguiensis* fed a diet containing no added cholesterol but 0.6% sterol esters had similar weight gain to those fed diets supplemented with 0.5% or 1% cholesterol. In contrast, a dietary cholesterol level as low as 0.12% and 0.19% was satisfactory for good growth and survival of American lobster (D'Abramo et al., 1984) and *P. monodon* (Sheen et al., 1994), respectively. Therefore, the optimum dietary cholesterol requirement of mud crab is higher than that of *P. monodon* (Sheen et al., 1994) and *P. vannamei* (Duerr and Walsh, 1996) and lower than that of larval *P. monodon*, which is 1% dietary cholesterol (Paibulkichakul et al., 1998). These studies with crustacean species indicate that an optimal dietary cholesterol ranges from 0.2% to 0.8%.

The digestion and assimilation of dietary cholesterol for crustacean are affected by dietary lipid and phospholipids (Teshima and Kanazawa, 1983; D'Abramo et al., 1985a). Cholesterol added to a lipid-free diet is absorbed only slightly. The addition of dietary phospholipids enhanced 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 was required for *P. japonicus* (Deshimaru and Kuroki, 1974) and 0.5% cholesterol was required for *H. americanus* (Castell et al., 1975). With varying level of lecithin supplements, Chen (1993) and Chen and Jenn (1991) indicated that 0.5% or higher dietary cholesterol was required for *P. monodon* or *P. penicillatus*; however, 0.25% cholesterol was sufficient for *H. americanus* (Kean et al., 1985). With no supplemental lecithin, 0.2% and 0.5% dietary cholesterol are minimum requirements for *P. monodon* (Sheen et al., 1994) and for mud crab in this study, respectively. Therefore, the physiological effect of phospholipid on the cholesterol requirement of crustacean needs further investigation.

Growth of crustacean is due to an increase in weight gain at molt and molting frequency. The low molting frequency may in some way be related to the low biosynthesis of ecdysone. Cholesterol is an important metabolic precursor for ecdysone biosynthesis in crustaceans (Watson and Spaziani, 1982). Spaziani and Kater (1973) indicated that in the shore crab, *Hemigrapsus nudus*, Y-organ uptake of cholesterol is greatly increased about the time that a molting sequence is initiated. Also, Vensel et al. (1984) pointed out that the specific activity of cholesterol in the hemolymph of eyestalkless *Cancer antennarius* is higher in postmolt or premolt stages. Clearly, crabs in this study fed diets supplemented with no cholesterol had lower mean molting frequency and the highest mortality. Therefore, the relationship between the amount of dietary cholesterol and ecdysone biosynthesis of crustacean needs further investigation.

It had been reported that *H. americanus* (Castell et al., 1975), *P. monodon* (Sheen et al., 1994) and *P. vannamei* (Duerr and Walsh, 1996) fed diets containing high levels of dietary cholesterol showed inferior growth. D'Abramo et al. (1984) suggested 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 of mud crab occurred when they were fed the diets containing 1.12% and 1.44% dietary cholesterol. Thus, excessive levels of dietary cholesterol may adversely affect growth and survival of crustaceans. Mercer (1982) stated that physiological responses to nutrients are graded and produce a characteristic nutrient–response curve, which increases to a point and tends to level off. The high levels of dietary cholesterol which caused the negative growth response in this study may be a nutrient–response characteristic rather than toxicity. However, the exact mechanism that produces this adverse response is still unknown and further investigation on this metabolism is needed.

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