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Cholesterol Content of Tiger Shrimp *Penaeus monodon* at Various Sizes, Moulting and Maturity Stages from Pazhayar Coast (South East Coast of India)

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ABSTRACT

A quantitative study was made on the occurrence of cholesterol in the muscle of different maturity stages of the wilder tiger shrimp *Penaeus monodon* collected from Pazhayar coast, Nagapattinam, Tamil Nadu. As the size increased from 8-10 cm to 14-16 cm the cholesterol content increased from 1.4-4.5 mg g^{-1}. The shrimp contained almost the same level of cholesterol in both sexes. The variation in the cholesterol level in relation to moulting in the male and female shrimp revealed that in both the sexes high cholesterol content was noticed in the intermoult stage. Subsequently, in the premoult stage the cholesterol content decreased from 2.0-1.8 mg g^{-1} and it was found to be the lowest among all the moult stages. In the matured stages the shrimp contained higher level of cholesterol (2.1 mg g^{-1}) and very less amount of cholesterol level (1.2 mg g^{-1}) in the immature and early mature stages. It is inferred from this study that the cholesterol level was more in the intermoult stage suggesting that cholesterol may be converted to some moulting hormones.

Key words: Cholesterol, moulting, *Penaeus monodon*, Pazhayar

INTRODUCTION

Cholesterol is a non-polar lipid constituent belonging to sterol group. It is an alcohol with a cyclic nucleus, found either free or esterified with fatty acids. Cholesterol is an essential precursor of bile acids, steroid hormones, molting hormones, vitamin D3 and prostaglandins, which are involved in the molting process in shrimp (Akiyama *et al*., 1992). Most animals can synthesize sterols from acetate, but crustaceans, like other arthropods, are incapable of de novo sterol synthesis from acetate (Fox *et al*., 1994). Therefore, dietary cholesterol is considered essential for good growth and survival of crustaceans. For example, *Penaeus japonicas* (Kanazawa *et al*., 1979), larval *P. japonicas* (Teshima and Kanazawa, 1983), *P. monodon* (Sheen *et al*., 1994) and *Cherax quadricarinatus* (Hernandez *et al*., 2004).

Polyunsaturated omega-3 (n-3) fatty acids; eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) show preventive effects against cancer, diabetes, cardiovascular and immune diseases (Vitale and Broitman, 1981; Logniskar *et al*., 1983). These fatty acids occur mostly in high amounts in seafoods. Therefore, to determine fatty acid compositions, total lipid and cholesterol contents of seafoods will be beneficial for recommendation of a preventive diet (Gordon, 1982; Krzynowek *et al*., 1982).

MATERIALS AND METHODS

Chemicals: Following chemical were used in this study absolute ethanol-acetone (1:1), chloroform, acetic anhydride-sulphuric acid reagent (Sigma chemical USA).

Collection: The shrimp *Penaeus monodon* was collected from the fisherman using trawler net. They were brought to the laboratory and divided into various groups in relation to size (8-16 cm) moulting and spawning stage. The animals were dissected and the tissues were kept in oven for drying (1100 rpm). The dried samples were powdered, from this 100 g were taken for the extraction of cholesterol.

Preparation of cholesterol standard: A stock solution of cholesterol was prepared by dissolving 100 mg in 100 mL of chloroform. This stock solution was diluted to 1:10 with chloroform for a working standard. Both the solutions were stored in the refrigerator until use. From the working standard 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg cholesterol equivalent to volumes i.e., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mL were taken and made to 5 mL using chloroform. Further estimation was made as mentioned earlier. The optical density at 625 nm was plotted against the concentration of cholesterol.

Extraction and estimation of cholesterol: The method of Stadtman (1957) Libermann-Burchard reaction was followed for the estimation cholesterol.

The 100 g tissue sample was ground with 80% (v/v) ethanol acetone (1:1) using pestle and mortar for deprotenization. The reaction mixture was centrifuged and the supernatant was decanted into a clean test tube. The precipitate was extracted with ethanol-acetone for 10 min in a water bath 40-50°C to effect complete recovery of the cholesterol. The sample was again centrifuged and the supernatant solution was pooled. The combined ethanol-acetone extracts of the samples were then evaporated to dryness.

The residue of each sample was dissolved in 5 mL of chloroform. Then, 2 mL of cold acetic anhydride-sulphuric acid reagent was added to the sample and mixed by stiring. The tubes were placed at 18°C and the colour was allowed to develop for 15 min. The blue green colour was measured immediately at 625 nm is spectrophotometer (Hitachi model 220 S). The quantity of cholesterol was determined by using cholesterol standard.

RESULTS

Table 1 shows the content of cholesterol expressed as mg g^{-1} of tissue in the male and female shrimp *Penaeus monodon* in relation to size groups. As the size increases, the level of cholesterol was also increased. The maximum level of cholesterol were observed in 14-16 cm size animals at the level of 4.5 in male and 4.0 g mg^{-1} in female animals. The minimum cholesterol levels were observed in 8-10 cm animals at the level of 1.6 in male and female 1.4 g mg^{-1}. *Penaeus monodon* contained almost the same level of cholesterol in both the sexes.

Table 2 indicates the variation in the cholesterol levels at various moulting stages in the male and female shrimp *P. monodon*. In this results, higher amount of cholesterol levels were observed in male animals compared to female animals in different moulting stages. In both the sexes, a high cholesterol content was noticed in the premoult stage (male 2.5 and femal 2.1). Subsequently in the postmoult stage the cholesterol content were decreased and it was found to be the lowest among all the moult stages.

Table 1: Cholesterol content in the male and female Shrimp *Penaeus monodon* of various size groups

	Cholesterol content (g mg^{-1})	
Animal length (cm)	Male	Female
8-10	1.6	1.4
10-12	2.1	2.0
12-14	3.9	3.1
14-16	4.5	4.0

Table 2: Cholesterol content of *Penaeus monodon* at several moulting stages

	Cholesterol content (g mg^{-1})	
Moult stages	Male	Female
A	2.00	1.9
B	2.20	2.0
C	2.50	2.1
D	1.90	1.8

Table 3: Cholesterol content in *Penaeus monodon* at various maturity stages

	Cholesterol content (g mg^{-1})	
Maturity stages	Male	Female
Immature stages	1.6	1.2
Early mature	1.9	1.5
Late mature	2.3	1.6
Mature	2.5	2.1

Table 3 showed the cholesterol levels in varies maturity stages of *P. monodon* in male and female animals. Among the varies maturity stages cholesterol levels highly found in mature stage. Among the animals, male animals have high cholesterol level compare to females. The maximum cholesterol levels were observed in mature stage of both sexes male 2.5 and female 2.1 g mg^{-1}. The minimum levels of cholesterol were observed in immature stages male 1.6 and female 1.2 g mg^{-1}.

DISCUSSION

In the present study, the cholesterol content was studied in the shrimp *P. monodon* in relation to sex, size moulting and maturity stages. There was wide differences in cholesterol levels between the sexes, mostly higher in male compare to female. As the size increased the level of cholesterol also increased. Regarding moulting the cholesterol content was more in the intermoult stage. Regarding the maturity stages maximum level of cholesterol was noticed in the mature stage and the minimum level of cholesterol in the immature and early mature stages. Sheen *et al.* (1994) reported that diets containing less than 0.8% cholesterol improved growth and survival of *P. monodon* (Thongrod and Boonyaratpalin, 1998).

Previous results suggest that cholesterol is an essential factor for crustaceans. Also, it has been demonstrated that some crustaceans are capable of converting exogenous ergosterol (Teshima and Kanazawa, 1971). However, previous studies on the sterol metabolism in crustaceans have very seldom been carried out by using individuals at specified stages of the moulting cycle. Sheen and D'Abramo (1991) reported that the level of dietary lipids including phospholipids

and cholesterol should be optimum and balanced in order to obtain maximum growth and survival of shrimps and that high dietary lipid levels may have a detrimental effect on growth performance of crustaceans. Mercer (1982) stated that physiological responses to nutrients were graded and produced a characteristic nutrient-response curve which increased to a point and then tended to level off the high levels of dietary cholesterol (D2, D3 and D4) which caused the negative growth response in this study may be a nutrient-response characteristic rather than toxicity.

There is much information regarding variations in cholesterol during the moult cycle of crustaceans. The moulting of crustaceans has been known to be controlled by hormones from the Y-organ, a secretary organ for moulting hormones and the X-organ, a secretary organ for moulting inhibiting hormone. Spaziani and Kater (1973) studied the uptake and turnover of cholesterol in the Y-organ of crab, *Hemigrapsus nudus* during moulting cycle and reported that the concentration of cholesterol was high in the Y-organ as compared with the haemolymph or the mass of peripheral tissue and did not change markedly with moulting cycle. Moreover, they demonstrated that the incorporation of cholesterol into the Y-organ proceeded actively at intermoult stage. In the present study the cholesterol level was more in the intermoult stage suggesting that cholesterol may be converted to some moulting hormones.

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