CHOLESTEROL REQUIREMENTS OF JUVENILE INDIAN WHITE PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

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ABSTRACT

Cholesterol is an essential nutrient in the diet of juvenile *P. indicus*. Survival, growth FCR (Feed conversion ratio), PER (Protein efficiency ratio) and protein retention of juveniles were greatly affected by cholesterol deficiency. The growth, FCR, PER and protein retention in the body were significantly improved on inclusion of 0.5% cholesterol in the diet of prawn which resulted in more protein deposition in the body. Cholesterol requirement for juvenile prawn seems to be 0.5% of the diet. No harmful effect was observed in the prawn on addition of higher level (4%) cholesterol in its diet.

Key words: Cholesterol requirements, *Penaeus indicus*, *Penaeus japonicus*, growth, diet, juvenile prawn.

Earlier investigations established cholesterol to be the most abundant sterol in crustaceans (Teshima and Kanazawa, 1971b, Gagosian, 1975). Cholesterol occurs in plasmamembranes of many animal cells in the lipoprotein of plasma and large quantities occur in the brain and nerve tissues (Lehninger, 1984). In crustaceans cholesterol is the precursor for various physiologically important compounds like steroid hormones, brain and moulting hormones and vitamin D (Kanazawa *et al.*, 1971 a, New, 1976) and sterols are important components in cellular and subcellular membrances, particularly in the hypodermis (Gilbert and O'Connor, 1970; Guary and Kanazawa, 1973, New, 1976). While vertebrates are known to biosynthesize cholesterol from precursors such as acetate and mavalonate, crustaceans do not have the ability to biosynthesize cholesterol (Van den Oord, 1964; Zandee, 1966; Kanazawa *et al.*, 1971 a; Teshima *et al.*, 1983; D'Abramo *et al.*, 1984). But cholesterol is found to be an essential nutrient for growth and survival of several crustaceans (Kanazawa *et al.*, 1971 a and b; Deshimaru and Kuroki, 1974; Castell *et al.*, 1975; D'Abramo

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et al., 1984). Teshima and Kanazawa (1971 a and b) have demonstrated the inability of a brine shrimp (*Artemia salina*), a prawn (*Penaeus japonicus*), a lobster (*Panulirus japonica*) and a crab (*Portunus trituberculatus*) to synthesize sterols.

Studies have also shown variation in quantitative requirements of cholesterol by crustaceans. While Shudo *et al.* (1971) reported fastest growth in juvenile *P. japonicus* fed diet containing 0.1% cholesterol, Kanazawa *et al.* (1971a) found 0.5% cholesterol to be best for the juveniles of the same species. In contrast, Deshimaru and Kuroki (1974) found that the best relative growth was achieved with 2% cholesterol in juvenile *P. japonicus.* Similarly the survival and growth rate of the prawn, *Artemisia longinaris* was improved by feeding a diet containing 0.5% cholesterol (Petriella *et al.*, 1984).

Inspite of the importance of cholesterol in prawns there is no information on the sterol requirement of the Indian white prawn *P. indicus* and hence the present study was undertaken to determine the dietary cholesterol requirement of juvenile *P. indicus.*

MATERIALS AND METHODS

Experimental set-up

Feeding experiments of 30 days duration were conducted in triplicate in the laboratory at the Central Marine Fisheries Research Institute, Kochi. Fifty litre capacity, round bottom plastic, non-reactive tubs were used as experimental containers. About 40 litres of sea water of salinity 20 ± 2‰ was used in each container and continuous aeration was provided. Water was siphoned and filtered through a biological filter and used for two subsequent days. On the fourth day, the entire water was replaced with fresh sea water of the same salinity. Details about environmental factors are given in Table 1.

Table 1. Various parameters used for the experiments on the cholesterol requirements of juvenile *Penaeus indicus*

Parameters	
Salinity (‰)	20.0 ± 2
Temperature (°C)	28 to 31
pH	7.9 to 8.3
Dissolved oxygen in water (mg/l)	4.8 to 6.2
Total ammonia–N in seawater (ppm)	0.02 to 0.11
Initial number of prawn per treatment	30
Average initial length range (mm)	25 to 30
Average initial wet weight range (mg)	81.90 to 172
Average initial dry weight (mg)	27.16
Feeding level of % of biomass	15

Formulation and preparation of diets

Seven isonitrogenous and isocaloric purified diets were formulated and prepared employing the methods of Kanazawa *et al.* (1970, and 1977) and Deshimaru *et al.* (1979) with slight modifications. Composition of diets are given in Table 2.

Table 2. Ingredients composition in percentage of test diets for cholesterol requirements of *Penaeus indicus*

Ingredients	Diet						
	1	2	3	4	5	6	7
Casein	31.0	31.0	31.0	31.0	31.0	31.0	31.0
Egg albumin	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Amino acids mixture (1)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Glucosamine	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Starch	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Glucose	4.9	4.4	4.0	4.0	4.0	3.5	3.0
Sucrose	11.5	11.5	11.4	10.9	10.4	9.9	9.4
Cholesterol	0.0	0.5	1.0	1.5	2.0	3.0	4.0
Oil mixture (2)	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Lecithin	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Mineral mixture (4)	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Cellulose powder	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Carageenan	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Distilled water (ml)	100.0	100.0	100.0	100.0	100.0	100.0	100.0

1) Amino acids mixture (g /100 g diet): Arginine—1.0, Methionine—0.5, Glycine—2.0, Taurine—0.5, Glutamic acid—1.0.
2) Oil mixture (g/100 g diet) Cod liver oil—5.34, Soyabean oil—2.66.
3) Vitamin mixture (mg/100 g diet): Thiamine HCl (B)—4.9, Riboflavin (B2)—8.0, Para Amino Benzoic acid—10.9, Inositol—400.0, Niacin—40.0, calcium pantothenate—60.0, Pyridoxine HCl—12.0, Menadione—4.0, β-Carotene—9.6, Tocopheral (Vitamin E)—20.0, Calciferol—1.2, Cynacobalamin (B12)—0.08, Sodium Ascorbate—2000.0, Folic acid—0.8, Choline chloride 600.0.
4) Mineral mixture (g/100 g diet), K_2HPO_4—2.00, Ca $(PO_4)_2$—2.720, $MgSO_4 \cdot 7H_2O$–3.02, $NaH_2PO_4 \cdot 2H_2O$—0.790, $MnSo_4 \cdot 5H_2O$—0.004, $FeSO_4 \cdot 7H_2O$—0.015.

Studies by Kanazawa *et al.* (1971 a) and Teshima *et al.* (1983) clearly established the superiority of cholesterol among sterols in promoting growth in prawn, *P. japonicus* and therefore, among the sterols cholesterol was selected for the present study. The cholesterol levels ranged from zero to four per cent in the diets (Table 2). The levels of glucose and sucrose were adjusted

to maintain isocaloric level in each of the diets (Table 2). A mixture of soyabean oil and codliver oil (8%) was used as source of basal lipid in the ratio of 1:2 as this ratio provided better growth in *P. indicus* (Chandge, 1993). Since soya lecithin enhance the cholesterol solubilization and transport in crustaceans (Lester *et al.*, 1975) and it was found to be essential for survival and growth of *P. japonicus* (Kanazawa *et al.*, 1985) and *P. indicus* (Chandge, 1987). Therefore, soya lecithin was incorporated at 4% level in each diet. Agar, agar was used as a binder. Purified ingredients (Table 2) were purchased from ICN Biochemicals USA, SIGMA USA, BDH England, Applied Science Laboratories USA, Suppleco Switzerland and British Codliver Oil Ltd. Hull.

Juveniles of *P. indicus* ranging from 25 to 30 mm in mean length and 81.9 to 172 mg in mean weight, from the same brood reared in Prawn Culture Laboratory of CIBA Narakkal were randomly selected for the experiment. Ten juveniles per replicate and 30 juveniles per treatment were stocked. Feeding rate was 15% of the total body weight which was divided into two doses ¼ in the morning at 8 hrs and ¾ in the evening at 18 hrs. Everyday morning leftover feed as well as a fecal strands were separately collected by siphoning and dried in electric oven. The dry weight of leftover feed and fecal matter was recorded for calculation of FCR and PER.

The proximate composition of juvenile prawn, diet and fecal matter was determined by using standard methods of Lowry's *et al.* (1951) for proteins, Bligh and Dyer (1959) for lipids, Dubois *et al.* (1956) for carbohydrates, Hestrin (1949) for cholesterol, and AOAC (1975) for dry weight and ash content.

Experimental results obtained on survival, gain in length, wet weight, dry weight, Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and similarly on percentage of moisture, protein, lipid, carbohydrate, ash, cholesterol content of post-experimental prawn were subjected to statistical analysis

Analysis of variance (ANOVA) was done on the means of each parameter to find out if the dietary treatments hold any significant influence on the observed parameters. When significant influence was observed the data were processed to find out if the differences observed between the treatments were significant or not by the least significant difference test.

RESULTS

The results of the feeding experiment conducted in juveniles *P. indicus* are shown in Figs. 1A–1F and 2A–2F. The survival rate was not significantly affected by dietary cholesterol level. The per cent mean gains in length, wet weight and dry weight were considered for assessing growth of animals and will be referred as 'growth' hereafter. Analysis of variance of data showed that the dietary level of cholesterol significantly ($P < 0.05$) affected the growth of juvenile prawns. From Figs. 1A–1F it is evident that the cholesterol deficient diet (Diet 1) produced relatively poor growth and that the inclusion of

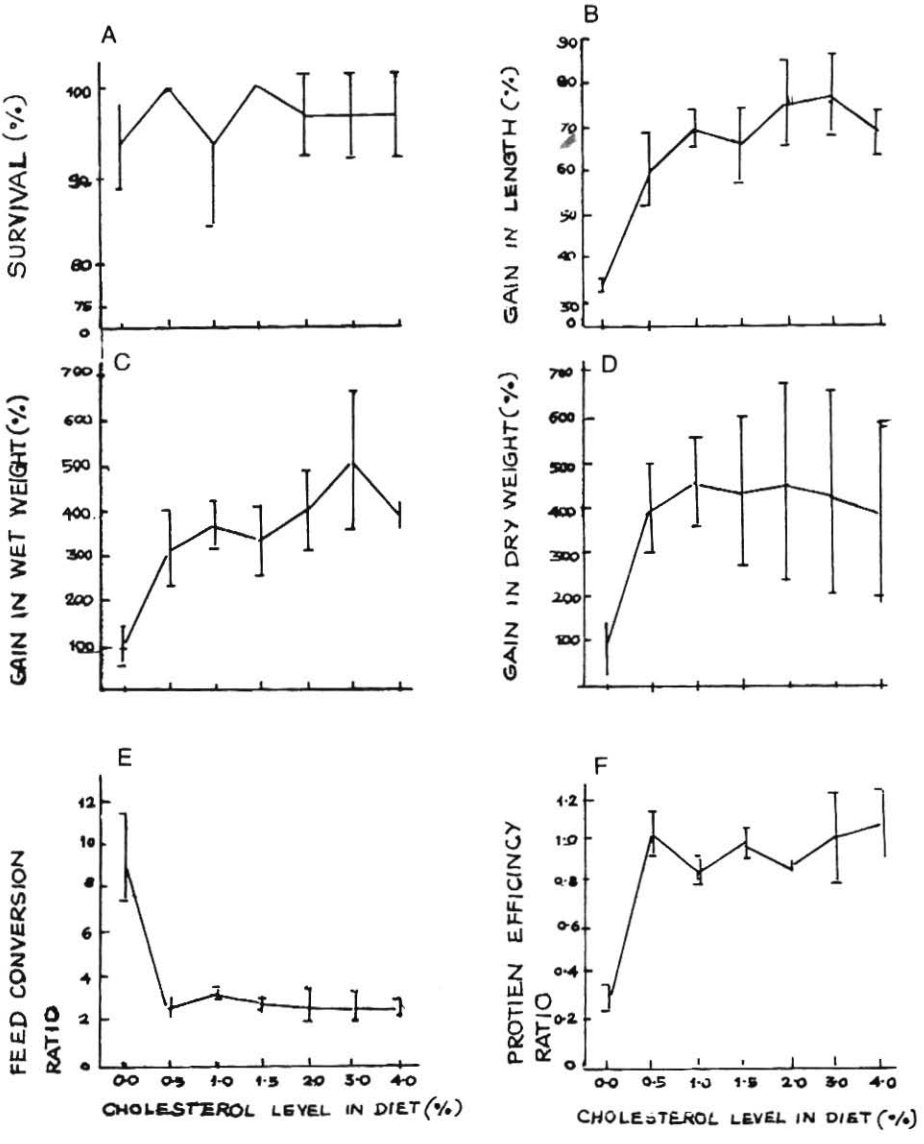

Fig. 1. Survival rate and growth of *P. indicus* fed on a diet containing graded levels of cholesterol. (A)—Survival (%). (B)—Gain in length (%). (C)—Gain in wet weight (%). (D)—Gain in dry weight (%). (E)—Feed Conversion Ratio. (F)—Protein Efficiency Ratio.

cholesterol at a level of 0.5% (Diet 2) significantly increased the growth of juvenile prawns. Least significant difference test showed that the growth of prawns fed the cholesterol deficient diet were significantly lower than those fed diets containing various levels of cholesterol (Diet 2 to 7). Though the gain

in length and wet weight were significantly ($P < 0.05$) higher at 3% cholesterol in the diet than that at 0.5% cholesterol, there was no significant difference in the dry weight gains of prawns between these two dietary treatments. However, inclusion of 4% cholesterol in the diet resulted in reduced growth (Fig. 1B, C, D). The observed differences in the growth (Figs. 1B, C, D) among prawns receiving diets containing cholesterol level ranging from 0.5 to 4% were not statistically significant.

While FCR (Fig. 1E) was significantly higher ($P < 0.05$) and the PER was significantly lower ($P < 0.05$) for the Diet 1. The FCR and PER were significantly ($P < 0.05$) improved by the inclusion of cholesterol at a level of 0.5% in the diet (Diet 2). However, inclusion of increasing level of cholesterol in the Diet 3 to 7 did not significantly influence the FCR and PER (Figs. 1E and F).

The proximate composition of the juvenile prawns subjected to various experimental diets are shown in Figs. 2A–2F, and was significantly affected by dietary levels of cholesterol. Compared to the prawns fed the diets containing various concentration of cholesterol, the cholesterol deficient diet fed prawns had significantly higher ($P < 0.05$) moisture, ash and carbohydrate content. Inclusion of cholesterol in the diet even at lower level of 0.5% resulted in reduced moisture and ash content but caused increased accumulation of protein, lipid and cholesterol content. Although significant differences in the proximate composition of prawns were not observed between diets containing more than 0.5% cholesterol, the protein content of prawn from treatment 2 was significantly greater than that of treatment 4, 5, 6 and 7 (Figs. 2A–2F).

DISCUSSION

The results of the present experiment clearly demonstrate that cholesterol is an indispensable nutrient in the diet for the juvenile *P. indicus*.

Although growth of prawn was significantly affected by cholesterol deficient diet but survival was not affected much, indicate that trace amounts of cholesterol present in the ingredient might have sustained such a high rate of survival in prawn. The poor growth, FCR, PER and protein retention in the body in the juvenile prawns fed on cholesterol free diet indicate *P. indicus* require cholesterol in the diet as indispensable nutrient and 0.5% level of cholesterol in the diet appears to be most effective for promoting growth, food and protein utilization and for protein deposition in the body (Figs. 1A–1F and 2A).

Feeding experiments using artificial diets have shown that *P. japonicus* juveniles require an optimum level of 0.5% cholesterol in the diet (Kanazawa *et al.*, 1971 a) for normal growth and survival. Supplementation of 0.05 or 0.1% cholesterol resulted in poor growth, while 1% cholesterol produced no improvement over 0.5% level, 5% dietary cholesterol depressed growth. These findings agree with the present results on juvenile *P. indicus*. In contrast to the above observations, Deshimaru and Kuroki (1974) reported relatively higher

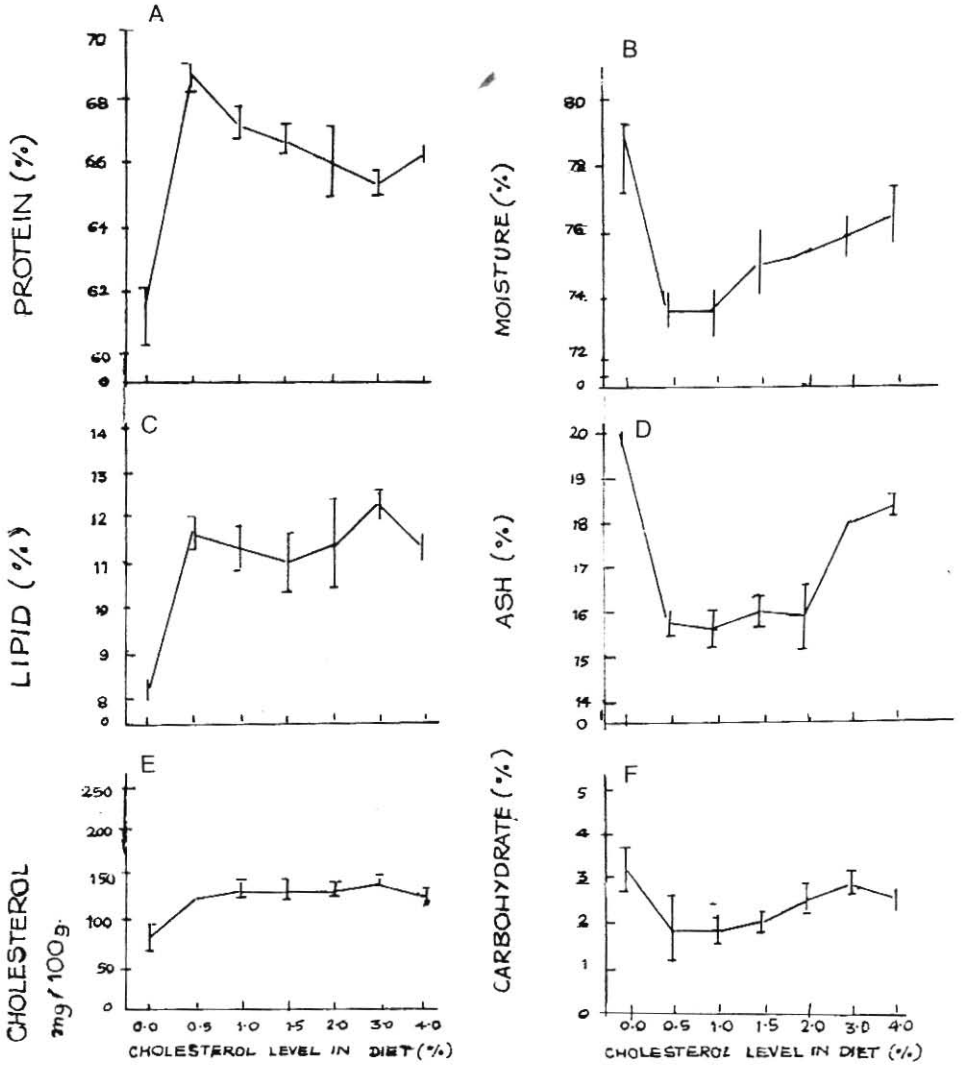

Fig. 2. Biochemical composition of juvenile prawns *P. indicus* fed on diets containing graded levels of cholesterol. (A)—Protein (%). (B)—Moisture (%). (C)—Lipid (%). (D)—Ash (%). (E)—Cholesterol (%). (F)—Carbohydrate (%).

level of (2.1%) dietary cholesterol for promoting best growth in juvenile *P. japonicus*. Read (1981) also empirically used 2% cholesterol in his compounded diet prepared for *P. indicus* and observed better growth with this diet, though no comparison was made to find out the influence of inclusion of

cholesterol at lower levels. Whereas Shudo *et al.* (1971) reported relatively lower level (0.1%) as dietary cholesterol requirement for juvenile *P. japonicus*. All these observations indicate the need for optimum cholesterol in the diet of prawn. However, these differences in dietary cholesterol requirement can be attributed to the differences in the composition of the basal diet used as well as due to differences in quality and quantity of basal lipid used in the diet (D'Abramo *et al.*, 1984). Kanazawa *et al.* (1971 a) used 8% lipid in the diet as compared to 6% lipid used by Deshimaru and Kuroki (1974). The increased requirement of cholesterol (2.1%) reported by Deshimaru and Kuroki (1974) for juvenile *P. japonicus* may be due to the relatively low lipid content in their diets. Whereas the relatively lower dietary cholesterol requirements reported by Kanazawa *et al.* (1971 a) may be due to relatively higher lipid in the diet. Dietary lipid is presumed to contain certain level of cholesterol (Kanazawa, 1985). Thus optimum cholesterol requirement in the diet also depends upon other ingredients used in the diet. These observations indicate that in the presence of adequate lipid level in the diet, about 0.5% of cholesterol would be adequate to promote maximum growth and survival of prawn *P. indicus*. In the present experiment with *P. indicus*, the author used a basal lipid level of 8% constituting 5.34% codliver oil and 2.66% soyabean oil. It is assumed that 0.5% cholesterol alongwith the mixture of lipid used in the present study appears to be sufficient enough for producing maximum growth in the prawn *P. indicus*.

Teshima and Kanazawa (1983) have also demonstrated that the absorption rate of dietary cholesterol is improved by the presence of other lipids. The high content of dietary lecithin in lobster diet has been presumed to facilitate uptake of cholesterol (D'Abramo *et al.*, 1982). Lester *et al.* (1975) observed that lecithin enhanced cholesterol solubilization when associated with the crustacean emulsifier N.N-dodecanosacrosyl taurine (DST). Absence of the phospholipid (phosphotidylcholine) has been found to restrict the effective transport of cholesterol within the body of prawn. In the present study, diets had 4% lecithin which certainly would have helped in the effective utilization of cholesterol by the prawns. Thus effective utilization of cholesterol depends upon the presence of phospholipids in the diet, as well as on the presence of polyunsaturated fatty acids (PUFA) (D'Abramo *et al.*, 1982). These observations (D'Abramo *et al.*, 1982) further support the use of codliver oil (a source of PUFA) and lecithin (phospholipid) as a basal lipid source for the present study to determine the cholesterol requirement of *P. indicus.*

The proximate composition of *P. indicus* was also influenced by the dietary level of cholesterol. The rate of deposition of protein, lipid and cholesterol was relatively low in prawns fed on the cholesterol deficient diet, when compared to prawns fed on cholesterol diets. But there were no significant differences in the chemical composition of prawns between diets containing cholesterol levels from 0.5 to 4%. The FCR and PER significantly improved on inclusion of cholesterol in the diet of prawn which resulted in more deposition of protein in the body. It appears that at optimal concentrations of cholesterol has protein

sparing action, as the protein content of prawn increased when fed on the diet containing cholesterol (0.5%). The increased protein deposition may be due to the acceleration in the anabolic processes in the tissues as a result of stimulating effects of the steroid hormones synthesized from the dietary cholesterol. Thus the enhanced growth attained on addition of 0.5 cholesterol in the diet might be because of the better utilization of food and protein (Figs. 1E and F).

Studies have shown cholesterol is used in hypodermis formation (Guary and Kanazawa, 1973; New, 1976). Besides the sterols are important as elements of cellular and subcellular structures in arthropods (Gilbert and O'Connor, 1970). Several workers have also reported sterols are found to be precursor of moulting hormone in arthropoda (Gilbert and O'Connor, 1970) as well as brain hormone in prawns (Kanazawa *et al.*, 1971 a; New, 1976). Kanazawa *et al.* (1971 a) reported that frequency of moulting increased in *P. japonicus* when fed on a diet containing cholesterol indicating the involvement of cholesterol in moulting. Further studies by Kanazawa *et al.* (1972) have demonstrated that ecdysterone induce moulting in *P. japonicus* and sterols are found to be precursor of ecdysterone, a moulting hormone (Gilbert and O'-Connor, 1970). Deficiency of cholesterol in tissues has been shown to cause moult death syndrome in the lobster (D'Abramo *et al.*, 1982). Since moulting is an essential physiological process in prawns, preceding synthesis of new tissues in the body, the significant increase in growth as well as in protein content, as observed in the present study in prawns, can be expected by the addition of cholesterol which is the precursor for the steroid hormones.

Prawns fed on the cholesterol free diet retained relatively lower levels of tissue cholesterol than those fed on cholesterol supplemented diets in the case of juveniles of *P. indicus*. This observation is similar to that observed in the prawn *P. japonicus* (Kanazawa *et al.*, 1971 a; New, 1976) and lobster, *Homarus* sp. (D'Abramo *et al.*, 1984). Thompson (1964) reported that total cholesterol content of the body of various prawns was around 156 mg/100 g (*P. aztecus*) and 157 mg/100 g (*P. setiferus*). The quantity of cholesterol found in *P. indicus* during the present study also agrees with the cholesterol content of the above prawns.

The results of these experiments clearly indicate the need of cholesterol for proper survival and growth of *P. indicus* and 0.5% of cholesterol in the diet was found to be most effective for promoting growth, FCR, PER and for more protein retention in juvenile *P. indicus*.

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