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DIETARY CHOLESTEROL REQUIREMENT OF LARVAE AND POSTLARVAE OF THE PRAWN *PENAEUS INDICUS* (H. MILNE EDWARDS)

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ABSTRACT

Two sets of experiments were conducted to determine the dietary cholesterol requirement of larvae and postlarvae 1-10 of *Penaeus indicus*. Seven approximately isocaloric and isonitrogenous purified experimental diets were tried with graded levels of cholesterol ranging from 0 to 4%. The control feed for larvae and postlarvae 1-10 were phytoplankton and compounded feed NPCL-17, developed by CMFRI, Cochin respectively. Result of these experiments indicate that cholesterol is an essential nutrient in the diet of larvae and postlarvae 1-10. Survival and growth of larvae and postlarvae 1-10 were greatly affected by cholesterol deficiency in the diet. The optimal cholesterol requirement for larvae appeared to be 0.5% of the diet, while it was higher for postlarvae where inclusion of cholesterol at a level of 2% in the diet gave higher growth.

INTRODUCTION

Nutritional requirement of juvenile shrimp and prawn has been well worked out (New, 1976; Akiyama, 1992). As for the prawn *Penaeus indicus,* techniques on brood stock maturation, spawning and larval rearing have been established (James, 1990). The introduction of purified diets (Kanazawa *et al.,* 1970; Deshimaru and Kuriki, 1974) has led to the establishment of the requirement of proteins, lipids, carbohydrates, minerals and vitamins of juvenile *P. japonicus* (New, 1980; Kanazawa, 1985). However, nutritional requirement of the Indian penaeid prawns, except that of a few species, is not reported. While in case of juvenile *P. indicus,* protein and vitamin requirement was reported by Gopal (1986), lipid requirement by Chandge (1987) and carbohydrate by Ali (1982). Knowledge about nutritional requirement of larval stages except that of lipids (Chandge, 1990) of Indian penaeid prawns is still scanty (Rao, 1983). Teshima and Kanazawa (1971) have shown that the adult of the prawn *P. japonicus* was incapable of synthesizing sterol from (14^C) acetate, suggesting the necessity of dietary requirement for sterol for growth. Further it was confirmed by feeding trials using purified diet (Kanazawa *et al.,* 1971). They have shown that survival and growth of prawns fed on sterol free diet were poor while those fed diets containing 1.0 %

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cholesterol were good.

The importance of cholesterol as a diet ingredient has also been demonstrated for the juvenile of the American lobster *Homarus americanus* (Castell *et al.*, 1975), *P. japonicus* (Shudo *et al.*, 1971; Deshimaru and Kuroki, 1974) and Indian white prawn *P. indicus* (Chandge, 1987).

Sterol in the diet was reported to be essential for the larvae of the crabs *Rhithropanopeus harrisi* and *Libinia emerginals* (Whitney, 1969) and those of *P. japonicus* (Teshima *et al.*, 1983).

As the dietary cholesterol requirements of the larvae and postlarvae of *P. indicus* has not been worked out so far, the present study was undertaken to determine the same. Studies by Kanazawa *et al.* (1971 a & b) and Teshima *et al.* (1983) clearly established the superiority of cholesterol among sterols in promoting growth in the prawn *P. japonicus* and therefore among the sterols, cholesterol was selected for the present study.

MATERIAL AND METHODS

Two sets of experiments were conducted to determine the dietary cholesterol requirement of larvae and postlarvae PL1-10 by using three replicates for each treatment at the nutrition laboratory of the Central Marine Fisheries Research Institute, Cochin.

One litre capacity glass beakers were used for the larval rearing experiment with sea water of salinity 33 to 35‰ and pH 7.9 to 8.2 with vigorous aeration. A glass tube devised and fitted in the beaker helped to keep the water in motion along with the feed materials. Temperature was maintained between 30°C and 31.5°C by temperature control systems.

Round bottom blue colour plastic basins with five litres sea water of salinity 32±2‰ having pH 7.9 to 8.5 were used for post larval experiment. Continuous aeration was provided.

Water was changed daily with sea water of same salinity. Experimental larvae and postlarvae, from the same brood, were obtained from Prawn Hatchery of the Central Marine Fisheries Research Institute at Narakkal. Each of the experimental aquaria had either protozoeae-1 which were reared upto the postlarval-1 stage, or twenty postlarvae-1 which were reared upto PL-10 stage (upto 10 days) which is regarded as marine phase of the life history of the prawn.

Formulation and preparation of diet:-

Seven purified diets were formulated and prepared following the formula provided and methods used by Kanazawa *et al.* (1970, 1977, 1982), and Teshima *et al.* (1983) with little modification, the omposition of which is given in Table 1.

Seven isonitrogenous diets were formulated by using graded level of cholesterol ranging from 0 to 4% (Table 1). Chemical ingredients used for preparation of diets, of Sigma USA, BDH, SRL, HiMedia and Merck etc. were

Table 1 : *Ingredients composition in percentage of test diets for cholesterol requirements of larvae and postlarvae of P. indicus.*

Ingredients	Diet						
	1	2	3	4	5	6	7
Casein	37.0	37.0	37.0	37.0	37.0	37.0	37.0
Egg albumin	9.0	9.0	9.0	9.0	9.0	9.0	9.0
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	3.5	3.5	3.5	3.5	3.5	2.5	2.0
Sucrose	8.4	7.9	7.4	6.9	6.4	6.4	5.4
Cholesterol	0.0	0.5	1.0	1.5	2.0	3.0	4.0
Basal lipids (2)	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Lecithin	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin mixture (3)	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	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Carrageenan	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) Lipid mixture - Cod liver oil - 5.34, Soyabean oil - 2.66.

3) Vitamin mixture (mg/100 g diet) Thiamine HCl(B) - 4.9, Raboflavin (B_2) - 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, B-Carotene - 9.6, Tocopherol (Vitamin E) -20.0, Calciferol - 1.2, Cynacobalamin (B12) - 0.08, Sodium ascorbate - 2000.0, Folic acid - 0.08, Choline chbride - 600.0.

4) Mineral mixture (g/100 g diet), K_2HFO_4 - 2.00, Ca $(Po_4)_2$ - 2.720 $MgSO_4$, $7H_2O$ - 3.02, NaH_2PO_4 $2H_2O$ - 0.790, Mn SO_4. $5H_2O$ - 0.004, $FeSO_4$. $7H_2O$ - 0.015.

procured locally. Cod liver oil was purchased from British Cod Liver Oil Ltd., Hull, Lecithin from Sigma, USA and cholesterol from BDH, England. A mixture of Soyabean oil and codliver oil (8%) was used as source of basal lipid in the ratio of 1 : 2, since this ratio of codliver oil and soyabean oil have been shown to be necessary for good growth and survival of juvenile and larvae of *P. indicus* (Chandge, 1987). Since soyalecithin enhances the cholesterol solubilization and transport in crustaceans (Lester *et al.*, 1975) and it is found to be essential for growth of juvenile of *P. japonicus* (Kanazawa *et al.*, 1979 and 1985) and *P. indicus* (Chandge, 1987), Soyalecithin was incorporated at 2% level in each diet. Carrageenan was used as binder.

The prepared diets were freeze dried, powdered and sieved to obtain the desired particle sizes, less than 37μ for larvae and 300 to 1000μ for postlarvae.

Phytoplankton dominated with the *Chaetoceros* @ 20,000 cells/ml was used as control for larval rearing and compounded diet NPCL-17 prepared and used regularly at CMFRI laboratory was used as control for postlarval experiments.

Feeding rate was 0.16 mg/larvae/day (Villagas and Kanazawa, 1980). Feeding was done five times daily in equal doses at intervals of 4-5 hours. Feeding rate for postlarvae was about 30 to 40% of the live body weight which was distributed into three doses. 1/4 dose in the morning, 1/4 in the afternoon and 1/2 dose in the evening.

Parameters like salinity, dissolved oxygen (4 to 6.5 mg/lt) ammonia (less than 0.08 ppm/lt) pH (7.9 to 8.5) and temperature (30°C to 31.5°C) were monitored regularly.

Statistical analysis of survival and growth data obtained was conducted using ANOVA and least significant difference test for significant difference at the 5% level (Snedecor and Cochran, 1973).

RESULTS

Experiment - 1

Larvae : The results of the feeding experiment involving larvae of *P. indicus* are shown in Table 2A and 2B. All the protozoea I larvae in treatment 9, where food was not supplied, died within 3 days without metamorphosis. In treatment 8 (conrol), where phytoplankton was fed, 34% of the larvae (protozoea) attained the postlarval stage 1 in 10 days. However the cholesterol deficient diet (Diet 1), when fed to the larvae, induced mortality at various larval stages with complete mortality at mysis stage-1 on the 7^{th} day of the experiment. Maximum mortality of larvae occurred at protozoea-II stage and it was relatively less in protozoea III stage and increased again resulting in complete mortality at mysis stage-1. The larvae grew to postlarvae-1 in 9 days with 20.6 % survival on the diet containing 0.5% cholesterol (Diet 2). Similarly, the diet with 1% cholesterol (Diet 3) produced 17.6% survival at the postlarval stage-1. The survival of larvae was relatively low in treatments 4 to 7

Table - 2A *Growth and survival of P. indicus larvae fed on diets containing graded levels of cholesterol*

Diet No.	Cholesterol (%)	Survival rates (%) of various developmental stages of prawn larvae							
		P1	P2	P3	M1	M2	M3	PL1	Feeding period days.
1	0.0	100	27.34	14.67	2.00	-	-	-	6
2	0.5	100	52.00	41.34	38.67	36.67	33.34	20.60	9
3	1.0	100	52.67	40.67	36.00	30.00	24.00	17.34	9
4	1.5	100	33.33	23.33	20.66	18.00	16.00	10.67	10
5	2.0	100	38.67	28.67	23.33	18.00	14.67	9.34	10
6	3.0	100	40.67	21.33	12.67	10.67	6.67	5.34	11
7	4.0	100	29.34	25.34	21.34	10.00	9.34	8.67	12
8	Control	100	80.67	80.67	66.00	57.34	45.34	34.00	9
9	No food	100	--	--	--	--	--	--	--

P1, P2, P3 = Protozoeal stages of larvae
M1, M2, M3 = Mysis stages of larvae
PL1 = Postlarvael

Table - 2B : *Survival rate (%) of postlarvae at various development stages during metamorphosis*

Diet No.	Cholesterol level (%)	Survival rate (%) of larvae at various developmental stages				
		P1	From P1 to P3	From P3 to M1	From M1 to M3	From M3 to PL1
1	0.0	100	14.67	13.63	--	--
2	0.5	100	41.33	93.54	86.20	62.00
3	1.0	100	40.67	88.52	66.67	72.22
4	1.5	100	23.33	88.57	77.41	66.67
5	2.0	100	28.66	23.33	62.85	63.63
6	3.0	100	21.33	59.37	52.63	80.00
7	4.0	100	25.33	21.33	43.75	93.00
8	Control	100	80.67	81.81	68.68	75.00
9	No food	100	--	--	--	--

as it ranged from 10.6 % to 5.34 %. Thus inclusion of cholesterol at levels above 1 % in the diet did not promote larval growth and survival, but rather resulted in decreased rate of survival.

Survival of larvae in treatment 2 and 3 was significantly ($p < 0.05$) higher than all other treatments, but significantly lower than treatment 8 (control). There was no significant difference in the survival rate between treatments 2 and 3, as well as among treatments 3, 4, 5 and 6.

Experiment - 2

Postlarve 1-10 : The results of the feeding experiment involving postlarvae-1 of *P. indicus* are shown in Fig. 1. Survival rate of postlarvae was uniformly high in all the treatments and it ranged from 91.65% to 100 %. Statistical analysis of data did not reveal any significant differences in survival rates among most of the treatments. Cholesterol concentration in the diet significantly ($p<0.05$) influenced the growth of postlarvae. The cholesterol deficient diet (Treatment 1) produced significantly ($P<0.05$) lower mean percent gains in length, live weight and dry weight than all other diets. Supplementation of cholesterol in the diets resulted in significant increases in length, wet weight and dry weight of the postlarvae. The diet containing 0.05% cholesterol produced significantly greater ($P<0.05$) growth than the cholesterol free diet. The wet weight gain of the postlarvae was significantly ($p<0.05$) higher with the diet containing 1% cholesterol, but gain in length and dry weights were not significantly higher than the postlarvae fed diet containing 0.05% cholesterol. Although the growth of postlarvae increased continuously corresponding to the increase in cholesterol level in diet, this trend in growth did not differ significantly between diets containing various levels of cholesterol. However, inclusion of cholesterol at a level of 2 % in the diet gave significantly higher mean wet weight gain and dry weight gain than the diet with 0.5% cholesterol.

DISCUSSION

The present results demonstrate that cholesterol is an indispensable nutrient in the diet of larvae and postlarvae of the prawn *P. indicus*. The growth, survival and metamorphosis of larvae and postlarvae seems to be greatly affected by cholesterol deficiency in diet. Growth increased significantly ($p<0.05\%$) when 0.5% cholesterol was added in the diet while there was no beneficial effect when diets containing more than 0.5% were fed to the larvae. Protozoeal stages were the worst affected by cholesterol deficiency, since the highest mortality rates occured at this stage when compared to mysis stage in almost all the treatments. The purified nature of the diet and its particle size could have had some adverse effect on the survival of protozoeae. The non-availability of adequate quantity of the desired particle size of the food in the vicinity of the mouth of the larvae, thus subjecting them to obligatory fast, and leaching of

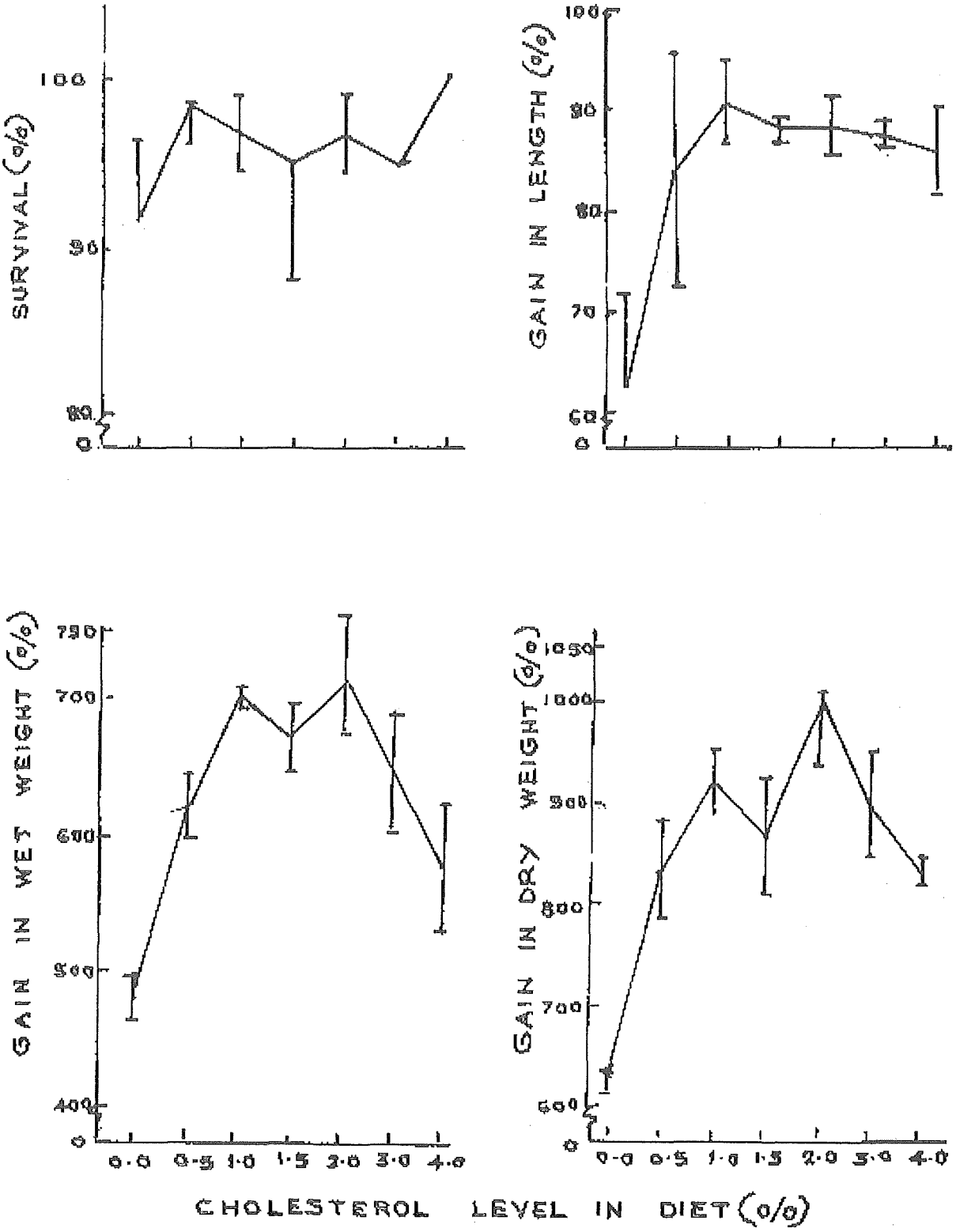

Fig. 1 *Survival rate and growth of postlarvae 1-10 P.indicus fed on diets containing graded levels of cholesterol.*

essential nutrients from micro-particulate diets, are factors suspected to have caused high mortality. As compared to protozoeae, mysis larvae are bigger and have appendages to collect and hold the food and ingest it more efficiently (Muthu, 1983) which perhaps resulted in relatively less mortality during mysis stage. Earlier studies (Teshima *et al.,* 1983) have shown that the larvae of the prawn *P. japonicus* also require an exogenous source of sterol in the diet for normal survival, growth and metamorphosis. Teshima *et al.* (1983) reported that larvae grew and survived better on a diet supplemented with 1% cholesterol diet, but the results of the present experiments agree to some extent with the report of Teshima *et al.* (1983).

The survival of postlarvae was not significantly influenced by the cholesterol content of diet; even the cholesterol deficient diet produced good survival. It is suspected that trace levels of cholesterol present in the ingredients might have sustained such high survival rate even in the prawns fed on the cholesterol deficient diets. Growth of post larvae was increased significantly when 0.5% cholesterol was added in the diet. The live weight gain of the post larvae was significantly ($P<0.05$) higher with the diet containing 1 % cholesterol but gain in length and dry weights were not significantly higher than the post larvae fed diet containing 0.5% cholesterol. Although the growth of postlarvae increased proportionate to the increase in cholesterol level in diet, this trend in growth did not differ significantly between diets containing various levels of cholesterol. These results indicate that postlarvae, like larvae of *P. indicus,* require cholesterol in the diet and 0.5% level of cholesterol in the diet appears to be most effective for promoting optimal growth.

Thus it is apparent that *P. indicus* do not have the capacity to synthesize cholesterol *de novo* and dietary cholesterol is essential as had been observed in *P. japonicus* by Kanazawa *et al.* (1971 a), Teshima and Kanazawa (1971 a and b) and Teshima *et al.* (1983).

P. japonicus juveniles are reported to require an optimum level of 0.5% cholesterol in the diet (Kanazawa *et al.,* 1971a) for normal growth and survival but Deshimaru and Kuroki (1974 b) reported a relatively higher level (2.1%) whereas Shudo *et al.,* (1971) reported a relatively lower level (0.1%).

These differences in dietary cholesterol requirement can be attributed to the difference in life cycle stage, 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 a 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 penaeid prawns. In the present experiment, a basal lipid level of 8% constituting 5.34% codliver oil and 2.66% soyabean oil with 2% lecithin was maintained and thus 0.5% cholesterol along with the mixture of lipid used appears to be sufficient enough for producing maximum growth in *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 purified 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-dodecano-sacrosly 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 has 2 % 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'Abrmo *et al.*, 1982) further support the use of cod-liver oil (a source of PUFA) and lecithin (phospholipid) as a basal lipid source in the present study.

Cholesterol is used in hypodermis formation (Guary and Kanazawa, 1973; Goad, 1976) and sterols are important as elements of cellular and sub-cellular structures in arthropods (Lasser *et al.*, 1966). Sterols are found to be precursors of moulting hormone in arthropods (Gilbert, 1969) as well as brain hormone in prawns (Kanazawa *et al.*, 1971a; New, 1976). Frequency of moulting increased in *P. japonicus* when fed on a diet containing cholesterol indicating the involvement of cholesterol in moulting (Kanazawa *et al.*, 1971a). Sterols are found to be precusor of ecdysterone, a moulting hormone (Gilbert, 1969) which induce moulting in *P. japonicus* (Kanazawa *et al.*, 1992 b). 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 larval and postlarval prawns, preceding synthesis of new tissues in the body, the significant increase in growth as observed in the present study in prawns, can be expected by the addition of cholesterol which is the precursor for the steroid hormones.

The results of these experiments clearly indicate the essentiality of cholesterol for proper survival and growth of larvae, and growth of

postlarvae of *P. indicus* and 0.5% of cholesterol in the diet was found to be most effective for promoting growth significantly in larvae and postlarvae of *P. indicus*.

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