EFFECT OF DIFFERENT DIETARY LEVELS OF LECITHIN ON GROWTH, SURVIVAL, MOULTING AND BODY PHOSPHOLIPID LEVELS IN *MACROBRACHIUM ROSENBERGII* POSTLARVAE

By

MAHESH.G, B.F.Sc.

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE

MASTER OF FISHERIES SCIENCE

FACULTY OF FISHERIES

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

PANANGAD, COCHIN

1996

DECLARATION

I hereby declare that this thesis entitled "**EFFECT OF DIFFERENT DIETARY LEVELS OF LECITHIN ON GROWTH, SURVIVAL, MOULTING AND BODY PHOSPHOLIPID LEVELS IN *MACROBRACHIUM ROSENBERGII* POSTLARVAE**" is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship or other similar title of any other University or Society.

Panangad,

20/05/1996.

MAHESH.S.

CERTIFICATE

Certified that this thesis entitled "**EFFECT OF DIFFERENT DIETARY LEVELS OF LECITHIN ON GROWTH, SURVIVAL, MOULTING AND BODY PHOSPHOLIPID LEVELS IN *MACROBRACHIUM ROSENBERGII* POSTLARVAE**" is a record of research work done independently by Sri.**MAHESH.S.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Panagad
21/05/1996

21.5.96

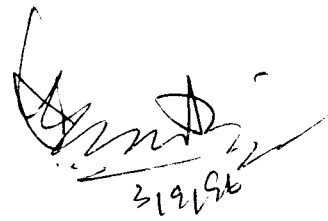
Dr.M.J.SEBASTIAN
(Chairman, Advisory Board)
Former Professor and Head,
Department of Aquaculture,
College of Fisheries,
Panangad,
Cochin.

ADVISORY COMMITTEE

NAME AND DESIGNATION **SIGNATURE**

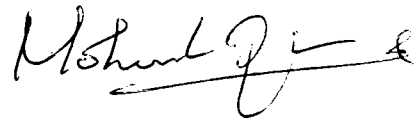
CHAIRMAN

Dr. M.J. SEBASTIAN,
Rtd. PROFESSOR AND HEAD,
DEPARTMENT OF AQUACULTURE,
COLLEGE OF FISHERIES,
PANANGAD, KOCHI.

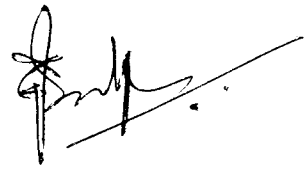


MEMBERS

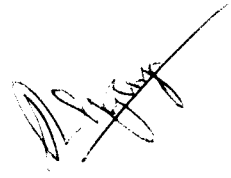
Dr. C. MOHANAKUMARAN NAIR,
ASSISTANT PROFESSOR,
DEPARTMENT OF AQUACULTURE,
COLLEGE OF FISHERIES,
PANANGAD, KOCHI.



Sri. T.M. SANKARAN,
ASSOCIATE PROFESSOR AND HEAD,
DEPARTMENT OF MANAGEMENT STUDIES,
COLLEGE OF FISHERIES,
PANANGAD, KOCHI.

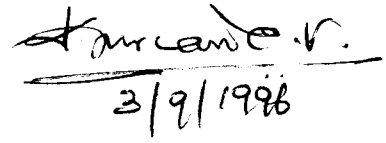


Sri. P.S. MRITHUNJAYAN,
ASSOCIATE PROFESSOR,
DEPARTMENT OF FISHERY HYDROGRAPHY,
COLLEGE OF FISHERIES,
PANANGAD, KOCHI.



EXTERNAL EXAMINER

Prof. Dr. C.V. KURIAN,
FORMER HEAD OF THE DEPARTMENT OF
MARINE SCIENCES, COCHIN UNIVERSITY.



ACKNOWLEDGMENT

I extend my sincere gratitude to **Dr. M.J. SEBASTIAN,** Chairman of the advisory committee and former Professor and Head, Department of Aquaculture, College of Fisheries, for his valuable guidance and helpful advice during the course of the study and preparation of the thesis. His constant encouragement and relentless support helped me to a great extent in the smooth execution and timely completion of the research programme. I am indebted to him for arranging sufficient funds at the right time and providing all facilities necessary for the experiment.

I owe a great deal to **Dr. C. MOHANA KUMARAN NAIR,** Assistant Professor, Department of Aquaculture, College of Fisheries, for being a constant source of support in my efforts and also for his sincere help and constructive suggestion during the thesis preparation.

My heartfelt thanks are due to **Sri. T.M. SANKARAN,** Associate Professor and Head, Department of Management Studies, College of Fisheries for his helpful assistance in designing the experiment and analysis of the results. I also remain grateful to him for valuable suggestions and critical comments he made at the time of thesis writing.

Thanks are also due to **Sri. P.S. MRITHUNJAYAN,** Associate Professor, Department of Fishery Hydrography, College of Fisheries, for all sorts of assistance extended to my research work.

I am ever thankful to **Dr. D.M. THAMPI,** Professor and head, Department of

Aquaculture and Dean in charge of the College of Fisheries for providing necessary facilities towards the successful completion of the experiment. I wish my special thanks to **Dr. SUSHEELA JOSE,** Associate professor of same department for arranging glucosamime for the experiment.

It is with great sense of gratitude I remember the name of **Dr. P.M. SHERIEF,** Assistant Professor, department of processing Technology. His help and assistance was always there from beginning till to the end of the experiment and his critical suggestions and comments help me a lot in the preparation of the thesis. My special thanks are also due to **Dr.D.D.NAMBOOTHIRI,** Head of the Department of Processing Technology for permitting me to use the facilities of biochemistry lab.

The assistance rendered by the library staff of the College of Fisheries and CMFRI Cochin in the collection of necessary literature is thankfully acknowledged.

The inspiration and help of my colleagues particularly from **SAJU.M.S.** and **GIJO ITTOOP** was with me through out the period of this study. At this juncture I would like to appreciate their restless efforts and record my sincere thanks.

The service rendered by **Mr.R.RAJEEV** and all the staff of KOPPARA ENTERPRISES towards the final processing of thesis is thankfully acknowledged.

MAHESH.S.

CONTENTS

LIST OF TABLES

LIST OF PLATES AND FIGURES

PLATES

FIGURES

1. INTRODUCTION

Aquaculture, especially the culture of shrimps and prawns, has shown tremendous advancement in the past one decade due to a steady increase in demand for these organisms in the world market. A number of species of shrimps belonging to the genera, *Penaeus* and *Metapenaeus,* and the giant freshwater prawn, *Macrobrachium rosenbergii* are being cultured in many parts of the world. *Macrobrachium* spp. commonly known as freshwater prawns (Jayachandran and Joseph, 1992) include more than 100 species distributed throughout the tropical and subtropical regions (New, 1990). Among these species only a very few are suitable for aquaculture purposes (New, 1995) and the giant freshwater prawn *Macrobrachium rosenbergii* is considered as the most favoured and economically important one as far as commercial production is concerned. *Macrobrachium rosenbergii* offers high farming potential and is supposed to have all the qualities of a commercial species (Durairaj *et al.*, 1992). Hence it is regarded as a candidate species for aquaculture purposes in many regions of the world.

In recent years, much attention has been given to the hatchery production of its seed and intensive farming (Nair and Thampy, 1987; Sebastian, 1990). At present it is cultured in a variety of conditions ranging from freshwater to brackish water of 25ppt salinity and in different systems like cages, pens and ponds (New, 1995), stocking with hatchery reared postlarvae and juveniles.

For optimum growth and survival of any organism adequate nutrition is a pre-requisite. This is especially true in the context of culturing organisms under restrictive and rigidly controlled conditions. So the success and failure of all farming operations depend on the availability and supply of a nutritionally balanced and acceptable diet. Effective formulation of a nutritionally balanced diet for a particular species requires a detailed understanding of the nutrient requirement of the concerned species. Nutritional research carried out in the past few decades reveals that a number of nutrients are essential in the diet of shrimps and prawns for sustaining growth and survival (New, 1976, 1987; Biddle, 1977). In the absence or low levels of these essential nutrients in the diet, organisms show abnormalities in behaviour, growth, moulting and survival; whereas excess inclusion, in addition to causing abnormalities, is an unnecessary wastage of nutrients and increases the feed cost which in turn affects the operational cost.

Among the different groups of nutrients required by the crustaceans, phospholipids are important and have got a considerable influence on growth and survival. It was Shiek [1969] who paved the way to phospholipid nutrition research in crustaceans. He noticed that the crustaceans can synthesis PL *de novo* and stated that the biosynthetic capacity is very limited during the early phases of development. Subsequent to this finding, several studies were initiated in different groups of crustaceans and it is proved beyond doubt that PL is an essential nutrient factor for penaeid shrimps and lobsters.

There are only a very few published papers on the requirement of freshwater prawn for dietary phospholipid. Previous studies of Hilton *et al.* (1984) Briggs *et al.* (1988) and Koshio *et al.* (1992) indicated that the addition of lecithin at levels from 0 to 10% dry weight of the diet is not required for *Macrobrachium rosenbergii* juveniles. But Koshio *et al.* (1992) in another

experiment found out that 0-2% of soylecithin in the diet seems to promote growth in very young postlarval prawns of 0.05g size. Further more, in the larval phase of *Macrobrachium rosenbergii,* some times, heavy mortalities are noticed during the metamorphosis moult at the 11th stage and early post-larval stage (Exuvia entrapment disease, EED). The affected larvae in large numbers die during the process of ecdysis getting entrapped in the exuvia. Aetiology of this disease is not yet conclusively found. In view of the involvement of PL in the dietary absorption and utilization of cholesterol (Teshima and Kanazawa, 1980; Teshima *et al.,* 1986 b,d) which acts as a precursor of moulting hormone, lack of sufficient PL was considered to be one of the reasons for this disease. Feeding trial conducted with lecithin incorporated diets indicated that the incidence of this disease can be controlled to some extent. Similarly in the American lobster *Homarus americanus* also supplementation of lecithin through the diet considerably reduces this type of disease like moult death syndrome reported by Bowser and Rosemark (1980) and Conklin *et al. (*1980).

In view of this, in the present study an attempt is made to evaluate the influence of lecithin on growth, survival, moulting and changes in body PL content brought about by different dietary inclusions of PL in the semi-purified diet of *Macrobrachium rosenbergii* post-larvae. A semi-purified diet based on casein egg albumin, incorporating different levels of lecithin was selected because the effect of varying levels of one ingredient can be studied more precisely than when a formulated feed using food stuff of unknown composition is used. Two week old *Macrobrachium rosenbergii* post larvae were selected as the experimental animal, since it is reported that the incidence of EED was found to occur at the 11th larval and early post larval stages. The study is expected to show whether the absence or low levels of lecithin is responsible for EED. In addition, the present study will also provide information about the dietary requirement of PL and its influence on growth, survival and moulting at different

post-larval phases. Further, it will help in identifying and characterising PL deficiency and excess symptoms. Thus, the results of the present study will be useful in formulating a balanced feed for the commercial production of this species of prawn.

2. REVIEW OF LITERATURE

2.1 Lipid requirement of crustaceans

Lipids play an important role as an energy source in the diet of animals. It is the most energy rich molecule of the nutrient classes providing approximately 9 Cal/g energy compared with 4-5- cal/g for carbohydrates and protein (Castell, 1982). This water insoluble biomolecules are involved in many aspects of metabolism in crustaceans such as synthesis of steroid hormones and prostaglandins (Paulraj, 1987; Watanabe, 1991; Rudolph *et al.,* 1992; Rudolph and Spaziani, 1992), activation of enzyme system (Kimelberg and Pupahadjopolus, 1972; Chappelle and Zwingelstein, 1984), brain and nerve functioning (Nicol, 1975), ovarian maturation (Middleditch *et al.*, 1980; Teshima *et al.,* 1988 a, b; Xu *et al.*, 1994) and also play a vital role in the structure of biomembrane at both the cellular and subcellular levels (Paulraj, 1987). Besides this, it has been found that dietary lipids have highest protein sparing action. The protein sparing action of lipids was extensively investigated in a number of fish species (Lee and Putnam, 1973; Takeda *et al.,* 1975; Takeuchi *et al.,* 1978 a, b). Studies in *Penaeus indicus* (Chandge, 1987)and *Macrobrachium rosenbergii (*Clifford and Brick, 1978) have also indicated improved protein utilization when the diet contained sufficient lipid.

For crustaceans as also for many fishes, lipids are required in the diet, not only fo their energy values, but also as a source of essential fatty acids, fat soluble vitamins, sterols and phospholipids. In addiition, lipids are important in determining the flavour and textural quality of the feed (Paulraj, 1987). However, the unique aspect of lipid nutrition in crustaceans is related to the requirement of EFA, sterols and phospholipids (Kanazawa and Teshima, 1977;

Kanazawa *et al.*, 1979 b, c; Teshima, 1982; Teshima *et al.*, 1982; Kanazawa, 1982, 1993).

As far as crustaceans are concerned, the quantitative dietary lipid requirements have been worked out mainly in shrimps and prawns. Since the early works of Kanazawa *et al.* (1970), Andrews *et al.* (1972), Foster and Beard (1973) and Sick and Andrews (1973) several studies have been carried out in different species of crustaceans to determine the optimum dietary requirement of lipids.

Table I gives an idea of optimum dietary lipid requirements of different species of crustaceans with reference to the source of lipid used.

According to the information presented in Table I, it would appear that the dietary lipid requirement of crustaceans does not exceed 12% of the dry weight of feed. Unlike fishes, crustaceans cannot tolerate high levels of fat content in the diet. Based on the observations of Foster and Beard (1973), Deshimaru and Kuroki (1974), Andrews *et al.* (1972), Sheen and D'Abramo (1993) and Davis and Robinson (1986) high levels of lipid are usually associated with significant retardation of growth and survival. Andrews *et al.* (1972) while studying the lipid requirement of *Penaeus setiferus* stated that lipases activity of shrimps is very limited and attributed this as the major reason for reduced growth rate in crustaceans fed with higher lipid diets.

As per Table I, the quantitative dietary lipid requirement of shrimps and prawns ranges from 3 to 12% of the dry weight. New (1976), Biddle (1977) and D'Abramo (1989) mentioned that this variation in optimal level could mainly be due to the inter relationship between different classes of lipids that influences requirement as well as the difference due to age and

TABLE I

Lipid requirement of some commercially important species of crustaceans with special reference to the source of lipid used

SPECIES	LIPID SOURCE	SUGGESTED OPTIMUMLEVEL IN PERCENTAGE	REFERENCE
Penaeus monodon	—	11.75	Mendoza(1982)
		10-11	Catacuttanand Kanazawa (1985)
	Cod liver oil	12	Catacuttan (1991)
	Cod liver oil and corn oil in 2:1 ratio	8-12	Sheen and Chen (1992)
	Cod liver oil soybean oiland lecithin	6.5	Briggs *et al.* (1994)
	Cod liver oil or cod liver oil + soybean oil or Soyabean oil	8	Chen (1986)
P. japonicus	Cod liver oil and soybean oil in 1:1 ratio	6	Deshimaru and Kuroki (1974)
	Short necked clam oil	8	Kanazawa *et al.* (1979 a)
	Pollack liver oil and cod liver oil in the ratio 3:1 or 1:1	6	Deshimaru *et al.* (1979)

[Cont.....]

	Pollack residual oil	5	Kayama *et al.* (1980)
	——	6.5	Teshima and Kanazawa (1984)
P.indicus	——	10-12	Chandge (1987)
	Cod liver oil soybean oil and lecithin	10	Chandge and Raja (1993)
	Cod liver oil prawn head oil sardine oil and soybean lecithin in 1:1:1:1 ratio	6	Ali (1990)
P.setiferus	Beef thallow Menhaden oil corn oil in 1:1:1 proportion	10	Andrews *et al.* (1972)
	—	3.9	Doken and Lawerence (1987)
Palaemon serratus	Corn oil and cod liver oil	> 7.5	Foster and Beard (1973)
Penaeus dourarum	Beef thallow or linseed oil	10	Sick and Andrews (1973)
P.merguiensis	Cod liver oil	7	Aquacop (1978)
P.vannamei	—	8.9	Doken andLawerence (1987)
Macrobrachium rosenbergii	Shrimp head oil	3	Joseph and William (1975) Sandifer and Joseph (1976)
		> 10	Biddle *et al.* (1977)

[Cont.....]

Macrobrachium rosenbergii	Cod liver oil, corn oil and lecithin	>10	Hilton *et al.*(1984)
	Cod liver oil corn oil in 2:1ratio	>8	Sheen and D'Abramo (1991)
Homarus americanus	Cod liver oil	5	Castell and Covey (1976)
	—	8	Tridle and Castell (1980)
	Tuna oil, corn oil and cod liver oil	6	D'Abramo *et al.* (1980)
Procambarus acutus acutus	Manhaden oil	6	Davis and Robinson (1986)
Astacus astacus	—	7	Ackefors *et al.* (1992)
Carcinus maenas	Cod liver oil and cholestron	7.4 - 11.1	Ponat and Adelung (1983)

species.

In the case of penaeid shrimps optimum lipid content in the diet required for growth and survivial is between 3.9 and 12% (Table 1). While reviewing the nutritional requirements of shrimps Foster (1976), New (1976), Biddle (1977) and D'Abramo (1989) observed that penaeid shrimps do not require higher levels of fat and suggested that optimum level may fall between 5 and 10% in various species. Even in the same species wide fluctuations are noticed with respect to the optimum level. Sheen and Chen (1992) proposed that this variation in absolute level of dietary lipid noticed in these studies could be due to the differences in the amount and quality of dietary protein employed, the differences in lipid sources used, and also on the stage of the experimental animals. Besides this, New (1976) has pointed out that the variations in the experimental procedures followed, including the non-standardized way of expressing the proximate composition in the nutrition studies has limited the value of published information for comparison.

Penaeid shrimps like *Penaeus japonicus* and *P. monodon* which are carnivorous in feeding habit (Deshimaru and Yone 1978) require higher fat content when compared to other species. Based on the available literature dietary lipid requirement of *Penaeus monodon* lies in between 6 and 12%. Mendoza 1982 found that a diet containing 11.7% lipid gave maximum growth and feed efficiency for *Penaeus monodon* juveniles. Cutacuttan and Kanazawa (1985) showed best growth with lipid source rich in highly unsaturated fatty acid of n-3 series using semi purified diets containing 10 to 11% of lipid. Irrespective of sources , juvenile *Penaeus monodon* did not show differences in growth among dietary treatment incorporated with 8% codliver oil, 4% each of codliver and soyabean oil, or 8% soyabean oil alone (Chen 1986). According to Sheen and Chen (1992) juvenile tiger shrimps do not have any absolute level of

dietary lipid requirement and the optimal amount varies according to the protein level, available dietary energy and satisfaction of specific fatty acid requirements. On the other hand, Briggs *et al.* (1994) recommended the optimum level as 6.5% for this species when codliver oil and soyabean oil in 3:1 proportion alongwith 3.5% of lecithin were used as the source.

In *Penaeus japonicus*, another carnivoros species,the requirement of lipid lies around the range of *Penaeus monodon*. Deshimaru and Kuroki(1974) found that lipid supplementation to an extent of 6% in the casein-based purified diet produced significantly higher growth rate than either 0 or 12% addition. In the same species Kanazawa *et al.* (1977 a) obtained best growth with 8% inclusion of short-necked clam oil and suggested that excess oil to an extent of 16% inhibited growth of the shrimp among the 3 levels (8, 12 & 16%) tested. Deshimaru *et al.* (1979) assessed that 6% lipid content was appropriate for the growth of *Penaeus japonicus* when pollock liver oil and soyabean oil mixed in 3:1 or 1:1 ratio was used as the source. Using a purified diet with carragenin as a binder Teshima and Kanazawa (1984) had conducted a series of experiments in this species and concluded that elevation of lipid levels from 6.5 to 16.5% did not improve growth or survival when the diet contained sufficient carbo hydrate. A similar range of requirements (7%) was noticed in *Penaeus merguensis by* (AQUACOP, 1978).

Studies carried out in *Penaeus indicus* have demonstrated that larvae and post larvae require almost 10% lipid and advanced post larvae and juveniles need about 10 - 12% for promoting maximum growth, food and protein conversion and protein retention (Chandge, 1987). This was further confirmed by Chandge and Raja (1993) in another study using cod liver oil and soyabean oil combination with lecithin. In contrast to this, Ali (1990) reported that *Penaeus indicus* needs only 6% lipid in the purified diet, when cod liver oil, prawn head oil,

sardine oil and soyabean oil were used in equal proportion. Optimum lipid content for *Penaeus setiferus* was 3.9% according to Doken and Lawrence (1987).

Higher levels of lipid was found to supress growth and survival rate in many species of shrimps like *Penaeus setiferus* (Andrews *et al* 1972), *Palaemon serratus* (Foster and Beard, 1973) and *Penaeus duorarum* (Sick and Andrews, 1973).

Addition of 10% supplemented lipid consisting of equal parts of beef thallow, menhaden oil and corn oil to the diet of *Penaeus setiferus* had adverse effect on growth and survival (Andrews *et al., 1972),* Foster and Beard (1973) found depression in growth when 15% of cod liver oil or corn oil was supplemented in the diet of *Palaemon serratus;* whereas Sick and Andrews (1973) noticed high mortality and reduced growth with 10% corn oil incorporated diet in *Penaeus duorarum.*

Unlike penaeid shrimps, the lipid requirement of freshwater prawn, *Macrobrachium rosenbergii* is comparatively low. Earlier investigations of Joseph and William (1975) and Sandifer and Joseph (1976) reveals that the lipid requirement of post larval prawn can be satisfied by supplementing the diets [either a commercial shrimp feed or semi purified diet] with 3% shrimp head oil. Recently, Sheen and D'Abramo (1991) while studying the response of juvenile freshwater prawn, *Macrobrachium rosenbergii,* to different levels of lipid in the diet observed significant reduction in weight when 10-12% was included. However, they recommended the optimum level of 8% with cod liver oil and corn oil in 2:1 ratio. Biddle (1977) and Hilton *et al.*(1984)also indicated that the total lipid content in the diet of *Macrobrachium rosenbergii* should not exceed 10%.

Like in the case of shrimps and prawns, dietary lipid constitutes an essential nutrient of other groups of crustaceans as well. Requirement of lipids has been clearly demonstrated in several groups like lobsters, crayfish and crabs. Based on the feeding experiments with adult lobster *Homarus americanus* Castell and Covey (1976) reported superior growth rate at 5% lipid content; while D'Abramo *et al.* (1980) reported that inclusion of various oils at 6% of dry weight in the purified diet produced maximum growth in the same species of lobster.

In the case of *Procambarus acutus actutus* a cray fish, Davis and Robinson (1986) reported that 6% of lipid was the optimum level. Ackefors *et al.* (1992) observed that *Astacus astacus* requires 7% of lipid. In both these studies, growth rate and survival rate were considerably lower when higher percentage oil lipid was added to the feed.

Very little information is available concerning the lipid nutrition of crabs. Ponot and Adelung (1983) in their attempt to establish an optimal diet for crab *Carcinus maenas* found that 7.4 - 11.1% of lipid is essential for this species and reported that too high concentration slightly reduces the growth and survival.

Based on the information presented above it is obvious that the total lipid content of the diet for crustaceans should not exceed more than 10%. A critical need exists for further lipid nutrition research to define the energy requirement of lobsters, cray fish and crabs. In addition, emphasis must be placed on the differential needs of shrimps and prawns at various stages of growth and development.

2.2 Lipid classes required for crustaceans

Lipid groups which are important in the nutrition of crustaceans include fatty acids, phospholipids and cholesterol.. In the following section a brief review of some important metabolic functions and the requirements of these lipid classes are given.

2.2.1 Fatty acids

The principal component of most lipids is fatty acids and are considered to be the building block component of all classess of lipids in animals. They occur in very large amount in different forms and only traces are present in the free form. About 100 different kinds of fatty acids have been isolated from various lipids of animals and plants (Paulraj, 1987). Basically, fatty acids are of two types, saturated and unsaturated ones. Crustacean lipids have both saturated and unsaturated fatty acids particularly a greater concentration of unsaturated ones belonging to $\omega 3$ series. (Gopakumar and Nair, 1975; Krzynowek *et al.*, 1982). Castell (1982) grouped fatty acids in crustaceans into three categories viz., fatty acids synthesised in the body *de novo* from acetate, unusual fatty acids unique to crustaceans, and fatty acids which are not synthesised in the body, but are very essential for growth, survival and other physiological functions. Among the three, the most important one is the third category called as essential fatty acids (EFA), which are mainly derived from diet to meet the requirements.

2.2.1.1 Fatty acid synthesis in crustaceans

Crustaceans are known to synthesis certain groups of fatty acids *de novo* in their body. Clear indication for the bio-synthesis of saturated monoenoic fatty acids such as palmetic (16:0), palmetioleic (16:1), steric (18:0), and oleic acids (18:1 $\omega 9$) from the precursor acetate has been evident from the radio tracer experiments in many species of shrimps like

Penaeus japonicus (Kanazawa and Teshima, 1977), *Penaeus monodon* and *Penaeus merguensis* (Kanazawa *et al.*, 1979 d). The same pathway of synthesis of fatty acids from acetate has been found to exist in other crustaceans also like the freshwater cray fish, *Astacus astacus* (Zandee, 1966) and the lobster, *Homarus gammarus* (Zandee, 1967). Kanazawa *et al.* (1979 a) and Jones *et al.* (1979) also indicated the formation of corresponding monoenoic fatty acids from C14 palmetic and steric acids in the prawn; *Penaeus japonicus.* However, the ability for the synthesis of long chain fatty acids with double bond is absent or very limited in crustaceans (Kanazawa and Teshima, 1977 ; Kanazawa *et al.,* 1979 a , c; Jones *et al .,* 1979; D'Abramo *et al.,* 1980; Reigh and Stickney, 1989; D'Abramo and Sheen, 1993; Kanazawa and Koshio, 1993). Further more, Teshima *et al.*(1992a) examined the ability for bioconversion of 18:3n-3 to other fatty acids, specifically highly unsaturated HUFAS like eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA) by crustaceans. It was found that *Penaeus japonicus, Penaeus chinensis*, *Macrobrachium rosenbergii* and *Palaemon paucidens* had only limited ability for bioconversion of 18:3n-3 to 20:5n-3 and 22:6n-3 (EPA and DHA) fatty acids. They noticed that among the species compared *Penaeus japonicus* larvae had a greater ability to convert 18:3n-3 to other n-3 HUFAS than juveniles suggesting a possible change in fatty acid metabolism before and after metomorphosis. A similar phenomenon was noticed in brine shrimp *Artemia* salina by Kayama *et al.* (1963). Analysis of fatty acid profiles in the tissue of white shrimp *Penaeus setiferus,* brown shrimp *Penaeus aztecus* and pink shrimp *Penaeus duorarum* by Bottino *et al.* (1980) and *Penaeus indicus* by Read (1981) also indicated that these species possess only limited capacity to bioconvert C18 PUFA to C>20 PUFAS. The turnover of dietary EPA and DHA to other fatty acids are also very limited in penaeid shrimps and the lobster, *Panulirus japonicus* (Teshima *et al.,* 1992, a,b; Kanzawa and Koshio, 1993). These findings supports a need for supplementing fatty acid in the diet of crustaceans to enable them to grow well.

2.2.1.2 Fatty acid requirement in crustaceans

Table II, depicts the picture of essential fatty acid requirement of crustaceans. Polyunsaturated fatty acid (PUFA) belonging to linolenic n-3 and linoleic n-6 families (18:2n-6, 18:3n-3, 20:5n-3 and 22:6n-3) have been recognized as the most indispensible EFA supporting normal growth and survival in all groups of crustaceans (Kanazawa 1985; D'Abramo 1989; D'Abramo and Sheen 1993; Kanazawa and Koshio 1993).

Requirement of fatty acid in crustaceans depends on different factors. According to Castell (1982) these factors include, *de novo* synthetic ability of each lipid class, ability of the organism to alter fatty acids, dietary lipid composition as well as seasonal variation brought about by changes in environmental factors like temperature and salinity, and changes in the organism such as growth, reproduction, moulting stages etc. Reliable information regarding the EFA requirement of crustaceans especially shrimps and prawns is available from the feeding experiments conducted in the past few decades with semipurified or purified diets using saturated and unsaturated fatty acids.

In penaeid shrimp several authors reported that the growth has been enhanced through the dietary addition of 18:3n-3 or 18:2n-6 fatty acids (Shewbart and Miels, 1973; Guray *et al.*, 1976; Colvin, 1976; Kanazawa *et al.*, 1977 b; 1979, b;c; Kayama *et al.*, 1980; Martin, 1980; Read, 1981; Chandge 1987; Xu *et al.*, 1993 a;b; 1994). *Penaeus japonicus* is the species in which Kanazawa *et al.* (1977 a,b, 1978, 1979 a,c and d) have carried out extensive work on lipid and fatty acid requirements. In this species optimum weight gain was attained when the diet was supplemented with 1% each of linoleic and linolenic acid (Kanazawa *et al.*, 1977 b,

TABLE - II

Essential fatty acid requirement of crustaceans

SPECIES	SUGGESTED EFA REQUIREMENT	REFERENCE
Penaeus monodon	0.6 - 1.6% 0f PUFA	Chen (1986)
	PUFA particularly 20:5 ω3 and 22:6 ω3	Millamena and Quinitio (1985)
	ω3 PUFA such as EPA and DHA	Liao and Liu (1989)
	ω3 PUFA or Fish oil rich in 20:5 ω3 fatty acid	Kontara (1986) Kontara and Nardjana(1992) Rees *et al.* (1994)
Penaeus japonicus	20: 5 ω3 and 22: 6 ω3 more essential than 18: 2 ω3 or 18: 3 ω3	Kanazawa *et al.* (1977a)
	1% of 18: 2 ω3 and 18: 3 ω3	Kanazawa *et al.* (1977b)
	18: 3n-3 produced better growth than 18: 2n6	Guray *et al.* (1976)
	Microencapsulated diet provided with 3 series of PUFA such as 20: 5n-3, 22: 6n-3 and 21: 4n-6 recorded high growth and survival	Jones *et al.* (1979)
	1% of 18: 2w6 or 18: 3w3, more effective is1% of either 20: 5w3 or 22: 6w3	Kanazawa *et al.* (1979b) Kayama *et al.* (1980)
	Both linoleic and linolinic acid are essential linoleic acid inferior to linolenic	Kanazawa (1979d)

[Cont.....]

Penaeus indicus	Linoleic and lenolenic acid in the ratio 1:1 is essential	Colvin (1976)
	18: 3n-3, 18: 2n-6 with 20: 5n-3 and 22: 6n-3 gave superior growth	Read (1981)
	Diet containing a mixture of fatty acid such as 18: 2 ω6, 18: 3 ω3, 20: 5 ω3 and 22: 6 ω3 are essential for growth survival and metamorphosis	Chandge (1987)
Penaeus aztecus	1% 18: 3n-3	Shewbart and Miles (1973)
Palaemon serratus	Required both ω6 and ω3 fatty acid 18: 2 ω6 and 18: 3 ω3 in the ratio 2:2 optimum 20: 5 ω3 plus 22:6 ω3 fatty acid superior to 18 carbon PUFA	Martin (1980)
Penaeus vannamei	ω3 HUFA enables faster growth higher survival and increased stress resistance	Yates *et al.*(1987)
Penaeus stylirostris	18: 3 ω3 or 18: 6 ω6	Fenuecci (1981)
Penaeus chinensis	18: 3n-3, 20: 4n-6 and 22: 6n-3 re essential for growth and survival. But 1% of 22: 6n-3 along with 2% each of 16: 0 and 18: 1n-1 fatty acid recorded better growth fatty acid value in the diet 18: 2n-6 < 18: 3n-3 < 20: 4n-6 < 22: 6n-3	Xu *et al.*(1993a;b) (1994)

[Cont.....]

Macrobrachium rosenbergii	Require 18: 2n-6 and 18: 3n-3 fatty acid in 15: 1 ratio 18: 3n-3 alone in the diet was found to depress growth	Reigh and Stickney (1989)
	High concentration of ω3: ω6 fatty acids, found beneficial	Sandifer and Joseph (1976)
	Both 18: 2n-6 and 18: 3n-3 are best result was obtined with 18: 2n-6, 18: 3n-3 fatty acids in 18: 1 ratio	Teshima *et al.* (1992)
	22: 6n-3 or 20: 4n-6 HUFA at levels between 0.075% - 0.6%	D'Abramo and Sheen (1993)
	Enriched artemia with n-3 HUFA improves growth survival and metamorphosis rate and stress resistance	Devresse *et al.* (1990) Romdhane *et al.* (1994)
Homarus americanus	Fish oil high in ω3 PUFA superior to corn oil rich in 18: 2 ω6	Castell and Convey (1976) D'Abramo *et al.* (1980)
Panulirus japonicus	EPA and DHA	Kanazawa and Koshio (1993)
Carcinus maenas	18: 3 ω3 is preffered over 18: 2 ω6	Ponat and Adelung (1980)

1979 a,b,d, Kayama, *et al.*, 1980). In the other species like *Penaeus aztecus* (Shewbart and Miles, 1973), *Penaeus indicus* (Colvin, 1976; Read, 1981; Chandge 1987), *Palaemon serratus* (Martin, 1980) , *Penaeus stylirostris* (Fenucci, 1981) , *Penaeus monodon* (Millamena and Quinitio, 1985), *Penaeus vannamei* (Yates *et al.*, 1987) , and *Penaeus chinensis* (Xu *et al.*, 1993 a,b) linoleic and linolenic acids have been found effective in accelerating growth and survival.

Generally measured response of linolenic acid exceed that of linoleic acid . This was evident from the works of Shewbart and Miles (1973), Guray *et al.* (1976), Kanazawa *et al.* (1979 e) and Xu *et al.* (1993 b). Guray *et al.* (1976) and Kanazawa *et al.* (1979 e) proposed that even though linoleic and linolenic acids are essential in the diet, the growth rate produced by linoleic acid was inferior to that of linolenic acid in *Penaeus japonicus* when they are tested separately using semipurified diets. Shewbart and Miles (1973) recorded a similar result in *Penaeus aztecus.* Supporting these evidences Xu *et al.* (1993 a,b) indicated that the nutritive value of dietary n3 and n6 fatty acids in *Penaeus chinensis* increases in the order of 18:2n-6 < 18:3n-3< 20:4n-6< 22:6n-3.

In contrast to these observations in marine penaeid shrimps, the fresh water prawn *Macrobrachium rosenbergii* showed preference to 18:2n-6 fatty acids over 18:3n-3 in satisfying C18 PUFA requirements from the diet. Reigh and Stickney (1989) and Teshima *et al.* (1992 a) noticed a higher ratio of $\omega 6$ fatty acids 15:1 and 18:1 respectively in the diet for good growth rate.

Essential fatty acids of $\omega 3$ and $\omega 6$ series are found to produce faster growth rate and improved survival in the American lobster, *Homarus americanus,* the spiny lobster *Panulirus*

japonicus (Castell and Covey, 1976; D'Abramo *et al.*, 1980; Kanazawa and Koshio, 1993) and the crab, *Carcinus maenas* (Ponat and Adelung, 1980).

Eventhough addition of fatty acids like 18:3n-3 and 18:2n-6 in the diet fulfills the EFA requirement of crustaceans, several authors reported that the effect was inferior to that of long chain, highly unsaturated fatty acids like EPA and DHA (Kanazawa *et al.*, 1977 a,1978,1979 g;Jones *et al.*, 1979; Read, 1981; Chandge, 1987; Xu *et al.*, 1993 a,b, 1994; D'Abramo and Sheen, 1993). Shrimps and prawns, although possess the ability to elongate and desaturase dietary C18 fatty acids to HUFAS like EPA and DHA (Kanazawa and Teshima, 1977; Kanazawa, *et al., 1979 a,c)* the capacity was not sufficient enough to meet the requirements (Kanazawa *et al.*, 1979 f; Jones *et al.*, 1979; Teshima *et al.*, 1992 a; D'Abramo and Sheen, 1993). Superiority of long chain fatty acids was very well demonstrated in many species of shrimps.

Jones *et al.* (1979) by feeding a microencapsulated diet provided with 3 series of PUFA like 20:5n-3, 22:6n-3 and 20:4n-6 to *Penaeus japonicus* larvae recorded superior performance in terms of growth and survival than 18:3n-6 and 18:2n-6 fatty acids included diets. In the same species Kanazawa *et al.*, (1978, 1979 f) and Kayama *et al.* (1980) also noticed that the weight gain resulting from the diet contaning 18:2 ω-6 and 18:3 ω-3 fatty acid (even at the optimum levels) was lower compared to that from diet supplimented with EPA or DHA. Xu *et al.* (1993 a,b,1994) in a series of experiments with *Penaeus chinensis* got higher growth, survival and moulting frequency by the inclusion of lower level of EPA and DHA than 1% each of linoleic and linolenic acids. He noted that the fatty acid value of EPA and DHA is higher to that of linoleic and lenolenic acids. However, Read (1981) found that supplementing 18:3n-3, and 18:2n-6 fatty acids with dietary 20:5n-3 and 22:6n-3 gave better growth and survival than low fat diet with these long chain n-3 HUFA alone. Studies conducted in shrimps such as *Penaeus indicus* (Chandge, 1987), *Palaemon serratus* (Martin, 1980), *Penaeus monodon*

(Millamena and Quinitio,1985) and *Penaeus vannamei* (Yates *et al.*, 1987) also showed that long chain fatty acids are more beneficial than linoleic and linolenic acid. In *Macrobrachium rosenbergii* a smilar observation was made by D'Abramo and Sheen (1993) who found that the mean weight gain of the juvenile prawn fed a diet containing EPA and DHA was 30-50% higher than the control group fed with 18:3n-3 or 18:2n-6. The suggested requirement level of these fatty acids in the freshwater prawn ranges between 0.075 and 0.6%.

Fatty acid especially HUFA is very critical in the larval development stage of crustaceans. Sarac *et al.* (1993) indicated that small prawns appeared to be more sensitive to the differences in fatty acid levels of the diet. Feeding experiments of Liao and Liu (1989), Xu *et al.* (1993 a,b), Kontara (1986) Kontara and Nardjana (1992) and Rees *et al.* (1994) in *Penaeus monodon* , and Devresse *et al.* (1990) and Romdhane *et al.* (1994) in *Macrobrachium rosenbrgii* reveal that the growth and survival rate of the larvae were very low when reared on artemia or microbound diet with low $\omega 3$ HUFA profile. Enrichment of artemia or the microbound diet was found to produce good results. According to Sarac *et al.* (1993) a decreasing tendency in food intake was noticed when the diet was deficient in EFA and attributed that this was one of the major reason for reduced growth and survival rates. Chandge (1987) working with *Penaeus indicus* reported that low EFA content not only lead to poor growth and survival rate, but also delayed larval metamorphosis and food conversion efficiency. In addition to this, Castell and Covey (1976) found that EFA deficiency reduces blood cell number and serum protein level, and lengthens the intermolt period in the lobster, *Homarus americanus*.

Research in nutritional requirements of brood stock have established essential fatty acids as a key nutritional factor influencing ovarian maturation, spawning, egg production and hatching and larval development in crustaceans (Primavera, 1985; Cahu *et al.*, 1987; Xu *et*

al., 1994). EFA in particular HUFA which are very essential to promote ovarian growth of penaeids include, arachidonic acid AA (20:4n-6), EPA (20:5n-3) and DHA (22:6n-3) (Primavera, 1985). During the maturation process body lipid reserves specifically in the hepatopancreas are transferred to the ovary (Van derhorst, 1973). Middleditch *et al.*, (1979, 1980) demostrated that *Penaeus setiferus* would not produce eggs unless the diet contained EPA and DHA and implicated that these fatty acids are very essential for vitellogenesis. Besides this, arachidonic acid (AA) act as precursor for prostaglandin synthesis which in turn influences the reproductive organs and brings about necessary changes for the production of eggs and sperms (Middledetch *et al.*, 1979). EFA not only influences the maturation of ovary and egg production in penaeid shrimps, but also is essential for the hatchability of egg and larval development. Alava *et al.* (1993), Xu *et al.* (1994) and Cahu *et al.* (1994) have reported that the fecundity and hatchability of the egg produced by the shrimps *Penaeus japonicus, P. chinensis* and *P. vannamei* fed with a diet containing oils rich in $\omega 3$ HUFA was significantly greater than those of a control group maintained on a feed provided with low HUFA content.

Findings of the experiments discussed above, point to the need of EFA rich in HUFA for growth, survival , maturation, egg production, hatching and subsequent larval development in crustaceans. EFA requirement of any crustacean will be the sum total of fatty acid needed for synthesizing each important lipid class in various tissue and organs that makes the organism. So the essential fatty acids should be supplied through the diet in adequate quantities for the normal physiological functions of these organisms.

2.2.2. Phospholipids

Phospholipids is another important category of lipids required by fishes and crustaceans (Kanazawa, 1993).They are involved in several aspects of metabolism and constitute the major share of lipids in the tissue and haemolymph of all decapod crustaceans (Bliggs and Scott, 1966; Gopakumar and Nair, 1975; Teshima and Kanazawa, 1978 a; Clarke and Wickins, 1980 and Chapelle, 1986; Gonzalez - Baro and Pollero, 1988). Recent research has pointed out that phospholipid is an indispensible crustacean dietary factor essential for growth, survival and reproduction. In many of the physiological functions of crustacea phospholipids are very critical. Among these, the most important and unique function is the emulsification, absorption and transportation of dietary lipids (Teshima and Kanazawa, 1979; D'Abramo *et al.*, 1982; 1985 a and Teshima *et al.*, 1986 b). Since the crustaceans have an open vessel system, (Teshima and Kanazawa, 1979) absorption and interorgan transport of nutrients including lipids is persumed to be operated through the body fluid (haemolymph) associated with phospholipids of high density lipoprotein complex (Teshima and Kanazawa, 1980). Besides this, retention of lipids like cholesterol and triglycerides (TG) in the body is also the function of phospholipid (D'Abramo, *et al.*, 1982, 1985 b; Teshima *et al.*, 1986 b). Regarding the fact that crustaceans are incapable of synthesizing cholesterol (Zandee, 1966, 1967; Teshima and Kanazawa, 1971a; Teshima, 1982; and D'Abramo *et al.*, 1984) the PL transport mechanism is thought to have a substantial influence in the epidermal membrane synthesis and moulting, by limiting cholesterol, the precursor of moulting hormone (New, 1976; Conklin, 1985; Lanchaise *et al.*, 1993; and Sheen *et al.*, 1994).

Another aspect of crustacean metabolism in which the PL are suppossed to have a greater influence is the reproductive physiology (Middleditch *et al.*, 1980; Teshima and

Kanazawa, 1983; Bray *et al.*, 1990). Successful gonadal development and spawning take place only when the tissue phospholipids are present in adequate quantity (Teshima *et al.*,1988b) or when fed with PL supplemented diet (Millamena, 1989; Cahu *et al.*, 1994 and Alava *et al.*, 1993). It is implicated that PL act as a lipid moiety in the synthesis of vitellogenin, a lipoprotein constituting the yolk during the process of ovarian maturation (Meusy, 1980).

Generally, crustaceans can synthesis phospholipids via the same pathway as those in mammals (Shiek, 1969). In spite of this, it is found that an exogenous source of PL is very essential for sustaining growth and survival for many groups including penaeid shrimps and homarid lobsters (Kanazawa *et al.*, 1979a, 1982a; Conklin *et al.*, 1980; Teshima *et al.*, 1986a; Baum *et al.*, 1990; Camara *et al.*,1993, Kanazawa and Koshio, 1993 and Briggs *et al.*,1994).

The synthetic ability of PL is thought to proceed at a slow rate (D'Abramo *et al.*, 1981; Kanazawa *et al.*, 1985) and it is persumed that the requirement of PL for membrane building and renewal is very high during the faster growing, early developmental stages (Geurden *et al.*, 1995). Hence the requirement exceeds the endogenous synthetic capacity.

In addition to this, in crustacea PL is essential for several adaptive mechanisms brought about by the changes in environmental conditions like temperature (Cossino, 1976; Chapelle, 1978; Chapelle *et al.*, 1979; Farkas and Nevinzel, 1981) and Salinity (Pe'queux and Gillies, 1981; Pe'queux and Chappelle, 1982). Above all it acts as a crtical constituent in maintaining the structure and physiological integrity of cellular membranes (O'Conner and Gilbert, 1968; Gilbert and O'Conner, 1970; Chang and O'Corner, 1983).

As far as the nutritional aspect of PL is concerned, apart from growth promotion; the

phospholipid inclusion in the pelletized feed have the unique property of reducing the rapid leaching of water soluble nutrients and thereby increasing the available level of essential nutrients in the feed (Conklin, 1985; Chen and Jenn, 1992).

In view of this slow biosynthetic rate and its importance in the physiological functions like lipid absorption and transportation, adaptation mechanisms, growth, survival and reproduction, as well as being the critical constituent of cellular membranes, much work has been carried out concerning the PL nutrition, its content, composition and functions in crustaceans. In the following sections a brief description of various aspects of metabolism of phospholipids and its nutritional importance is highlighted.

2.2.2.1 Phospholipid biosynthesis in crustaceans

The biosynthetic pathway leading to the formation of phospholipids in crustaceans was studied using radioactive labelling techniques of PL precursors like phosphorus, fatty acids, glycerol and specific isotopes like choline, ethanolamine, serine, inositol, etc. (Chapelle, 1986). The ability of crustacean to synthesis PL was first pointed out by Shiek (1969) who showed that the lobster *Homarus americanus* was able to synthesis phospholipids from 32P-disodium phosphate. But he did not explain the possible pathway of PL formation. The principle route of PL biosynthesis in crustaceans was described by Chapelle *et al.* (1976; 1977) and Brichon *et al.* (1980) in crabs *Eriocher sinensis* and *Carcinus maenas*. According to them PL synthesis in these organisms takes place similar to that of mammals and composed of two main routes. As a first step, phosphotidic acid (PA) is formed from phosphorus, glycerol and fatty acids which is then converted to 1,2 diglyceride and CDP-diglyceride. Both these, 1,2-diglyceride and CDP-diglyceride lead to the formation of phosphatidylcholine (PL) and phosphatidylethanolamine

(PE) whereas phosphatidylinositol (PI) is formed by another pathway from CDP digliceride only.

Since a significant part of the PL molecules is composed of fatty acids, the study of fatty acids incorporation in the phospholipids is more appropriate to trace the biosynthesis of this molecule (Chapelle, 1986). Most of the works concerning this have been made using acetate as the radio tracer, because fatty acids are believed to be synthesised from acetate the basic precurssor (Kanazawa and Teshima, 1977; Kanazawa *et al.*,1979b). O'Conner and Gilbert (1968, 1969) have demonstrated 14C-1 acetate uptake by PL in crayfish *O.virils* and crab *G.lateralis*. In *Homarus americanus* Shiek (1969) observed the incorporation of 14-C-1 acetate and other longer chain fatty acids. Similarly Whitney (1974) and Farkas and Nevenzel (1981) found that extracts of phospholipids from the hepatopancreas and gills of crab *Callinectes sapidus,Libnia emerginatus* and crayfish *P.clarki* are labelled from acetate. In contrast to this, Allen(1972) and Morris and Sargent (1973) indicated low radioactivity in total PL when analysed after feeding or directly injecting the C14 palmetic acid. Regarding the incorporation of unsaturated and long chain PUFA into crustacean phospholipids only sporadic information is available. Kanazawa *et al.* (1979 g) pointed out that 1-C14 linolenic acid is converted to 20:5 $\omega 3$ and 22:6 $\omega 3$ fatty acids and moderately incorporated into PL of the shrimp *Penaeus japonicus*. In crab *Carcinus maenas*, Chapelle *et al.* (1985) also found C14 linolenic acids in the phosphatidylinositol (PI) among the PL fraction.

Another component which consitute PL is glycerol. Studies conducted using 14C or 3H-glycerol demonstrated the uptake of glycerol into PL. O'Connor and Gilbert(1969) proposed that the major portion of the label from glycerol appears in phospholipids and suggested a possible formation of PL from glycerol. A similar uptake of glycerol at the levels of

PC and PE in intact lobster *Homarus americanus* was reported by Shiek (1969). In addition to this there are evidences supporting that crustacean ovary is capable of synthesizing PC and PE from labelled 3H-glycerol (Lui *et al.*, 1974).

Studies using specific precursors like choline, inositol, ethanolomine etc. precisely explains the pathway of synthesis of various phospholipids. Utilization of methyl 14-C-coline for PC formation was first demonstrated in the crab *Cancer magister* and lobster *Homarus americanus* by Bilinski (1962) Shiek (1969) also showed the incorporation of methyl 14C-choline and methyl 14C phosphorylcholine into PC of lobster *H.americanus.* However, the alternative pathways for the synthesis of PC and PE in crustaceans was described by Brichon, *et al.* (1980) and Chapelle *et al. (1981, 1982).* According to their studies the main phosphotide PC and PE are synthesized directly from 14C-choline and 3H-elthanolamine bases respectively. Chapelle *et al.* (1982) while studying the synthetic pathway of PL observed significant incorporation of 3H-ethanolamine into PC from hepatopancreas of *Carcinus maenus* and suggested that via N-methylation process PE got converted into PC in this organism. Evidence for the conversion of PS to PC via PE was also reported in other crab species. Chapelle and Zwingelsten (1984) who experimented with radio active serine concluded that PS synthesised from labelled serine in the hepatopancreas of crab, *Eriocheir sinensis*, gets transformed into PE by decarboxylation process first and finally to PC by N-methylation pathway. On the contrary, sphingomyelin (SP) is formed directly from phosphorus, fatty acid, serine and choline (Chapelle, 1986).

The literature cited above indicates that PL formation in crustacea requires phosphatidic acid (PA) as an essential precursor of glycerophospholipids and two principal routes of synthesis of phsopholipids exist from PA; a pathway synthesizing PI via CDP-diglyceride

and a direct route forming PC and PE from 1,2 diglyceride and CDP bases while, SP formation takes place with the addition of serine and choline to PA.

2.2.2.2. Nutritive aspect of phospholipids

Dietary phospholipid plays a critical role in crustacean nutrition. It is essential for growth, survival, absorption and transport of dietary lipids, reproduction and enzymatic activity, and is supposed to involve in many other metabolic functions as well.

2.2.2.2.1 Phospholipid requirement for growth and survival

Recent studies in crustacean nutrition have pointed out phospholipids as a most important dietary factor for sustaining growth and survival, specifically during the early stages (D'Abramo, 1989; Wang *et al.,* 1992; Kanazawa, 1993; Teshima, *et al.*, 1993; Camera *et al.*, 1993; Tackaert *et al.*, 1991 a,b; Kanazawa and Koshio, 1993). The study which shed light on phospholipid requirement of crustacean was that of Kanazawa *et al.* (1977 a) in which they noticed that the weight gain of *Penaeus japonicus* was higher when reared on a diet supplemented with short necked clam lipid rich in PL (more than 65% of total lipid). To clarify this Kanazawa *et al.* (1979a) isolated and purified the phospholipid fraction from short necked clam and included in the diet at a level of 1% alongwith 7% of pollack liver oil. Following this, several studies were initiated and demonstrated that dietary source of PL is very essential for normal growth and survival of crustaceans like lobsters (Boghen and Castell, 1980; Conklin *et al.*, 1980; D'Abramo *et al.*, 1980; Tridel and Castell, 1980; Kanazawa and Koshio, 1993) and penaeid shrimps (Kanazawa, 1982; Pascual, 1985, 1986; Chen,1993; Kompiang,1992; Camera *et al.*, 1993; Briggs *et al.*, 1994; Chandge and Raja, 1993, 1995).

TABLE - III

Phospholipid requirements of crustaceans

SPECIES	REQUIREMENT	REFERENCE
Penaeus japonicus	1% purified Tapes lecithin or soyabean lecithin	Kanazawa *et al.* (1979a) Kanazawa (1982)
	6% soyabean PC	Kanazawa *et al.* (1985)
	3% soyabean lecithin	Teshima *et al.* (1986a)
	3% soyabean PC and PI	Teshima *et al.* (1986d)
	1.5% purified PC	Camara *et al.* (1993)
	> 2% PC	Tackaert *et al.* (1991b)
Penaeus monodon	0-2% soylecithin	Pascual (1985; 1986)
	Soylecithin	Meyers (1989)
	1.25% PC of 80% purity	Chen (1993)
	1% phospholipid	Kompiang (1992)
	3% soylecithin	Briggs *et al.* (1994)
Penaeus chinesis	Level not mentioned	Zhou and Wang (1992)
	1-2% soylecithin containing PC and PI	Kanazawa (1993)

[Contd.....]

Penaeus indicus	1.5% soylecithin 2% soylecithin	Ali (1990)
	2% soylecithin	Chandge and Raj (1995)
Penaeus penicillatus	< 1.25% PC	Chen and Jenn, (1991)
Macrobrachium rosenbergii	> 0.5%	Briggs *et al.* (1988) Hilton *et al.* (1984)
	0-2%	Koshio *et al.* (1992)
Homarus americanus	4% lecithin	Boghen and Castell (1980)
	4-6%	Tridel and Castell (1980)
	8%	Conklin *et al.* (1980)
		Bowser and Rosemark (1980)
	8% prurified lecithin	Baum *et al.* (1990)

Table III represents the requirement of phospholipid in the diet of penaeid shrimps, freshwater prawn and lobster. The data suggest that precise requirement of PL is yet to be established in these species of crustaceans. Among the species mentioned in the Table, the requirement fluctuates between 0 and 8% of the dry weight of the feed. In the same species itself wide differences are noticed; for instance in *P. japonicus* larvae Kanazawa *et al.* (1985) observed that 6% soybean PC was essential for growth and survival whereas, Teshema *et al.* (1986 c) reported 3% soyabean PC and PI combination can satisfy the requirement. Tackaert *et al.* (1991a) attributed this disparity to the unspecified composition and purity of the phospholipid source used for the experiments. In many of the feeding trials phospholipids of unspecified composition such as crude lecithin had been used for estimating the requirement (Conklin *et al.*, 1980; Tridel and Castell, 1980; Bowser and Rosemark, 1980; Kanazawa *et al.*, 1985; Pascual, 1985,1986; Teshima *et al.*, 1986 c; Meyers, 1989 ;Zhou and Wang, 1992) . Besides this,D'Abramo (1989), Tackaert *et al.* (1991 a) and Camara *et al.* (1993) proposed that the requirement of phospholipid decreases with increasing age of the prawns. This may be the reason why Teshima *et al.* (1986 c) reported 3% of purified PC and PI for larval *P.japonicus* and 1.5% soylecithin for postlarvae reported by Kanazawa *et al.* (1982 a). In addition to this D'Abramo (1989) while reviewing lipid requirement of shrimps suggested a species specific requirement of various lipid classes. As a result , based on the available literature it is very difficult to draw a general conclusion regarding the optimal level of phospholipid needed in the diet .

In spite of this, it has been found that availability of phospholipids from the feed is very critical during the larval stages of penaeid shrimps (Teshima *et al.*, 1982 ; Kanazawa, 1983; Kanazawa *et al.*, 1985; Teshima, 1986 c).

Teshima *et al.* (1982) by using a microparticulated diet containing carragenin as a

binder noticed that phospholipids in the diet is indispensible for growth and survival of larval *P.japonicus*. Deficiency of dietary PL caused 100% mortality of the larvae prior to the metamorphosis from zoea 1 to zoea 2 (Kanazawa *et al.*, 1985;Teshima *et al.*, 1986 c). For *P.japonicus* larvae best growth and survival rate was reported when 6% soyabean phosphatidylcholine was added to the diet (Kanazawa *et al.*, 1985); whereas Teshima *et al.* (1986c) stated that 3% PC and PI was the appropriate level of PL for efficient growth and maximum survival. In the case of the juvenile and postlarval shrimps 3% is the optimum level if the source was commercial grade soylecithin (Teshima *et al.* 1986 a) and only 1-1.5% was sufficient if the PL source was highly purified PC (Tackaert *et al.*, 1991b; Camara *et al.*,1993). Pascual (1985, 1986) stated that *P.monodon* showed increased growth and feeding efficiency when lecithin level in the diet was increased from 0 to 2%. The absence of PL in the feed resulted in the poorest performance in growth of *P.monodon* and addition of soylecithin or purified phospholipid at the rate of 1-3% improved growth and feeding efficiency (Kompiang, 1992; Briggs *et al.*, 1994). Other penaeid shrimps such as *P.chinensis* (Zhou and Wang, 1992; Kanazawa, 1993), *P.penicillatus* (Chen and Jenn, 1991) and *P.indicus* (Ali, 1990; Chandge and Raj, 1993 , 1995) generally require 1-2% PL for supporting optimum growth and survival.

Freshwater prawn *Macrobrachium rosenbergii* requires only very less quantity of PL in the diet, probably less than 0.1%. Though Hilton, *et al.* (1984) noticed no additional benefits for the supplementation of soylecithin in the diet, a trend towards enhancement of growth rate was observed by Briggs *et al.* (1988) when the lecithin content was increased from 0-5%. Koshio *et al.* (1992) while evaluating the effect of protein source and rearing method stated that inclusion of 0-2% of soybean lecithin showed no significant effect on communally reared prawns having 800mg size, but influence was noticed in individually reared early post larvae of 50mg size. The finding indicates that in *M.rosenbergii* phospholipids are essential only during the early

phase of growth. In support of this statement a decreasing tendency of PL requirement with aging of shrimp was reported in *Penaeus japonicus* by Camara *et al.*(1993) when they were reared in two successive phases of 18 and 26 days.

Most of the works concerning the PL requirement of freshwater prawns are conducted in aged postlarvae and juveniles. In the present study two week old postlarvae were selected as the experimental animals in order to see whether phospholipids are required during the early growth periods.

Among the various classes of phospholipids, phosphatidycholine PC is the most essential one from the nutritional point of view in crustaceans (Sargent, 1976). Kanazawa, *et al.* (1985) opined that the efficiency of phospholipids in promoting growth and survival varies with the kind and source of PL. They found that bonito egg PC, soybean PC and PI had high efficiency than chicken egg PC for *P.japonicus.* This was further confirmed by Teshima *et al.* (1986c) in the same species. Recently Camara *et al.* (1993) also obtained similar results. In this experiment it was concluded that prufied egg PC or soy PC at a rate of 1.5% is equally effective to 6.5% soy lecithin. Commercial grade soyabean lecithin is composed of approximately 30% PE, 23% PC, 18% PI and other components (Chen and Jenn, 1991). According to Kanazawa *et al.* (1985) the growth promoting components in 40% pure soylecithin is 23% PC + 18% PI. Purified PC thus should be more than twice as effective as crude soylecithin in acclerating growth. Due to the non availability and the cost of purified phospholipids most of the data published so far used low purity phospholipid source. This inconsistency of the PL quality is another factor which make the comparison between different studies difficult.

Evidence suggesting the superiority of PL was demonstrated in several other species of shrimps. While studying the combined effect of dietary PC and cholesterol in *Penaeus penicillatus* Chen and Jenn (1991) stated that 1.25% of PC in the diet was sufficient to bring about good growth rate. Chen (1993) and Kompiang (1992) observed better growth rate in *P. monodon* at 1.25% and 1% of purified PC and PI respectively in the diet. Experiments of Kanazaw (1993) and Zhou and Wang(1992) with *P. chinensis* and Tackaert *et al.* (1991b) with *P. japonicus* also showed that PC at lower concentration (>2%) seemed highly benificial than crude lecithin in acclerating growth.

Investigations of Boghen and Castell (1980), Conklin *et al.* (1980), Tridel and Castell (1980) and D'Abramo *et al.* (1981) indicated that phospholipids in the diet is critical and its absence reduces growth and survival rate significantly in juvenile lobster *Homarus americanus*. Conklin *et al.* (1980) and D'Abramo *et al.* (1981) found the survival rate of lobster was remarkably improved by the addition of 8% soyabean lecithin to the diet. Tridel and Castell (1980) showed increased survival and growth rate by the addition of 4-6% lecithin in a casien based diet. Incidence of death like molt death syndrome was considerably brought down by feeding lecithin supplemented diet to *Homarjus americanus* juveniles (Bowser and Rosemark, 1980). In contrast to this Kean *et al.* (1985) explained that the absence of dietary lecithin is not exclusively related to low survival and growth retardation in this species when crab protein was used instead of casein. Recently Baum *et al.* (1990) conclusively proved that inclusion of lecithin to a level of 8% is a must for normal growth and survivial of American lobsters irrespective of the protein source. Requirement of dietary source of PL similar to *Homarus americanus* was demonstrated in spiny lobster *Panulirus japonicus* also (Kanazawa and Koshio, 1993).

Effect of PL on growth promotion and enhancement of survival in crustacean was due to various reasons. Chandge (1987) proposed that addition of lecithin in the diet may provide, choline, which act as a methyl donor, during trans-methylation reaction and thereby spare sulphur containing amino acid, methionin for protein synthesis. Teshima, and Kanazawa (1979, 1980), Teshima *et al.* (1986a,b), D'Abramo *et al.* (1982, 1985 a) and Baum *et al.* (1990) attributed that the increment in growth and survival was due to the active utilization and absorption of dietary lipids by the PL ; whereas Chen and Jenn (1992) proposed that water stability of feed incorporated with PL is more compared to the control and suggested this increased water stability of the feed as the major reason for the increased growth and survival rates.

2.2.2.2.2. Role of phospholipid in lipid absorption and transport mechanism.

Compared with the blood lipids of other animals, crustacean haemolymph lipid are generally rich in phospholipid (Bliggs and Scott, 1966; Teshima and Kanazawa, 1978b). Presence of this high concentration of phospholipids in the haemolymph paved the way to understand the lipid absorption and transportation mechanism in crustaceans. To clarify the role of PL in crustacean haemolymph several studies had been conducted in shrimp *P.japonicus* (Teshima and Kanazawa, 1979, 1980; Teshima *et al.,* 1986b) and lobster, *Homarus americanus* (D'Abramo *et al.,* 1982, 1985 a) and *Nephrops norvegicus* (Dall, 1981). Radio tracer experiment of Teshima and Kanazawa, (1979) showed that dietary free fatty acid, absorbed at the hepatopancreas and hindgut are resynthesised into phsopholipid and released into the haemolymph. On the other hand, in mammals dietary lipids which are mainly absorbed at the intestine are resynthesised into tryglycerides TG and released as chylomicron rich in TG into the lymph (Bliggs and Scott, 1966). Using the same labelling pattern Teshima and Kanazawa (1980) further demonstrated that the dietary lipids are incorporated in to the phospholipids of

lipoprotein complexes like HDL2 (High density lipoprotein 2), HDL3 (High density lipoprotein 3) and VHDL (very high density lipoprotein) in *P.japonicus* and suggested that lipid transport mechanism in crustacean are carried out by PL associated with high density lipoprotein. In addition to this PL is involved in the retention and utilization of dietary lipids. Teshima *et al.* (1986 b,d) while evaluating the dietary role of PL found that retention of neutral lipids like TG and cholesterol was higher in the body of shrimps fed 3% soyabean lecithin than the group fed with lecithin deficient diet. In American lobster *Homarus americanus* D'Abramo, *et al.* (1982; 1985 a) reported that the absence of dietary source of PC restrict the movement of cholesterol from hepatopancreas to the haemolymph and indicated that effective utilization of cholesterol is a must for the survival of lobsters. A similar observation was made by Baum *et al.* (1990) and opined that impaired cholesterol movement may not be a casual factor for the mortality of lobsters fed diets without lecithin.

On the basis of these observations it can be concluded that phospholipids are very essential for the absorptioon, utilization and interorgan transport of dietary lipids which in turn effect growth and survival of crustaceans.

2.2.2.2.3 Role of phospholipids in moulting and moult related abnormalities

According to the information provided by Kanazawa *et al.* (1979 a) and Conklin *et al.* (1980) it is believed that phospholipids are a necessary dietary factor influencing the exoskeleton formation in crustaceans. Many authors reported that phospholipids are essential for successful moulting and to reduce the incidence of moult related abnormalities in crustaceans. Bowser and Rosemark (1980) indicated that lack of lecithin in the diet can considerably increase the moult associated mortalities in lobster *Homarus americanus* (Moult

death syndrome). Wickins (1981) cited by and Brock (1983) have described an apparently similar condition and designated as Exuvia entrapment disease (EED) in shrimp *Palaemon serratus*. According to Brock (1983) this symptom primarly occurs in the 11th larval stage or early postlarval stages of *Macrobrachium rosenbergii* and the affected animals die during the process of metamorphosis moult as a result of entraping the old exuvia or due to the deformity in appendages occured at the time of moulting. Lecithin supplemented feeds were found beneficial in reducing this abnormal mortality. Although the exact role of PL in these exoskeletal abnormality was not clearly stated, the role of PL in the absorption utlization and transportation of fat soluable nutrients especially cholesterol has been implicated as one of the reasons for this phenomenon. (Teshima and Kanazawa, 1979, 1980 ; Teshima *et al.*, 1986 b,d; D'Abramo *et al.*,1982, 1985). D'Abramo *et al.* (1982) and Teshima *et al.* (1986 b,d) have shown that dietary phospholipid deficiency in homarid lobster and penaeid shrimp reduces the availability of cholesterol in the haemolymph. Since crustaceans cannot synthesis cholesterol (Teshima and Kanazawa, 1971 a; Teshima, 1982) the drop in haemolymph cholesterol is believed to affect epidermal membrane synthesis indirectly by limiting the synthesis of moulting hormone , ecdysteroid for which cholesterol is a precursor (D'Abramo *et al.*, 1982). Mortality asscociated with incomplete ecdysis in the crayfish *Orconectes virillis* was suggested to be the result of an imbalance in moulting hormone and molt inhibiting hormone (Aiken, 1969). D'Abramo *et al.* (1982) further explained that the absence of effective cholesterol transport mechanism caused by the deficiency of PL in the diet may be reponsible for the moult death syndrome as described by Bowser and Rosemark (1980) in *Homarus americanus*.

Later D'Abramo *et al.* (1985 a) in another study opioned that since only a small amount of ecdysteroids are associated with sucessful moulting the reduced amount of cholesterol does not act as a limiting factor on the hormone precursor. However, Petriella (1990) observed that

by increasing the cholesterol content of the diet moulting frequency can be increased in Argentine Prawn *Artemesia longinaris* and she suggested that cholesterol is the starting molecule for the synthesis of moulting hormone and its availability is critical for the moulting of crustacean. Baum *et al.*(1990) also attributed that impairment in stored cholesterol movement due to the deficiency of PL in the diet was the main reason for large scale mortality of 4 - 6 stage lobster, *H.americanus.*

As per the available literature it is not clear whether dietary PL play an active role in the process of moulting in crustaceans. Considering the low biosynthetic ability of PL in the early growth stages (Shiek, 1969) and involvement of PL in the cholesterol utilization (D'Abramo *et al.*, 1982; Teshima *et al.*, 1986 b,d) the possibility of PL indirectly influencing the moulting in crustacean cannot be ruled out.

2.2.2.2.4. Role of phospholipid in ovarian maturation

Lipids, in particular n3 fatty acids and phospholipids are the nutrients which are supposed to have a very great influence on the reproductive performance and egg quality of crustaceans (Middleditch *et al.*, 1980; Teshima and Kanazawa, 1983; Bray *et al.*, 1990). Primavera (1985) while reviewing the maturation aspects of penaeid shrimps indicated the importance of phospholipid in the ovarian maturation.

The gonadal development induced through eyestalk ablation occured only if tissue reserves of PL was adequate in *P.japonicus* (Teshima *et al.*, 1988b). Millamena (1989) and Cahu *et al.* (1994) obtained complete gonadal development and spawning performance in *P.monodon* and *P.indicus* when the diet supplemented with PL was fed to the animals. Through

another feeding study Alava *et al.* (1993) concluded that *P.Japonicus* requires phospholipid for ovarian growth and that PL is responsible for the increase in the ovarian lipid content.

Phospholipid not only influences ovarian growth and egg production but also is essential for successful spawning and hatching of eggs. Chau *et al.* (1994) suggested that a concentration of 2% PL in the diet is essential to increase spawning frequency and egg hatching in *P.vannamei.*

Phospholipids are essential for the formation of vitellogenin (Kanazawa, 1985). Vitellogenin is a lipoprotein complex synthesised in the hepatopancreas and used by the oocyte to constitute the yolk during ovarian development in crustaceans (Meussy, 1980). The lipid moiety of this vitellogenin consist of phospholipids like PL and PI containing high levels of PUFA has been stated by Kanazawa *et al.* (1985). The incorporation of vitellogenin into the oocytes was evident with the high rate of transference of lipid from hepatopancreas to ovary during maturation process with TG and PC as the major class (Teshima *et al.*,1988b; Alava, *et al.,* 1993).

2.2.2.3 . Adaptive role of phospholipids

Aquatic medium is described as dynamic in nature due to the wide fluctuation in physico- chemical parameters like termperature, salinity, pH, etc. In order to cope up with the changes in environmental conditions the organisms living in it possess certain adaptive mechanisms, and phospholipids are believed to play a very critical role in some of these phenomena (Chappelle, 1986).

Generally, it is stated that survival of crustaceans is a function of their ability to adapt successfully to various external temperatures (Chapelle, 1986). According to the finding of Farkas *et al.* (1984) membrane phospholipids are involved in these adaptive changes towards environmental temperatures. Cossino (1976), Chapelle *et al.* (1979) and Chapelle (1986) co-relate unsaturation of fatty acids present in the phospholipids of crayfish, *Austropotamabrus pallipes* and crab *Carcinus maenas* to describe this phenomenon. They indicated that the unsaturation of fattyacids in the muscle and gills phospholipids rises with the drop in environmental temperature. Further, Farkas and Nevenzel (1981) found significant incorporation of 14C - acetate into long chain PUFAS of total phospholipids of *P.clarki* and explained that degree of unsaturation of fatty acids in PL increases with decrease of temperature. These data suggest that at lower environmental temperature the degree of unsaturation of fattyacids in the PL increases and helps the membrane to maintain the proper physical properties.

Apart from this fatty acid mechanism, quantitative modification in different phosphatides is noticed in crustaceans according to the changes in environmental temperature. Chapelle *et al.* (1977, 1981) and Brichon *et al.* (1980) found that PC was the more abundant PL in crab, *Carcinus maenas* adapted to higher temperature whereas PE fraction contributed to greater percentage in animals adapted to lower temperature of $13^{o}C$.

Velocity of biosynthesis of certain PL is also affected by thermal stress. An increased incorporation of 32P into PC and SP (Spihingomyelin) accompanied by a negligible label entry into PE was noticed in warm acclimated crab *Carcinus maenas* (Chapelle *et al.*, 1979). Influence of thermal stress on the uptake of specific precursor like 34 - ethanolamine and 14 C - choline into PE and PC was analysed by Chapelle *et al.* (1982). The result demonstrated that

acclimation temperature does not affect ethanolamine incorporation into PE, while a rise in choline uptake into PC was noticed with the rise in temperature in the crab, *Eriocheir sinensis*. Beside this Chapelle *et al.* (1982) also reported that the stepwise methylation of PE to PC is greater in crustaceans living at higher temperature. According to these observations it is seen that in crustacea phospholipids are involved in maintaining the fluidity of cell membrane with the changes in environmental temperature.

Unlike temperature, phospholipid concentration in the body is not significantly modified by the salt concentration of the medium (Chapelle, 1986).

But the role of PL in the biomembrane structure related to ion permeability and enzymatic activity has been evident in crustaceans. In an euryhaline species of crab, *Eriocheir sinensis* acclimated to a dilute medium Pe'queux and Chapelle (1982) and Pe'queux *et al.* (1983) noticed variations in the level of PL concentration in the branchiae, especially posterior, compared to other tissues. Assuming that the posterior gills are responsible in Chinese crabs for the control of ions haemolymph level in freshwater (P'equeux and Gilles, 1981) the changes in PL concentration in the branchiae are essentially involved in the regulation of ionic movements with respect to the salinity of the media. Using 14 C - acetate a significant rise in PE and PS biosynthesis in the gills of *C.sapidus* following dilution has been reported by Whitney (1974). The incidence of increased biosynthesis of PS in the gills of crab *Eriocheir sinensis* and *Carcinus maenas* was associated with high Na^+_+ K^+ATPase activity(an enzyme system responsible for the transport of ion across the cellular membrane) and stablizes the internal organisation of the body with respect to the salinity of the medium (P'eqeux and Gilles, 1981; P'eqeux and Chapelle, 1982 and Chapelle and Zwingelstein, 1984).

The data presented suggest that phospholipids especially PS are involved in osmoregulatory function in crustacea during the fluctuation of environmental salinity.

2.2.3. Cholesterol and other sterol requirement

Like other arthropodes, crustaceans are unable to synthesis sterols *de novo* from precursors like acetate and mevalonate (Zandee, 1966, 1967; Teshima and Kanazawa, 1971a; Douglas *et al.*, 1981; Teshima, 1982; D'Abramo *et al.*, 1984). Therefore sterol is considered to be an essential nutrient supplied through the feed for the growth and survival of crustaceans. From the nutritional point of view among the sterols, the most important one is cholesterol since it is belived to be involved in many metabolic functions (Paulraj, 1987) and acts as a starting molecule for the synthesis of number of compounds like hormones and vitamins (Sheen *et al.*, 1994).

Acording to D'Abramo *et al.* (1984) crustaceans derive cholesterol either directly from the diet or via the metabolic conversion of other sterols present in the diet. Brine shrimp, *Artemia salina* (Teshima and Kanazawa, 1971a,b), penaeid shrimp, *Penaeus japonicus* (Teshima and Kanazawa, 1973) and the crab *Portunus trituberculatis* have been shown to possess this conversion ability. In spite of this, experiments of Teshima *et al.* (1983, 1989) and Teshima and Kanazawa (1986, 1987) to evaluate the nutritive value of different sterols reveal that cholesterol is superior to others in larval and postlarval development.

One of the important functions of cholesterol in crustacean is that it act as a precursor for various physiologically important compounds like sex hormones, moulting hormone,adrenal croticosteroids, bile acids and vitamins (New, 1976; Sheen *et al.*, 1994). It is also essential for

the elaboration of new subcellular membrane associated with rapid growth and moulting (Biddle, 1977; Petriella, 1990).

In spiny lobster *Panulirus japonicus* evidence of the conversion of exogenous cholesterol to steroid hormones such as progesterone, 17 α hydroxyprogesterone, androsteredione and testosterone was pointed out by Kanazawa and Teshima (1971). Lanchaise *et al.* (1993) in their review of moulting gland of crustaceans suggested that Y - organ secrets three different moulting hormones (ecdysteroids). The *in vitro* studies of Rudolph *et al.* (1992) and Rudolph and Spaziani (1992) in Xanthid crab *Menippe mercenaria*, also explain that Y - organ forms 7 dehydrocholesterol from cholesterol and it is then converted into secreation products like 3 dehydroxy ecdysterone and 25 deoxyecdyson without accumulation of other intermediates; whereas Kleinholz (1985) isolated 4 ecdysteroid, which are supposed to be the intermediate between cholesterol and ecdysone from the ovary of crab *Carcinus maenas*.

In the light of these evidences it has been recognised that the presence of higher levels of cholesterol in the diet increases the moulting rate. Feeding experiment of Petriella (1990) in Argentine prawn *Artemesia longinaris* confirms this hypothesis. She found that the diet supplemented with 1.3 to 2.1% cholesterol had increasing moulting frequency than lower levels in this species of prawn . Castell *et al.* (1975) in lobster *H.americanus* also reported higher moulting rate in 1% cholesterol supplemented diet.

Importance of cholesterol in sustaining growth and survival had been studied in several species of crustaceans. As per the available literature the cholesterol requirement of different groups of crustaceans is presented in the Table IV .

TABLE - IV

Cholesterol requirement of crustaceans

SPECIES	REQUIREMENT	REFERENCE
Penaeus japonicus	0.1% - 0.2%	Shudo *et al.* (1971) cited by Sherief *et al.* (1992)
	0.5%	Kanazawa *et al.* (1970, 1971), Teshima (1982), Teshima *et al.* (1983, 1989), Teshima and Kanazawa (1986).
	2.1%	Deshimaru and Kuroki (1974)
Penaeus monodon	0.2 - 0.8%	Sheen *et al.* (1994)
	0.5%	Chen (1993)
Penaeus indicus	0.5 - 1	Ramesh and Kathirasan (1992)
Penaeus kerathurus	1%	Bianchini (1984)
Penaeus penicillatus	0.5%	Chen and Jenn (1991)
Artemesia longinaris	1.3 - 2.1%	Petriella (1990)

[Contd.....]

Macrobrachium rosenbergii	0.12 - 0.5%	Sherief *et al.* (1992) Briggs *et al.* (1988)
Homarus americauns	0.12 - 0.5%	Castell *et al.* (1975), D'Abramo *et al.*(1984), Bordner *et al.* (1986), Kean *et al.*(1985)
Pacifastacus leniuusclus	0.4 - 1%	D'Abramo *et al.* (1985b)
Carcinus maenas	1.4 - 2.1%	Ponat and Adelang (1983)

The requirement ranges from between 0.1 to 2.1% in various groups. Among the crustaceans most of the studies regarding the cholesterol requirement was carried out in penaeid shrimps and lobsters. Optimum dietary cholesterol for supporting growth and survival was reported to be 0.5% in penaeid shrimps like *P.japonicus* (Kanazawa *et al.*, 1970, 1971; Teshima, 1982; Teshima, *et al.*, 1983; Teshima and Kanazawa, 1986), *P.monodon* (Chen, 1993) and *P.penicillatus* (Chen and Jenn, 1991). But species like, *Penaeus kerathurus* (Banchini, 1984), *Artemesia longinaris* (P'etriella, 1990) and *P.indicus* (Ramesh and Kathiresan, 1992) reported a higher requirement of 0.5 to 1%. There are also reports contradictory to this. Shudo *et al.* (1971) as cited by Sherief *et al.* (1992) proposed a level as low as 0.1 to 0.2% as satisfactory for good growth and survival of *P.japonicus*, whereas Deshimaru and Kuroki (1974) observed a higher level of 2.1% (dry weight) in the same species. Sheen, *et al.* (1994) also noticed a higher requirement of 0.8% for *P.monodon*. Sherief *et al.* (1992) and Briggs *et al.* (1988) using a semipurified diet pointed out that the level of supplementary cholesterol for *M.rosenbergii* ranges between 0.12 and 0.5% of the dry weight of the diet. According to Castell, *et al.* (1975), D'Abramo *et al.* (1984) and Bordner *et al.* (1986) the requirement of cholesterol for American lobster varies between 0.12 and 0.5%; while Kean *et al.* (1985) proposed a wider range of 0.25 to 1%. Similarly, freshwater crayfish *Pacifastacus leniusculus* and crab *Carcinus maenas* have been reported to require 0.4 to 1 and 1.4 to 2.1% cholesterol respectively in the diet.

Sheen *et al.* (1994) attributed that the differences in the design of experimental diet was the main reason for the lack of agreement with the result of various studies. Thus the effective dietary level of 2.1% of cholesterol reported in *P.japonicus* (Deshimaru and Kuroki, 1974) and in *Artemesia longinaris* (Petreilla, 1990) was very likely the level required for the dietary formulation used by them. Studies by D'Abramo *et al.* (1982,1985a), Teshima and Kanazawa (1983), Teshima *et al.* (1986a,b) and Baum *et al.* (1989) have shown that digestion,

absorption, assimilation and utilization of cholesterol from the diet in crustacea is affected by presence of PL in the feed. This can also be a reason for the variation observed in this studies. Castell *et al.* (1975) in *H.americanus*, Chen (1993) in *P.monodon* and Chenn and Jenn (1991) in *P.penicillatus* found higher levels of cholesterol requirement in the absence of lecithin. But Kean *et al.* (1985) irrespective of lecithin levels in the diet suggested that 0.25% of cholesterol was optimum for *Homarus americanus*. Therefore, the physiological effect of PL on the cholesterol requirement of crustacea needs further research.

3. MATERIAL AND METHODS

Macrobrachium rosenbergii postlarvae used in the experiment were obtained from the Freshwater Prawn Hatchery of the College of Fisheries. All the experimental animals were obtained from a single batch and were supposed to have a known nutritional history. The larvae were reared in the hatchery in 1.2 tonne capacity fibre glass tanks coupled with biofilters using a clear water recirculating system. The feeding schedule and rearing techniques of the larvae were similar to those reported by Nair and Thampy (1987), Nair *et al.* (1989) and Sebastian (1990).

3.1.0 Conditioning of experimental animals

Prior to the initiation of the experiments, the postlarvae were conditioned for a period of one week on artificial diets. When the PL have settled down completely, they were slowly acclimatized to freshwater conditions and maintained in a 1.2 tonne tank at a density of $500/m^2$. The prawns were fed with a pelletized diet twice daily, comprising clam meat as the major protein source. During the course of this conditioning procedure, the uneaten feed, excreta, and other waste materials settled at the bottom were siphoned out daily morning before giving feed and 50% water exchange was resorted. Tank was filled only to half the capacity. Aeration was provided through diffuser stones to keep the dissolved oxygen levels within the optimum range. In addition, some dry submerged twigs were kept submerged inside the tank to offer shelter for the newly moulted individuals and to reduce cannibalism among the prawns.

3.2 Experimental rearing facilities

The study was carried out in the *Macrobrachium* Hatchery of College of Fisheries. Circular flat bottom fibre glass tanks having the following specifications were used for the experiments.

Capacity	—	83 litres
Diameter	—	55cm
Height	—	35cm
Rim width	—	3cm
Thickness	—	4mm
Colour	—	Aquamarine

Tanks were housed inside the hatchery building which had provision for subdued light penetration (Plate I). Clear filtered fresh water drawn from a well was used for the experiment and the water was filled in the experimental tanks upto 75 litres capacity [20cm height] after subjecting to a fine filteration using nylon bolting silk. This level was maintained throughout the experimental period. Gentle aeration via diffuser stones and plastic tubes connected to the air distribution pipes of the hatchery was provided in the tanks and air supply was maintained in a uniform condition by using control valves.

Since it was reported that artificial substratum can considerably reduce cannibalism and increase survival rate of *Macrobrachium rosenbergii* during the nursery rearing phase (Smith and Sandifer, 1975; Ra'anan *et al.*, 1984; Gomez *et al.*, 1988) 20x20cm size flat, square shaped tiles were kept inside the tanks for this purpose. Tiles were placed in a slanting position over a small piece of stone (Plate II).

PLATE I

ARRANGEMENT OF EXPERIMENTAL TANKS

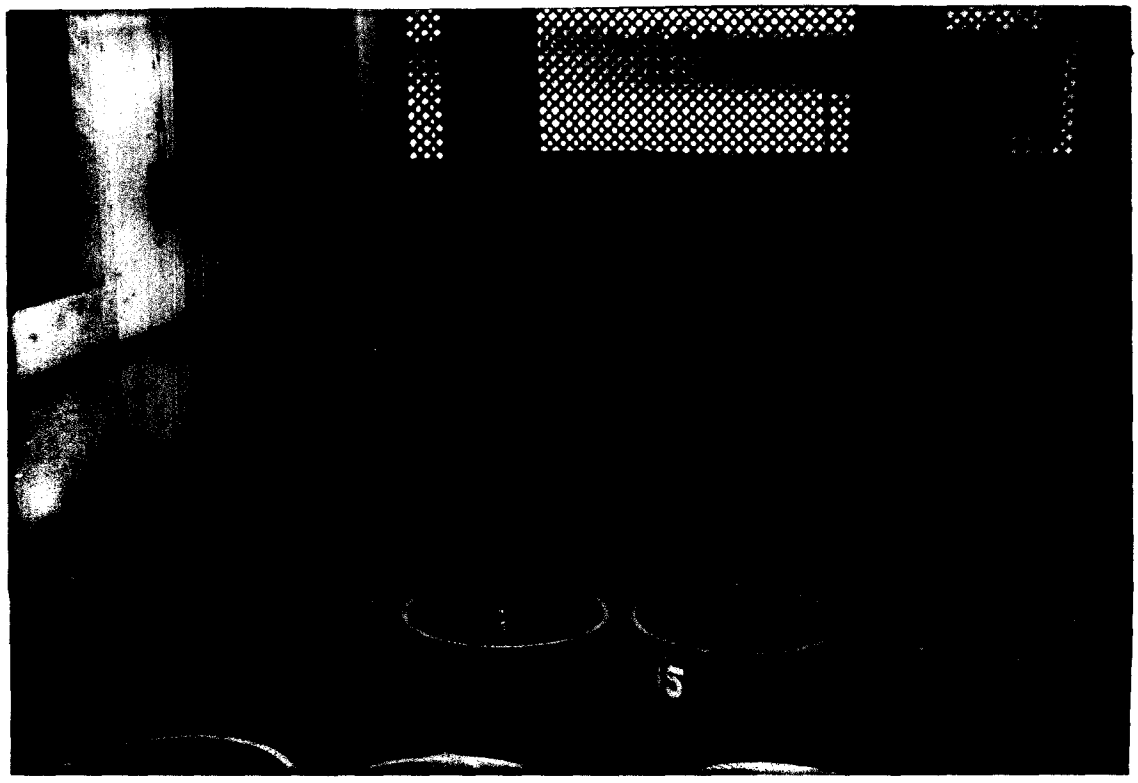


PLATE I

ARRANGEMENT OF EXPERIMENTAL TANKS

PLATE II

EXPERIMENTAL TANK USED FOR THE STUDY

3.3. Experimental diet formulation and processing

3.3.1 Diet formulation

On the basis of the diet formulated by Sherief *et al* (1992) for *Macrobrachium rosenbergii* postlarvae, five semipurified, isonitrogenous diets, incorporating different levels of soylecithin (Sigma chemicals, U.S.A.) and using casein and egg albumin as the protein source were prepared. The basal lipid content of 6% was combined with different levels of lecithin, viz., 0, 2.5, 5, 7.5 and 10 percentages. All other ingredients of the diet are similar to the formulation of Sherief *et al.* (1992)except the binder carageenin, which was replaced by carboxymethyl cellulose [CMC] and lipid source sunflower oil replaced by cornoil. The composition of the test diets are shown in Table V.

Fat free casein was used as the main protein source of the diets since it is available in highly purified form and found to be assimilated satisfactorily by *Macrobrachium rosenbergii* (Hilton *et al.*,1984; Briggs *et al.*, 1988). Polyunsaturated fatty acid, especially belonging to n3 and n6 series are very essential for the normal growth of *Macrobrachium rosenbergii* (Reigh and Stickney, 1989; D'Abramo and Sheen 1993). For satisfying this requirement a mixture of corn oil and cod liver oil was used as the lipid source.

Alpha corn starch was selected as the carbohydrate source because polysaccharides were found to be more efficiently utilized than monosaccharides by the prawns (Pascual *et al.*, 1983; Alava and Pascual, 1987).

Vitamin and mineral mixes used in the diet were prepared according to the composition and percentages given by Kanazawa *et al.* (1982) and Stahl and Aheran (1978) respectively.

Compostion of the test diets used for the study in percentage

Table - V

INGREDIENTS	DIETS				
	D1	D2	D3	D4	D5
CASEIN	31	31	31	31	31
EGG ALBUMIN	4	4	4	4	4
GLUCOSE	4	4	4	4	4
SUCROSE	4	4	4	4	4
ALPHA CORN STARCH	20.1	20.1	20.1	20.1	20.1
GLUCOSAMINE HYDROCHLORIDE	0.8	0.8	0.8	0.8	0.8
SODIUM CITRATE	0.3	0.3	0.3	0.3	0.3
SODIUM SUCCINATE	0.3	0.3	0.3	0.3	0.3
MINERAL MIXTUR	4	4	4	4	4
VITAMIN MIXTURE	4	4	4	4	4
COD LIVER OIL	2	2	2	2	2
CORN OIL	6	6	6	6	6
ALPHA CELLULOSE	14	11.5	9	6.5	4
LECITHIN	0	2.5	5	7.5	10
CHOLESTEROL	0.5	0.5	0.5	0.5	0.5
CARBOXY METHYL-CELLULOSE (CMC)	5	5	5	5	5

The studies of Briggs *et al.*(1988) and Sherief *et al.* (1992) reveal that cholesterol is an essential nutrient in the diet of *Macrobrachium rosenbergii*. Based on this cholesterol was included in the diet at the rate of 0.5%.

3.3.2 Diet preparation

Dry and crystalline ingredients of the diet were powdered seperately and sieved through a 250 micron sieve first. They were then accurately weighed in an electronic balance according to the percentage composition and mixed thoroughly in a Habot bowl mixer. Boiling water, around 350ml was then added to this and mixed well to get a dough like consistency. This was followed by the addition of vitamin mixture and oil and kneeded well for proper mixing. The dough was then extruded through a hand pellet mill using a 3mm die, collected in a tray and dried at 50^{o}c for 12hrs to a moisture content of <10%. These pellets were subsequently broken into pieces and sieved. Particles between 1 - 2mm diameter were retained, packed in air-tight containers and refrigerated at 4^{o}c until required.

3.4 Proximate analysis of the diet

Proximate composition of the diets was analysed to evaluate the nutrient status and the result is presented in Table XXV. All analysis were based upon the mean of three samples and were done according to the procedures of AOAC, 1980. The results are expressed in dry matter basis except for moisture content which was on feed basis.

[1] Moisture content: By drying the sample at 105^{o}c for 12hrs.

[2] Crude protein: By microkjeldahlas method

[3]	Crude fat:	By solvent extraction method using petroleum ether [B.P. 40-60^{o}c] in a soxhlet extraction apparatus for 6 hours.
[4]	Crude fibre:	By the method of Pearson (1976)
[5]	Ash:	By combustion at 450^{o}c for 12 hours.
[6]	Carbohydrate:	By difference in dry weight (Hasting, 1976)

3.5 Experimental design and procedure

Well pigmented healthy individuals of *Macrobrachium rosenbergii* postlavae were selected from the stock population and ten numbers of them randomly distributed to each of the 20 experimental tanks filled with water. (4 replications per treatment; total 5 treatments).

The respective treatments (feed with different levels of lecithin viz. 0, 2.5, 5, 7.5 and 10) were randomly allotted to each tank and animals in them were acclimatized to the diets for a week from the pelletized diet used for stock rearing. Before starting the trial, the prawns were starved for 24 hours with the aim of depleting the body reserves, blotted dry with tissue paper and weighed collectively from each tank using a electronic balance [SHIMADZU-LABROR-AEU-130V] with an accuracy of 0.0001g.

Average weight of the experimental prawns selected was 0.05386 $\pm$0.043g. The duration of the study was 56 days; during this period the prawns were subjected to growth assessment every fortnight.

The diets were given to the prawns in Petridish and fed *ad libitum* twice daily. Feeding schedules were fixed, one during the morning between 9.00 and 10.00 and the other during the evening between 5.00 and 6.00. The rate of feeding was 20% of the body weight during

the first 4 weeks and 10% for the next 4 weeks. Every day before giving the feed, unconsumed food remnants were collected prior to the first feeding and dried at 60^{o}c for estimating food conversion ratio. Similarly during the morning feeding hours, bottom and sides of the tanks were scrubbed well to prevent algal growth and excreta accumulation.

Moulting, mortality and abnormal syndroms were also monitored daily and recorded in charts maintained for this purpose. Experimental tanks were thoroughly scrubbed and cleaned with detergent prior to refilling during every growth assessment in order to restrict the build up of bacteria and other parasitic organisms.

While assessing the growth great care had been imparted to handle the prawns with minimum stress. Weighing was done in an electronic balance, similar to the procedure mentioned in the opening paragraph of this section after subjecting the prawns to starve for one day. This helped to get rid of the undigested feed remaining in the gut which might lead to erroneous results.

At the end of the feeding study, approximately 2g prawn sample was collected from each tank and analysed the body phospholipid content, after extracting the total lipids with 3:2 isoproponol hexane mixture. Spectrophotometric method of Zilversmith and Davis [1950] was employed for the estimation and optical density was measured at 660nm in SPECTRONIC-20 photometer.

3.5.1 Water quality measurement

Physiochemical parameters of the water filled in the tanks were monitored by the

following method.

1. Temperature - Measured with bulb thermometer with accuracy of $0.1^{o}c$
2. pH - Universal indicator solution.
3. Dissolved oxygen - Standard Winkler method.

Temperature and pH were measured daily and DO content once in a week. Three samples collected from randomly selected tanks were used for the estimation of DO during the study period.

3.6 Evaluation indices and criteria of the experiment

Performance of the prawn was measured in the following manner.

3.6.1 Growth rate

In the present study growth performance was measured in terms of specific growth rate [SGR] since it is a more refined and improved growth index than absolute weight gain or percentage growth rate (Hepher 1988).

$$SGR\% = \frac{\ln Wf - \ln Wi}{Tf - Ti} \times 100$$

where SGR% is the specific [relate] growth rate as percentage [assuming exponential growth] Wi is the initial weight of the experimental animal at the day Ti and Wf is the final weight at the

day Tf. In denotes logarithm [base e] (Briggs *et al.*, 1994).

SGR% were calculated for every 14 days interval. Similarly the weight gain also.

3.6.2 Feed efficiency

The efficiency of the different diets used in the trial was meausured by the most commonly used index, the feed conversion ratio [FCR]. It is defined as the dry weight of feed per unit wet weight gain.

$$FCR = \frac{\text{Feed intake on a dry matter basis}}{\text{Weight gain on wet matter basis}}$$

In the present study FCR was selected to evaluate the diets because it easily relates the quantity of feed and increase in weight to the cost of production of feed.(Chiu, 1989).

Phospholipid is believed to be a very expensive nutrient and determination of FCR in this experiment helps us to understand, whether the addition of phospholipid is beneficial or not in the practical diet of *Macrobrachium rosenbergii*. It also helps in the formulation of feed very economically.

3.6.3 Survival rate

Mortality of prawns during the course of the experiment was noted down for each replication of the treatment and survival rate was calculated with the termination of the prescribed period. Percentage survival rate was calculated as follows:

$$\text{Survival rate \%} = \frac{\text{Initial number of prawn - No: of dead prawns}}{\text{Initial number of prawns}} \times 100$$

3.6.4 Moulting rate

Moulting rate was estimated to study the influence of different levels of lecithin on the moulting frequency of experimental animals.

Moults noticed in each replication of the treatments throughout the experimental period were recorded and moulting rate was calculated according to Petriella (1990).

$$\text{Mouting rate [M]} = \frac{\text{Moult percentage}}{\text{Mean life of the group}}$$

where,

Moult percentage = m/n x 100

m = number of moults

n = initial number of animals.

The mean life of the group was calculated by adding the number of days each individual survived.

3.7 Statistical Methods

The experiment was carried out using a completely randomized design [CRD].

All indices, such a specific growth rate, food conversion ratio [FCR], survival rate, moulting rate and body phospholipid levels were subjected to comparison using Analysis of Varience (ANOVA, Snedecor and Cochran, 1968) and treatment difference studied at 1% level of significance. Pairwise comparison of treatment values were done using critical difference based on Students 't' test at 1% level of significance.

While analysing the percentage survival rates S.G.R. moulting rate and F.C.R. the observations were subjected to arc - sign transformation, in order to meet the theoretical requirement of analysis of varience (Snedecor and Cochran, 1968). The transformation was done using

$$Y = \text{Sin}^{-1} [x/100]^{0.5}$$

where 'Y' is the transformed value and 'X' is the calculated value of respective indices.

4. RESULT

Effect of different dietary levels of lecithin on *Macrobrachium rosenbergii* post larvae was evaluated in terms of growth rate [specific growth rate], survival rate, moulting rate, food conversion ratio [FCR] and variation in body phospholipid levels. Details of the observation made during the study period are discussed in the following sections.

The test diets with lecithin contents at 0, 2.5, 5, 7.5 and 10% levels are denoted as D_1, D_2, D_3, D_4 and D_5 respectively for convenience.

4.1 Effect of lecithin on growth rate

Growth rate was measured in terms of specific growth rate expressed in percentage.

Specfic growth rate of *Macrobrachium rosenbergii* postlarvae fed with semi-purified test diets differing in lecithin content was estimated every 14th day during the experiment. The observed values are presented in Table VI. Growth increment for these periods are also represented graphically in Fig.1-4.

Despite poor performance in growth a significant difference [$P<0.01$] in the effect of dietary levels of lecithin was noticed between the SGR percentages during the first fortnight. Subsequent pairwise comparison of the data revealed that the diet containing 2.5% of lecithin was different from all others. It is found that the prawns fed with this diet produced the highest

S.G.R of *Macrobrachium rosenbergii* fed with different test diets

Table - VI

DIET	R*	INITIAL WEIGHT OF PRAWNS IN GRAMS	SGR(%DAY-1) 14TH DAY	SGR(%DAY-1) 28TH DAY	SGR(%DAY-1) 42TH DAY	SGR(%DAY-1) 56TH DAY
D1	A	0.5124	2.0590	2.0247	1.8870	1.8528
	B	0.4672	2.7552	2.5748	2.1787	2.0197
	C	0.5214	2.5672	2.4757	2.1149	2.0116
	D	0.5914	2.1356	2.0560	1.6395	1.6003
MEAN		0.5231	2.3793	2.2828	1.9550	1.8711
D2	A	0.5238	3.3291	2.5010	2.0984	1.9194
	B	0.5130	3.5723	2.4732	2.1500	1.9391
	C	0.5911	2.8869	2.2154	2.0081	1.9375
	D	0.5043	2.9540	2.1373	1.9823	1.9814
MEAN		0.5331	3.1856	2.3317	2.0597	1.9444
D3	A	0.6482	2.1764	1.9793	1.9497	1.7440
	B	0.5834	2.7942	2.5284	2.2199	2.0131
	C	0.5492	2.6501	2.4267	2.1471	1.9422
	D	0.5386	2.1139	2.1331	2.1054	2.1000
MEAN		0.5799	2.4337	2.2669	2.1055	1.9498
D4	A	0.5302	2.1569	2.1511	2.0491	1.8939
	B	0.5637	2.2425	2.1157	2.0037	1.8440
	C	0.5760	2.4748	2.3177	2.1260	1.9940
	D	0.4830	2.2712	2.2218	2.2451	2.2017
MEAN		0.5382	2.2864	2.2016	2.1060	1.9834
D5	A	0.5621	2.0323	1.9753	1.9044	1.9003
	B	0.5173	2.1859	1.9769	1.8696	1.7585
	C	0.5143	2.5670	2.1519	2.1516	1.8777
	D	0.4816	2.1308	2.1179	2.0303	1.8169
MEAN		0.5188	2.2290	2.0555	1.9889	1.8384

* Replications

Table - VII

ANOVA of S.G.R. on14th day

SOURCE	SS	DF	MSS	F
DIETS	2.429	4	0.6073	7.49
ERROR	1.217	15	0.08113	
TOTAL	3.646	19	0.1919	

TABLE VALUE AT 1% LEVEL = 4.89

CD = 0.2695

X2 = 3.1856

X3 = 2.4337

X1 = 2.3793

X4 = 2.2864

X5 = 2.2290

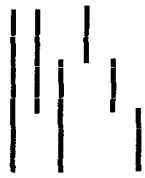


Table - VIII

ANOVA of S.G.R. on 28th day

SOURCE	SS	DF	MSS	F
DIETS	0.1874	4	0.0469	1.2018
ERROR	0.5847	15	0.0390	
TOTAL	0.7721	19	0.0406	

TABLE VALUE AT 1% LEVEL = 4.89

ANOVA of S.G.R. on 42nd day

Table - IX

SOURCE	SS	DF	MSS	F
DIETS	0.0782	4	0.0196	0.8112
ERROR	0.3608	15	0.0241	
TOTAL	0.4690	19	0.0247	

TABLE VALUE AT 1% LEVEL = 4.89

ANOVA of S.G.R on 56th day

Table - X

SOURCE	SS	DF	MSS	F
DIETS	0.058	4	0.0145	0.0792
ERROR	0.274	15	0.0183	
TOTAL	0.332	19	0.0175	

TABLE VALUE AT 1% LEVEL = 4.89

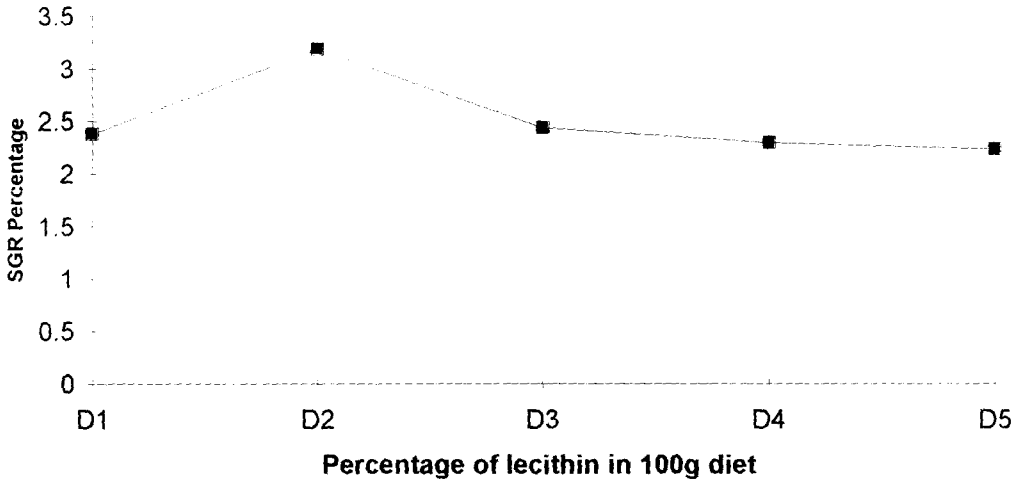


Fig: 1

SGR of the test diets on the 14th day

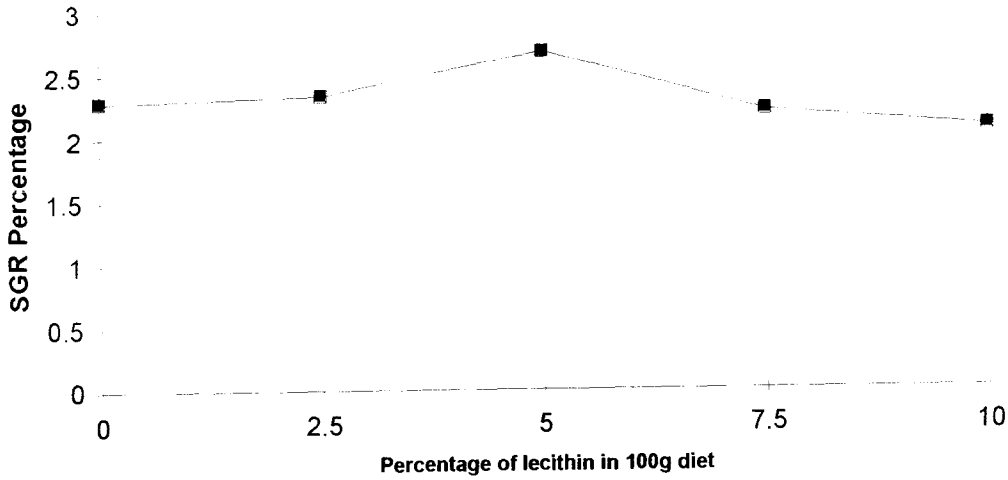


Fig: 2

SGR OF THE TEST DIETS ON THE 28th DAY

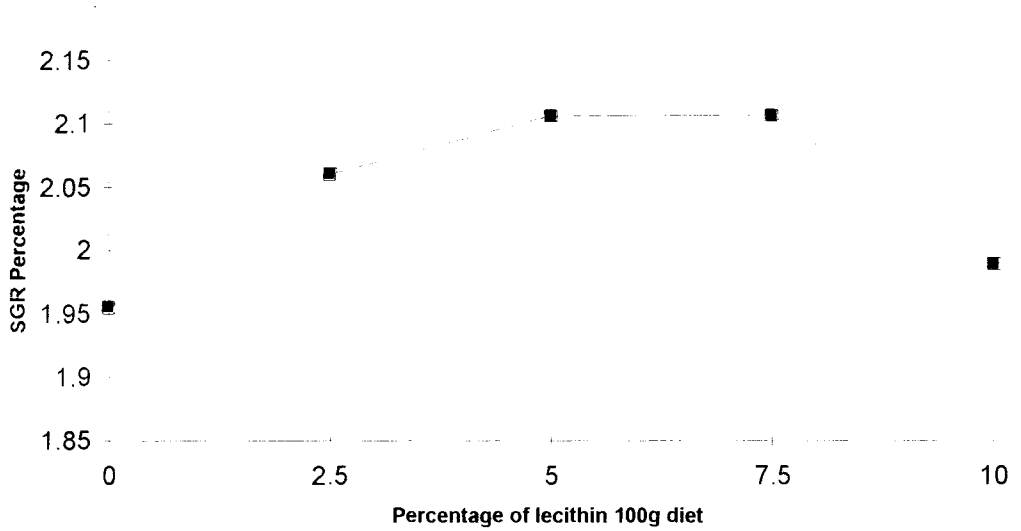


Fig: 3

SGR OF THE TEST DIETS ON THE 42nd DAY

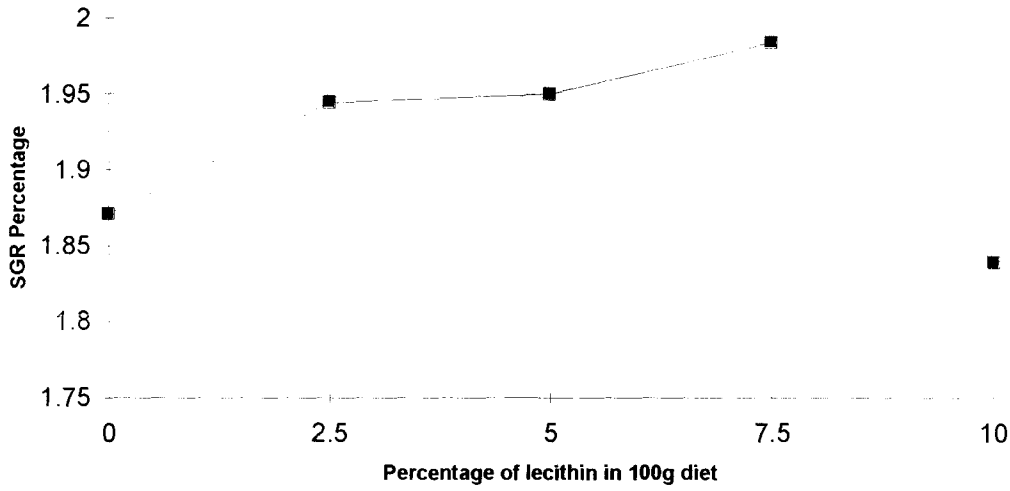


Fig: 4

SGR of the test diets on the 56th day

SGR [3.19] followed by the diet containing 5% [2.44] and diet without lecithin supplement [2.37]. Lowest SGR % were 2.28 and 2.20 recorded by the diets D_4 and D_5 respectively.

Analysis of variance of SGR values of the 2nd, 3rd and 4th fortnights did not show any significant difference among diets at 1% level. However, the specific growth rate of prawns grown on the diet D_2 continues to be the highest in the second fortnight also. Afterwards all the diets are giving almost the same specific growth rates.

4.2 Effect of lecithin on survival rate

The data on survival rate of *Macrobrachium rosenbergii* postlarvae fed with the test diets are given in Table XI and presented graphically in Fig.5.

Analysis of the data showed that supplementation of lecithin in the diet had no effect at all on the survival rate of the prawns. No significant variation was noticed between the percentage values of survival for differnt test diets. Highest rate of survival was 97.5% recorded for the diet D_4 and next 95% for D_3. The diet D_2 produced the lowest rate of 85% and the range was 80-100%.

Survival rate of *Macrobrachium* rosenbergii Post larvae fed with differen t test diets

Table - XI

DIETS	REPLICATION	SURVIVAL %	MEAN
D1	A	90	92.5
	B	90	
	C	90	
	D	100	
D2	A	80	85.0
	B	80	
	C	100	
	D	80	
D3	A	100	95.0
	B	80	
	C	100	
	D	100	
D4	A	100	97.5
	B	100	
	C	90	
	D	100	
D5	A	100	87.5
	B	80	
	C	90	
	D	80	

ANOVA of Survival rate

Table - XII

SOURCE	SS	DF	MSS	F
DIETS	730.218	4	182.555	1.343
ERROR	2039.472	15	135.965	
TOTAL	2769.69	19	145.773	

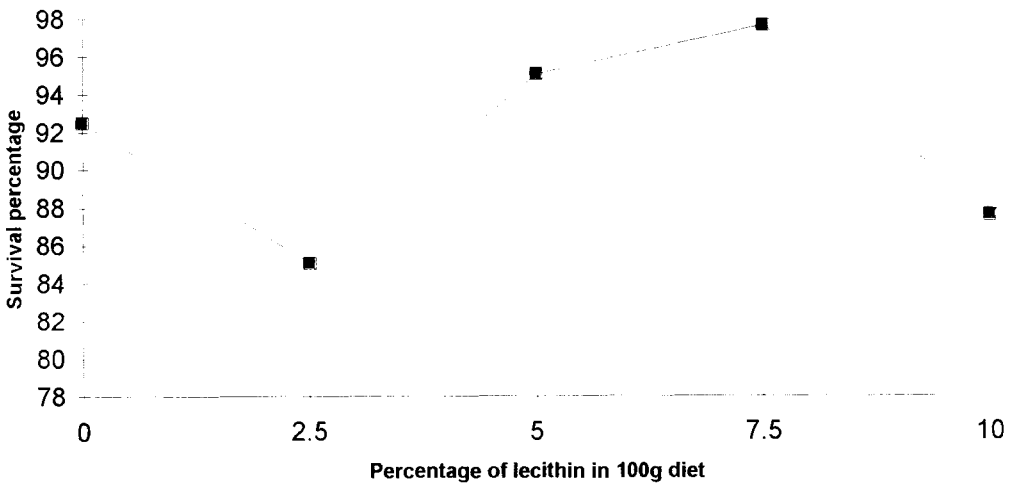


Fig: 5

Survival rate of *M.rosenbergii* Postlarvae fed with different test diets

4.3 Effect of lecithin on moulting rate

Moulting rate of *Macrobrachium rosenbergii* postlarvae throughout eight week period of the experiment is presented in Table XIII and also shown in Fig.6. The moulting rate was evaluated according to Petriella (1990).

ANOVA of the data showed no significant difference statistically at 1% level. Mean moulting rate of the group was 8.03±1.6. Each prawn achieved moulting once in every eight days and at least 6 - 7 times over the trial period. Though the differences among the moulting based on different diets were not statistically significant, the prawns which were exposed to the diet with 2.5% lecithin content showed a higher moulting rate [9.26] followed by the diet with 5% lecithin. For the rest of the diets it remains almost consistent with an average of 7.49.

No sign of moult death syndrome or any other unusual diet related exoskeletal abnormalities were noticed in any of the prawns fed with various test diets. Absence of significant difference between the diets tested in terms of moulting rate also proved to be unrelated to the growth under the conditions of this experiment.

Moulting rate of *Macrobrachium rosenbergii* fed with different test diets

Table - XIII

DIET	REPLICATION	NO: OF MOULTS	MOULTING RATE (DAYS)	MEAN
D1	A	20	3.7189	7.7166
	B	42	7.8096	
	C	54	10.0409	
	D	50	9.2971	
D2	A	55	10.2269	9.6226
	B	42	7.8096	
	C	50	9.2971	
	D	60	11.1566	
D3	A	44	8.1815	8.0421
	B	38	7.0658	
	C	43	7.9955	
	D	48	8.9253	
D4	A	38	7.0658	7.7632
	B	46	8.5537	
	C	40	7.4377	
	D	43	7.9955	
D5	A	39	7.2518	7.0194
	B	35	6.5081	
	C	31	5.7642	
	D	46	8.5534	

ANOVA of Moulting rate

Table - XIV

SOURCE	SS	DF	MSS	F
DIETS	16.6	4	4.15	1.3075
ERROR	47.61	15	3.174	
TOTAL	64.21	19	3.379	

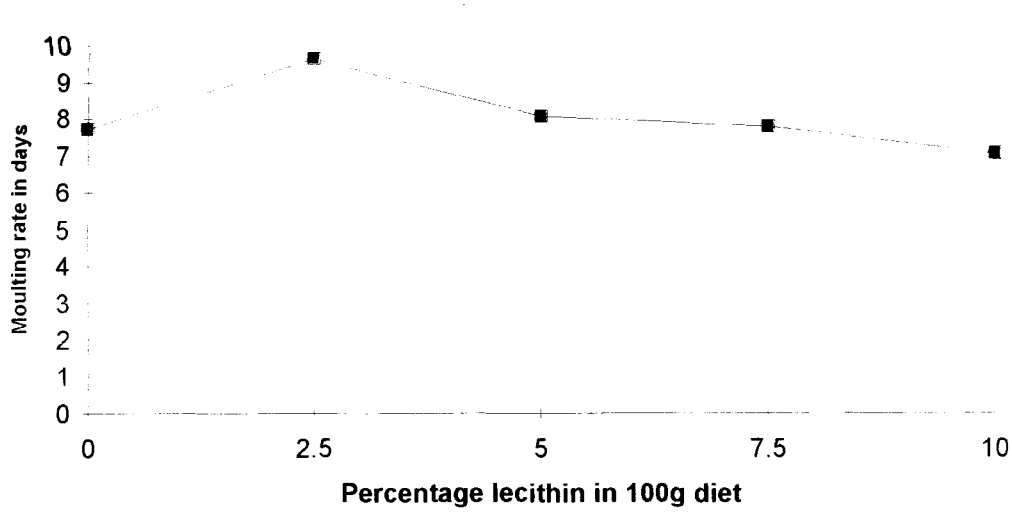


Fig: 6

Moulting rate of *M.rosenbergii* postlarvae fed with different test diets

4.4 Effect of lecithin on food conversion ratio

The food conversion ratio of *Macrobrachium rosenbergii* postlarvae in response to different dietary levels of lecithin was evaluated every two week during the study and the data are given in Table XV and Fig. 7-10.

The analysis of variance of data on FCR showed significant variation [$P<0.01$] between the diets with different lecithin contents during the first and second assessments, i.e., on the 14th and 28th day of the experiment. Comparison of mean FCR values during the first fourteen days of growth indicated that diet D_2 and D_3 differ significantly from all other treatments. FCR means of these two also showed difference.

Among the diet D_2 and D_3 it seems that the food conversion ratio of D_2 [4.94] is lower than that of D_3. Hence it can be suggested that incorporation of lecithin to a level of 2.5% in the diet is beneficial to the postlarval prawns during this period of growth. Diets D_1, D_4 and D_5 produced comparatively higher FCR than D_2 and D_3.

In the second assessment no difference was observed between the FCR means of first 3 diets, whereas the diet D_4 and D_5 showed significant difference. Higher levels of lecithin added in the diet was found to increase the food conversion ratio.

During the third and fourth assessment [i.e. in the 42nd and 56th day] FCR values of the diets were found close to each other and statistically not significant. Mean ranges between 4.66 — 6.22 and 3.85 — 4.50 respectively in this period. Supplementation of lecithin to any levels in the diet need not have any influence on FCR at this stage of growth.

Food Conversion ratio (FCR) of *Macrobrachium rosenbergii* fed with different test diets

Table - XV

DIETS	REPLICATION	FCR 14thDAY	FCR 14th DAY	FCR 42ndDAY	FCR 56thDAY
D1	A	8.5377	6.6078	6.3420	4.4302
	B	6.3528	5.3977	5.0294	3.4461
	C	6.6102	5.2052	4.5354	3.1623
	D	8.1315	6.2180	6.1228	4.3875
MEAN		7.3986	5.8572	5.5074	3.8563
D2	A	4.6293	5.3089	3.7311	3.8965
	B	4.5455	5.6739	5.3424	3.8710
	C	5.3916	5.3500	4.5056	3.2354
	D	5.1762	5.8707	5.8161	4.7366
MEAN		4.9357	5.5509	4.8486	3.9349
D3	A	5.8673	6.1646	5.1368	3.6545
	B	5.1682	5.1938	4.2360	3.9419
	C	5.5291	5.7410	4.2536	3.5848
	D	7.4815	6.5028	5.3685	3.8192
MEAN		6.0115	5.9006	4.7487	3.7501
D4	A	7.5351	7.7286	5.0950	4.1356
	B	6.9793	7.8392	5.0104	3.9019
	C	5.7920	6.1739	3.9990	3.5322
	D	7.7744	7.6140	4.5389	3.8355
MEAN		7.0202	7.3389	4.6608	3.8513
D5	A	8.0116	7.6412	5.6130	3.8168
	B	7.8018	7.8121	7.4639	5.2102
	C	6.6452	7.0644	4.7151	4.2477
	D	8.9928	7.6738	7.0185	4.8145
MEAN		7.8629	7.5478	6.2026	4.5223

ANOVA 0f F.C.R on 14th day

Table - XVI

SOURCE	SS	DF	MSS	F
DIETS	22.118	4	5.5295	6.734
ERROR	12.316	15	0.8211	
TOTAL	34.434	19	1.8120	

TABLE VALUE AT 1% LEVEL - 4.89

CD - 0.8592

TREATMENT MEANS

D5 7.8629

D1 7.4081

D4 7.0202

D3 6.0115

D2 4.9357

The treaments which are not significantly different are connected with vertical lines

ANOVA of F.C.R on 28th day

Table - XVII

SOURCE	SS	D.F	MSS	F
DIETS	13.830	4	3.4575	11.1209
ERROR	4.663	15	0.3109	
TOTAL	18.493	19	0.9733	

TABLE VALUE = 4.89
CD = 0.5287

TREATMENT MEAN

D5 = 7.5478

D4 = 7.3389

D3 = 5.9006

D1 = 5.8572

D2 = 5.5509

The treatments which are not significantly different are connected with vetical lines.

ANOVA of F.C.R on 42nd day

Table - XVIII

SOURCE	SS	D.F	MSS	F
DIETS	6.869	4	1.7173	2.256
ERROR	11.418	15	0.7612	
TOTAL	18.287	19	0.9625	

TABLE VALUE = 4.89

ANOVA of F.C.R on 56th day

Table - XIX

SOURCE	SS	DF	MSS	F
DIETS	1.52	4	0.380	1.5019
ERROR	3.80	15	0.253	
TOTAL	5.32	19	0.280	

TABLE VALUE = 4.89

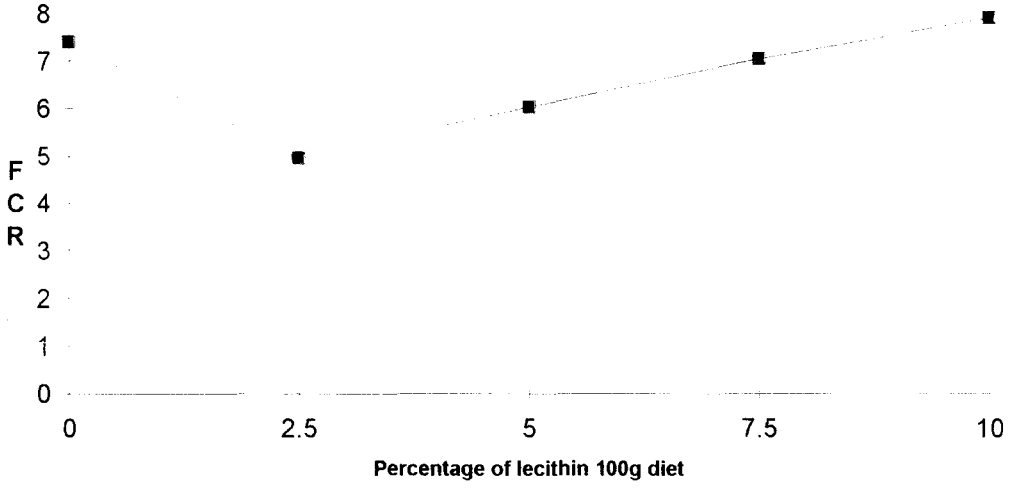


Fig: 7

FCR of the test diets on the 14th day

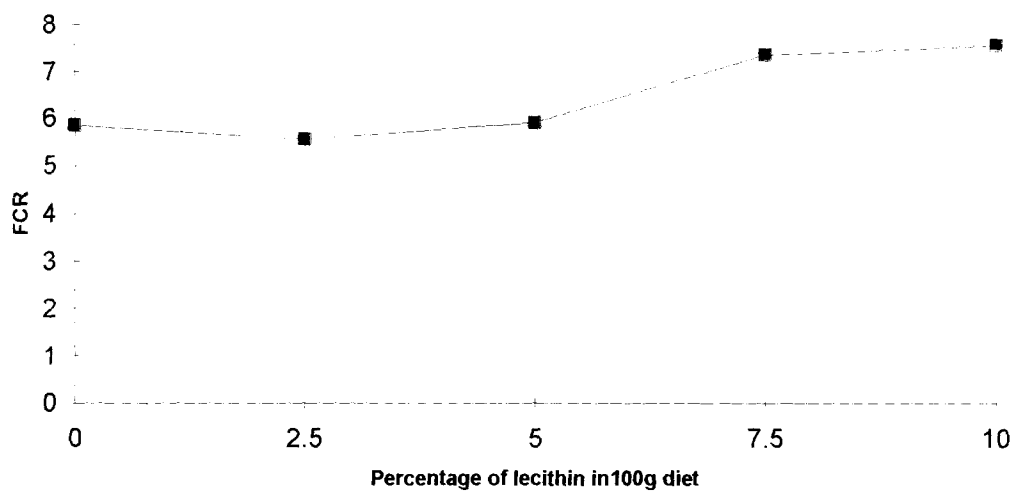


Fig: 8

FCR of the test diets on the 28th day

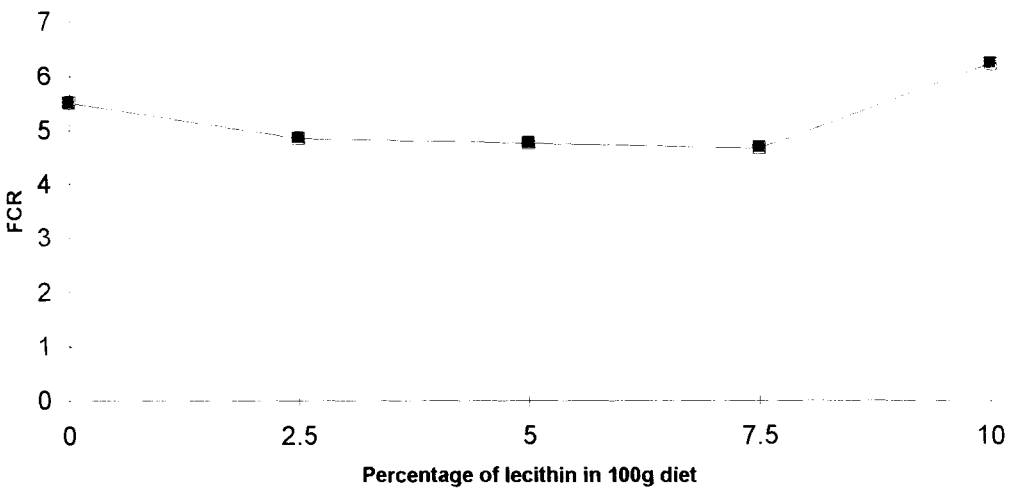


Fig: 9

FCR of the test diets on the 42nd day

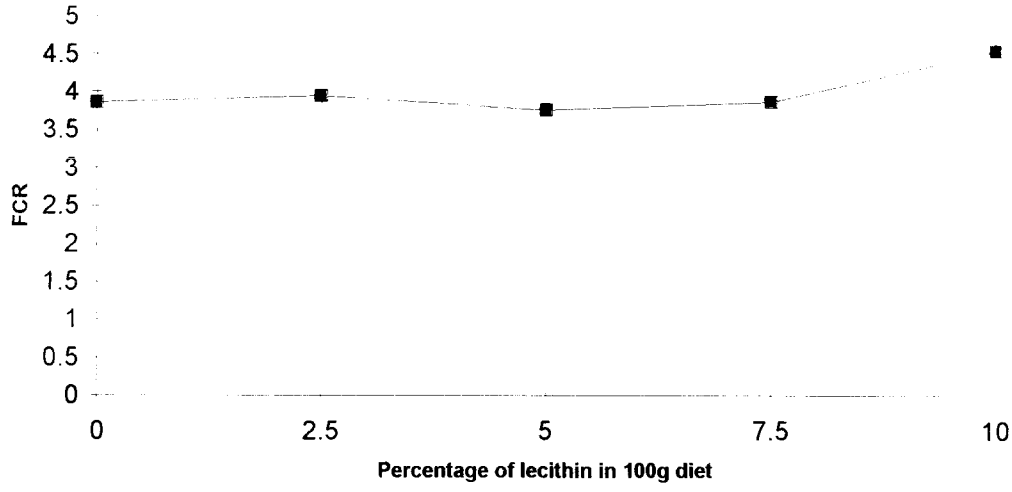


Fig: 10

FCR of the test diets on the 56th day

4.5 Effect of lecithin on body phospholipid levels

Body phospholipid contents of the prawnf fed with different test diets were determined by the method of Zilversmith and Davis [1950]. Table XX and Fig.11. show the respective results and its graphical representation.

ANOVA of the body phospholipid composition of the prawn with the termination of the trial indicated that the dietary formulation had no significant effect. However, the higher levels of dietary lecithin tend to result in increased carcass phospholipid levels. Body phospholipid of the prawn was raised from 0.1873% to 0.2144% when the content of lecithin in the diet was increased from 0 - 10%.

4.6 Abnormal symptoms

During the course of the study no diet related deficiency or excess symptoms were noticed in any of the prawns fed with different test diets. Overall performance especially in terms of growth, pigmentation etc. was very poor in all treatment groups, which could be attributed to purified nature of the diet used.

4.7 Water Quality

Water Quality was maintained relatively constant throughout the duration of the experiment. Details of physico-chemical parameters like temperature, pH and dissolved oxygen meausred during the study period are given in Table XXII - XXIV.

Body Phospholipid content of *Macrohrachium rosenbergii* fed with different test diets

Table - XX

DIETS	R*	OPTICAL DENSITY AT 660 NM	% OF BODY PHOSPHOLIPID	MEAN
D1	A B C D	0.070 0.120 0.090 0.110	0.1524 0.2209 0.1858 0.1901	0.1873
D2	A B C D	0.160 0.180 0.200 0.165	0.1804 0.2140 0.2327 0.1618	0.1972
D3	A B C D	0.180 0.210 0.160 0.190	0.2017 0.1946 0.2081 0.2079	0.2030
D4	A B C D	0.195 0.160 0.230 0.160	0.2245 0.2002 0.2419 0.1709	0.2094
D5	A B C D	0.220 0.172 0.120 0.260	0.2185 0.1952 0.2172 0.2265	0.2144

* Replication

ANOVA Body phospholipid content

Table - XXI

SOURCE	SS	DF	MSS	F
DIET	0.0018	4	0.00045	0.75
ERROR	0.0090	15	0.00060	
TOTAL	0.0108	19	0.00060	

TABLE VALUE AT 1% LEVEL = 4.89

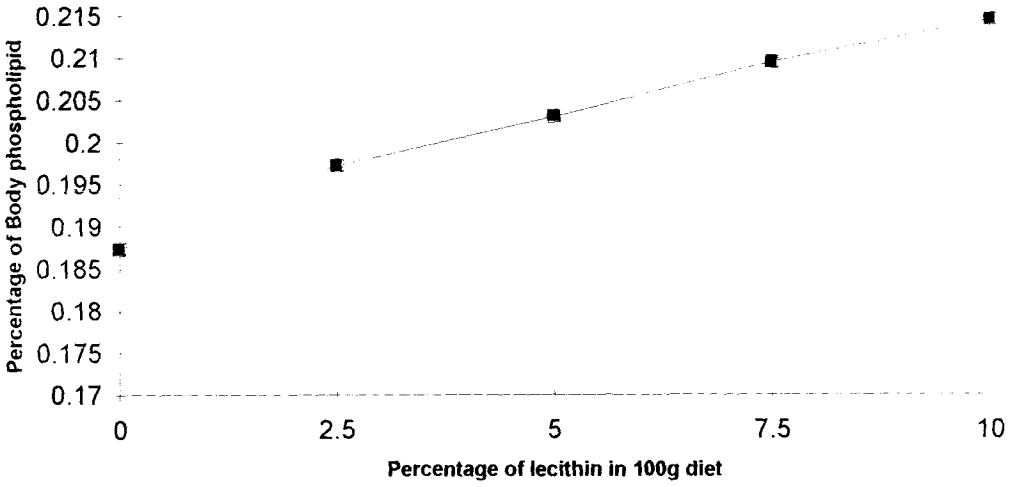


Fig: 11

Body phospholipid content of *M.rosenbergii* fed with different test diets

Water teperature in the experimental tanks during the study period

Table - XXII

TEMPERATURE	WEEKS							
	1	2	3	4	5	6	7	8
MEAN	27.540	27.560	27.160	27.040	28.400	27.900	27.400	26.760
+ - SE	0.863	0.534	0.440	0.424	0.470	0.410	0.447	0.370
RANGE	26.5-29	26.5-28.3	26.3-28	26.5-27.8	27.8-29.2	27.2-28.3	26.8-28.3	26.2-27.3

pH of water in the experimental tanks during the study period

Table - XXIII

pH	WEEKS							
	1	2	3	4	5	6	7	8
HEAN	8.80	7.90	7.80	8.02	8.07	7.71	8.90	7.85
+ SE -	0.70	0.21	0.42	0.38	0.32	0.23	0.58	0.44
RANGE	8-10	7.5-8.2	7-8.3	7.5-8.5	7.5-8.5	7.5-8	8.2-9	7-8.5

Dissolved oxygen in the experimental tanks during the study period

Table - XXIV

DISSOLVED OXYGEN	WEEKS							
	1	2	3	4	5	6	7	8
MEAN	6.67	6.63	7.80	6.40	7.80	8.10	6.60	7.30
+ SE -	0.29	0.54	0.24	1.07	0.33	0.24	0.38	0.31
RANGE	6.3-7.01	5.9-7.2	7.5-8.1	5.2-7.8	7.5-7.8	7.9-8.5	6.3-7.2	7.1-7.8

Water teperature in the experimental tanks during the study period

Table - XXII

TEMPERATURE	WEEKS							
	1	2	3	4	5	6	7	8
MEAN	27.540	27.560	27.160	27.040	28.400	27.900	27.400	26.760
+ - SE	0.863	0.534	0.440	0.424	0.470	0.410	0.447	0.370
RANGE	26.5-29	26.5-28.3	26.3-28	26.5-27.8	27.8-29.2	27.2-283	26.8-28.3	26.2-27.3

pH of water in the experimental tanks during the study period

Table - XXIII

pH	WEEKS							
	1	2	3	4	5	6	7	8
HEAN	8.80	7.90	7.80	8.02	8.07	7.71	8.90	7.85
± SE	0.70	0.21	0.42	0.38	0.32	0.23	0.58	0.44
RANGE	8-10	7.5-8.2	7-8.3	7.5-8.5	7.5-8.5	7.5-8	8.2-9	7-8.5

Dissolved oxygen in the experimental tanks during the study period

Table - XXIV

DISSOLVED OXYGEN	WEEKS							
	1	2	3	4	5	6	7	8
MEAN	6.67	6.63	7.80	6.40	7.80	8.10	6.60	7.30
± SE	0.29	0.54	0.24	1.07	0.33	0.24	0.38	0.31
RANGE	6.3-7.01	5.9-7.2	7.5-8.1	5.2-7.8	7.5-7.8	7.9-8.5	6.3-7.2	7.1-7.8

Temperature was maintained at 27.47 ± 0.48 and pH at 8.14 ± 0.42. Dissolved oxygen measured 7.16 ± 0.63 which was well above 100% saturation since mild aeration was provided in the tanks throughout the experimental period.

Water quality was seen to be not detrimental to the growth of the prawns in the present study.

4.8 Proximate composition of the test diets

Proximate composition of the test diet used in the study is given in Table XXV. The diets on an average contain 32.42% protein, 32.12% carbohydrate, 7.74% moisture and 7.78% of ash content. Lipid content varies between 6.2 and 17.2% and fibre content 3.27 and 12.5%.

Proximate composition of the test diets used for the experiment

Table - XXV

COMPOSITION	DIETS					MEAN
	D1	D2	D3	D4	D5	
PROTEIN	33.5	32.5	31.7	32.1	32.3	32.42 ± 0.601
LIPID	6.2	9.2	11.7	14.3	17.2	11.72 ± 3.8
CARBOHYDRATE	32.8	31.6	32.9	32.8	30.5	32.12 ± 0.94
MOISTURE	7.2	8.1	7.5	7.3	8.6	7.74 ± 0.531
FIBRE	12.5	10.8	8.2	5.9	3.7	8.22 ± 3.19
ASH	7.8	7.8	8.0	7.6	7.7	7.78 ± 0.132

5. DISCUSSION

The result of the present experiment conducted to evaluate the influence of dietary lecithin in the purified diet of Macrobrachium roserbergii postlarvae based on growth rate, survival rate, moulting rate, food conversion ratio and body phospholipid are discussed below.

5.1 Effect of lecithin on growth rate

Though the overall growth rate of the postlarvae was not affected by dietary lecithin, growth rate estimated during the first fortnight of the study showed significant influence. For this period the diet with 2.5% of lecithin content gave the highest SGR compared to other levels of inclusion and found that higher levels of lecithin did not have any beneficial effect. Specific growth rate produced by all the diets except D_2 can be considered more or less the same. In subsequent fortnight also SGR % estimated failed to show any influence of lecithin.

Lecithin which composed of phosphatides, polarlipids and carbohydrates has been found to be required by some crustaceans (Conklin *et al.,* 1980; D'Abramo *et al.,* 1981). The essentiality of dietary supplementation of phospholipids for growth was demonstrated in several species of penaeid shrimps such as *Penaeus japonicus* (Kanazawa *et al.*, 1979 a,1985; Kanazawa, 1982; Teshima *et al.*,1986 a, c; Camara *et al.,* 1993), *Penaeus monodon* (Pascual, 1985, 1986; Chen, 1993; Kompiang, 1992; Briggs *et al.*, 1994), *Penaeus indicus* (Ali 1990; Chandge and Raja, 1993, 1995), *Penaeus penicillatus* (Chen and Jenn, 1991, 1992), *Penaeus chinensis* (Zhou and Wang, 1992). Similarly phospholipids are also reported to be an essential

nutrient for supporting growth in lobsters like *Homarus americanus* (Boghen and Caslell, 1980; Conklin *et al.*, 1980; D'Abramo *et al.*, 1980) and *Panulirus japonicus* (Kanazawa and Koshio,1993). In contrast to this, Hilton *et al.* (1984), Briggs *et al.* (1988) and Koshio (1992) reported that supplementation of lecithin at different levels in the purified diet of *Macrobrachium rosenbergii* **were found to produce only insignificant result in terms of growth rate. However, a growth enhancing trend was noticed by Briggs** *et al.* (1988) when the lecithin content was increased from 0 to 5% in the diet.

Crustaceans have long been known to synthesise phospholipid *de novo* (Shiek, 1969; Chapelle, 1986), But this biosynthetic ability is limited or fall short of the amount actually required to satisfy the metabolic requirements during the rapid growing younger stages (D'Abramo *et al.*, 1981; Kanazawa, *et al.*, 1985 and Wang *et al.*, 1992). The deficiency of phospholipid which arises as a result of this seriously affects growth unless the required amount is derived from the diet. D'Abramo (1989) while reviewing the lipid requirement of shrimps speculates that the requirement of different lipid classes, including phospholipids varies according to the age of shrimps. Adding further evidence to this Camara *et al.* (1993) demonstrated a decreasing requirement for dietary phosphatidylcholine in *Penaeus japonicus* postlarvae. This age dependent requirement for phospholipid could be one of the reasons that the previous works of Hilton *et al.* (1984), Briggs *et al. (1988) and Koshio et al.* (1992) in *Macrobrachium resenbergii* failed to show any influence on growth rate. All these studies have been carried out in postlarvae of 6 weeks old or juveniles weighing 800mg. On the other hand, 2 weeks - old postlarvae weighing 0.053g were used in the present study.

Evidence supporting the suggestion of D'Abramo (1989) was noticed by Koshio *et al.* (1992) in another experiment with *Macrobrachium rosenbergii* postlarvae. According to their

study soylecithin at levels up to 2% enhances growth in very young postlarvae of 0.05g size, when reared individually. Importance of individual rearing method in nutrition studies was stressed by New (1976) while reviewing the dietary studies of shrimps and prawns. He opined that results of such experiments were more reliable, being particularly true when species like *Macrobrachium rosenbergii* are the experimental animals. However, Briggs *et al.* (1988) conducted the study by individually housing the prawns and the result obtained was not significant which may be due to the older age of the larval prawns used. Eventhough the protein source of the diet and the rearing method followed in the present experiment (Communal rearing method) were different from that of Koshio *et al.* (1992) the observed increase in specific growth rate of the prawns, fed a diet containing 2.5% lecithin, two weeks after the start of the experiment was a clear indication supporting the suggestion of D'Abramo (1989).

In the light of the findings discussed above and on the basis of the results of the present study it is suggested that with aging, *Macrobrachium rosenbergii* postlarvae gaind the ability for *de novo* phospholipid synthesis sufficient enough to sustain metabolic requirements and growth. It is also suggested that after a particular period of postlarval growth the requirement of phospholipid can be met with *de novo* synthesis and supplementation of lecithin in the diet is not needed for *Macrobrachium rosenbergii* beyond this critical stage.

Kanazawa (1982) indicated that the growth of crustaceans especially penaeid shrimps is not only affected with the dietary levels, but also with the kind and quality of phospholipids. Addition of soy phosphatidylcholine, phosphatidylinositol as well as phosphatidylcholine derived from bonito egg which contained higher percentage of essential fatty acids such as 18:2w6 or 22:6w3 was found very effective in promoting growth of larval *Penaeus japonicus*

(Kanazawa, *et al.*, 1985). According to Kanazawa *et al.* (1979 b and c) the biosynthetic and bioconversion ability of EFA in shrimps is very limited. Hence they rely on the diet to meet the requirement of EFA. Feeding studies of Kanazawa *et al.*(1979 a,b,c) in *Peanaus japonicus*, Xu *et al.* (1993 a,b and 1994) in *Panaeus monodon* and *Penaeus chinensis,* Kanazawa and Koshio (1993) in the lobster *Panulirus japonicus* and D'Abramo and Sheen (1993) in *Macrobrachium rosenbergii* very well established that these species of curstaceans require dietary source of essential fatty acid for supporting normal growth. Besides this Kanazawa *et al.* (1985) reported that the effect of phospholipid in promoting growth of curstaceans seems to vary with the kind of compound esterified at the C-3 position of phosphoric acid and crustaceans show some preference towards choline and Inisitol in this position. Convincing evidence supporting this theory was obtained in lobster *Homarus americanus* (D'Abramo *et al.*,1981) and shrimps like *Penaeus penicillatus* (Chen and Jenn, 1991) and *Penaeus monodon* (Chen, 1993). Recently it was reported that low levels of purified phosphoalidylcholine (1.5%) is more effective than higher levels (6.5%) of soy lecithin for young postlarval *Penaeus japonicus* (Camara, *et al.*,1993). Teshima *et al.* (1986b) also indicated that the effect of purified phosphatidylcholine was very much pronounced in the larval stage. Available literature suggest that among the various phospholipids required by crustaceans, phospholidycholine is the most essential one and is found superior to others in accelerating growth.

Soy lecithin used in the present experiment was more refined one containing 60% of phosphatidylcholine campared to those used by Hilton *et al.* (1984) and Briggs *et al.*(1988) in *Macrobrachium rosenbergii* containing 35% and 40% respectively. Therefore, it is believed that the presence of high percentage of phosphatidylcholine rich in w6 and w3 fatty acid, in the soy-lecithin is likely to be the reason for high SGR observed for the prawns fed with diet having 2.5% of lecithin.

Growth promoting effect of phospholipid in crustaceans is due to various reasons. Kanazawa *et al* (1979 a) suspects that the growth promoting effect of phospholipids in shrimp is due to the role played by them in the intestinal absorption and inter - organ transport of dietary lipids and cholesterol. Teshima and Kanazawa (1979) in another study with *Penaeus japonicus* confirmed that the EFA absorbed at the hind gut and hepatopancreas was resynthesized and released into the haemolymph in the form of phospholipids. Radio - tracer experiment of Teshima and Kanazawa (1980) clearly described the mechanism of absorption and transportation of lipids in the shrimp. They explained that the transportation of dietary lipids in crustacean was mainly carried out by phospholipids associated with very high density lipoprotein. Continuing the studies of lipid transport mechanism in *Penaeus japonius* Teshima *et al.* (1986 b,d)reported that the retention and utilization of dietary lipid, especially cholesterol in the body of shrimps was markedly reduced due to the absence of phospholipids in the diet. Similar observations on the requirement of phospholipids particularly phosphatidylcholine for efficient utilization and retention of cholesterol in the body were also reported in lobster *Homarus americanus* (D'Abramo *et al.*, 1982, 1985a; Baum *et al.*, 1990). Based on these studies it can be considered that the improved growth rate noticed for the diet D2 in the present experiment may be due to the effective absorption and utilization of dietary lipids. Besides this, Chen and Jenn (1992) attributed that the growth promoting effect of phospholipids was also due to the increased water stability, preventing leaching of essential nutrients from the feed. Considering the fact that the young postlarvae are more sensitive to imbalances of essential nutrients the explanation given by Chen and Jenn (1992) can also be the reason for improved SGR observed in the prawns fed with D_2 diet.

Growth rate of prawns reared on diets D_4 and D_5 consisting 7.5 and 10% of lecithin was slightly but not significantly lower than that of the growth rate obtained for the diet group

D_1, D_2 and D_3. Biddle (1977) and Sheen and D'Abramo (1991) reported that the total lipid content of the diet for *Macrobrachium rosenbergii,* should not exceed 10%. Therefore, it is possible that slight growth retardation noticed in prawns reared on these diets should have been caused by excess lipid content. In the present study the levels, 7.5 and 10% of lecithin was selected arbitrarily to study the adverse effect of lecithin on growth and also to characterise the excess symptoms, if any caused by it.

Specific growth rate estimated in the second, third and fourth biweekly intervals of the study period indicated no significant difference. The result reveals that there is no advantage in supplementing phospholipd when the biosynthetic ability of the prawns become sufficient enough to support growth. A similar suggestion was made by Briggs *et al.* (1988) that endogenous level of phospholipids present in the dietary formulation is sufficient for growth in freshwater prawns.

5.2 Effect of lecithin on survival rate

High survival recorded in all diet groups irrespective of lecithin content indicates that phospholipid has got a very little influence in the diet of *Macrobrachium rosenbergii* with respect to the survival rate. Hilton *et al.* (1984) and Briggs *et al.* (1988) reported that inclusion of lecithin in the semi-purified diet of *Macrobrachium rosenbergii* did not affect survival rate. These findings point that even if phospholipid is absent, or present in the diet in excess than the normally required quantity it will not cause any detrimental effect upon the survival of freshwater prawns.

Unlike in *Macrobrachium rosenbergii* phospholipid plays a vital role in the survival of

some marine crustaceans like penaeid shrimps and lobsters (D'Abramo, 1981;Kanazawa *et al.*, 1985;Kanazawa, 1993; Teshmia *et al.*, 1993]. Conklin *et al.*(1980) found that inclusion of soy-lecithin in the diet was critical for the survival of lobsters and the absence of it reduces the survival rate drastically. Similarly in *Homarus americanus* and hybrids of *Homarus americanus* vs *Homarus grammicus* juveniles, increasing the lecithin to 7.5% of the dry weight of the diet was found to decrease the incidence of mortality (Bowser and Rosemark, 1980). On the other hand, Kean *et al.* (1985) opined that phospholipids do not influence survival of lobsters when the primary protein source, casein was replaced by crab protein. But later Castell *et al.* (1989) while comparing two standard diet formulations for crustacean explained that endogenous level of phospholipids present in the crab protein concentrate was sufficient to overcome the requirement of crustaceans.

Essentiality of dietary phospolipid for survival, especially during the larval as well as the postlarval stages was demonstrated in penaeid shrimps *Penaeus japonicus* by several authors (Kanazawa *et al.*, 1985; Teshima *et al.*, 1986a,b;Tackaert *et al.*, 1991b; Camara *et al.*1993). Dietary source of phospholipid is indispensible for successful metamorphosis of larvae. In *Penaeus japonicus* Kanazawa *et al.* (1985) and Teshima *et al.* (1986 a) reported that deficiency of phospholipid in the diet caused marked death of the larvae prior to metamorphosis from zoea I to zoea II. In postlarvae Camara *et al.* (1993) showed that the presence of phosphatidylcholine in the feed not only produced higher survival rate but also improves the stress resistance. In penaeid shrimps like *Penaeus monodon* and *Penaeus chinensis* also phospholipids seem to increase survival rate significantly (Pascual, 1985, 1986; Zhou and Wang, 1992 ; Briggs *et al.*,1994).

The reason why phospholipids increases survival rate of crustaceans was investigated

by several workers. According to D'Abramo *et al.* (1981; 1982 and 1985 a) phosphatidylcholine molecule is an important component of lipoprotein complex that efficiently transfers cholesterol and other essential fat soluable materials for the survival of lobster *Homarus americanus.* In another study Baum *et a.*(1990) proved that impairment in cholesterol movement due to the absense of phospholipd in the diet was the main factor responsible for mortality of lobster *Homarus americanus.* In penaeid shrimps phospholipds are involved in the absorpiton and transportation of dietary lipids especially cholesterol and triglyceride in the body(Teshima and Kanazawa, 1979; Teshima *et al.*,1986b). These findings indicate that crustaceans like lobster and shimps require phospholipd for the emulsification, absorption and utilization of cholesterol and other dietary lipids. The absence of phospholipd in the diet restricts the movement and utilization of these lipids within the body and adversily affects the survival rate. In addition to this, phospholipids are also reported to increase the stress resistance of the animals according to Camara *et al.* (1993). Higher survival rate observed in crustaceans fed with phospholipid supplemented diets can be attributed to these reasons.

However, there are also studies in penaeid shrimps indicating insignificant role of phospholipid on survival rate. Chen and Jenn (1991), Kompiang (1992), Chen (1993) reported that inclusion of phsophatidylcholine in the diet had no effect on the survival rate of *Penaeus monodon* and *Penaeus penecillitus.* While reviewing the lipid requirement of shrimps D'Abramo (1989) speculates that the requirement of differnt classes of lipids varies according to the difference in age and species. This was supported by Camara *et al.*(1993) by showing that survival rate of shrimps varies not only with the nature and sources of phsopholipds used in the diet but also with the age differences of the experimental animals. May be due to the aging of the postlarvae used for the experiment , the studies of Kompiang (1992) and Chen (1993) in *Penaeus monodon* showed no influence. On the other hand the insignificant result recorded by

Chen and Jenn (1991) in *Penaeus penicillatus* may be the variation of species specific requirement as suspected by D'Abramo (1989).

The result of the present study is similar to that of Hilton *et al*, (1984) and Briggs *et al.* (1988) conducted in *Macrobrachium rosenbergii.* It is appreciated that the cod liver oil used in the study contained some amount of phospholipids and this much amount might be sufficient for sustaining the survival of *Macrobrachium rosenbergii* postlarvae. Therefore one cannot state on the basis of the study that lecithin is not an essential nutrient for fresh water prawns. However, it is concluded that supplementation of lecithin is not required in the semi-purified diet of *Macrobrachium rosenbergii* when held under the experimental conditions of this study.

5.3 **Effect of lecithin on moulting rate**

The result of the study indicated that different dietary levels of lecithin do not have any significant influence on moulting rate of the prawns. Average moulting rate of the groups was 8.03 which means that each prawn moulted every 8th day during the study period. Observation similar to this was made by Briggs *et al.* (1988) with *Macrobrachium rosenbergii* juveniles in which addition of lecithin in the diet irrespective of levels was seen to have no influence on the moulting rate.

Involvement of phospholipids in the exoskeletal formation of crustanceans has been evident from the investigation of Conklin *et al* . (1980). Bowser and Rosemark (1980) in American lobster *Homarus americanus* and Brock (1980) in *Macrobrachium rosenbergii* larvae noticed that lack of dietary phospholipid commonly resulted in death due to the animals inability to extricate itself at moult from the old exuvia. Based on the findings of Teshima and

Kanazawa (1979) the reason attributed for this phenomenon was the inhibition in the transport of fat soluable nutrients especially cholesterol through the haemolymph due to the absence of phospholipids. D'Abramo *et al.* (1982) and Teshima *et al.* (1986 a,c) reported that phospholipids are involved in the absorption,transportation and utilization of cholesterol in crustaceans. Since it is demonstrated that the crustaceans have only limited ability to synthesis this steroid, the drop in haemolymph cholesterol was believed to influence epidermal membrane synthesis either directly or indirectly by limiting the synthesis of moulting hormone ecdysteroid, for which cholesterol is a precurssor. Contradictory to this D'Abramo *et al.* (1985 a) while studying the effect of phospholipid on cholesterol transport mechanisms in *Homarus americanus* explained that only a small amount of ecdysteroids are involved with successful moulting, the reduced serum cholesterol and associated transport rate do not exert any limiting effect upon the amount of hormone precursor. This study reveals that eventhough phospholipid has on active role in the absorption and transportation of cholesterol, its influence on moulting is very limited. It might be due to this limited involvement of phospholipid, the average moulting rate estimated remains consistent in all the diet groups in the present study. Moreover the diet including the control (without lecithin supplements) contained an additional level of 0.02% of phospholipid derived from the codliver fraction of the basal lipid. Presence of this small quantity of phospholipid may be sufficient for the effective utilization of cholesterol and to induce moulting on freshwater prawn *Macrobrachium rosenbergii.* Based on the result, it is recommended that addition of lecithin over the endogenous level is not essential in the diet of *Macrobrachium resenbergii* to influence moulting.

5.4 **Effect of lecithin on food conversion ratio**

It is noticed that the diet with lower lecithin levels produced good FCR, during the initial

phase of the present feeding trial (ie. during the 14th and 28th day of the experiment). The better food conversion ratio noticed for the diet with 2.5% of lecithin content can be due to the requirement of lecithin for growth at this stage. Deshimaru and Shigueno (1972) and Colvin (1976) opined that the deficiency or presence of excess essential nutrients in the diet of shrimps and prawns will have adverse effect on FCR. Extremely high FCR recorded for the diet with higher levels or without lecithin in this study support this suggestion. Deshimaru and Shigueno (1972) also reported that even if the diet was nutritionally unbalanced, it is likely to be consumed more by prawns, provided they are palatible and this high diet intake does not necessarly bring about rapid growth. Phospholipid addition in the diet improving FCR has been demonstrated in several species of penaeid shrimps. Teshima *et al.* (1986 a) reported that food conversion efficiency of the diet supplemented with 3% of soy lecithin was higher than lecithin deficient diet when fed to *Penaeus japonicus.* In *Penaeus monodon Briggs* et al (1994) obtained best FCR when purified diet contained 3% of lecithin. The same species exhibited increasing growth and feeding efficiency when lecithin content was increased from 0 to 2% in a practical styled diet with 10% of total lipid (Pascual 1985).

Among the diets tested in this study better FCR got for the diet with lower percentage of lecithin during the first fortnight was mainly due to the utilization of lecithin for growth. The weight gain of the prawns fed with this diet showed a marked improvement during this period and hence the FCR also. As per the study of Shiek (1969) it is belived that the phospholipid synthesis takes place only to a limited level during younger stage of crustaceans, and dietary source of phospholipid might have influenced the growth rate and produced good FCR.

Against these benefical effect of phospholipids discussed above, Chen and Jenn (1991) indicated that inclusion of even purfied phosphotidylcholine did not improved the food

conversion ratio of juvenile *Penaeus penicillatus*. Dietary requirement of certain classes of lipid charges from species to species (D'Abramo, 1989). The requirment of dietary phosphatidylcoline in *Penaeus penicillatus* may be very less compared to other species or its influence on growth is less. That is why Chen and Jenn (1991)noticed insignificant result on FCR.

The result of the study, especially the FCR estimated subsequent to second fortnight did not exhibit any influence. For the second fortnight no difference was noticed between the FCR of the diets D_1, D_2 and D_3; whereas the diet D_4 and D_5 showed significant influence. The study reccommends that incorporation of lecithin in the diet is unnecessary since it is seen to have no beneficial effect on FCR.

5.5 **Effect of lecithin on body phospholipid levels**

Analysis of the body phospholipid composition of *Macrobrachium rosenbergii* postlarvae with the termination of the experiment did not show any significant effect on dietary formulations. However, an increasing tendency in whole body phospholipid was noticed as the lecithin content of the diet increases from 0 to 10%. The highest body phospholipid concentration was noticed in the diet D_5. The result suggests that some amount of phospholipid derived from the diet was incorporated into the body of the prawns.

According to the finding of Teshima *et al.* (1986 a, c) composition of phospholipid such as phasphotidylcholine and phosphatidylinositol in the whole body of *Penaeus japonicus* larvae and juvenile was higher when the shrimps are fed with 3% soy bean lecithin supplemented diet compared to the control. A similar increment in total body lipid with the increase of dietary lipid levels was noticed by Hilton *et al.* (1984) in *Macrobrachium rosenbergii,*

Chen (1993) and Briggs *et al.* (1994) in *Penaeus monodon* and Chen and Jenn (1991) in *Penaeus penicillatus.* All these studies are carried out in the context of evaluating the effect of dietary phospholipids. Above mentioned findings stress that in crustaceans dietary lipid composition seems to play an important role in influencing the total lipid level and some lipid class composition in the muscle. Further, radio tracer experiment of Teshima *et al.* (1989) found that labelled phosphatidylcholine was highly incorporated in the muscle within 24 hours after injection in *Penaeus japonicus* and to a lesser extent in *Macrobrachium rosenbergii.* Though Chen and Jenn (1991) pointed out that the retention of the phospholipids in the muscle cannot be related to the dietary supplements, the fattyacids profile of dietary phospholipds was strongly reflected in the polarlipid of the muscle. Since more than 60% of polarlipid is constituted by phospholipids a possible replacement of muscle phospholipid pool by the phospholipids of dietary origin was speculated by them. In the present study also this phenomenon might have happened; that is why analysis of whole body phospholipids of the prawn showed a slight increment with the increase of lecithin in the diet.

5.6 Abnormal symptoms

Specific diet related deficiency or excess symptoms were not observed in any of the prawns fed with various test diets. Overall performance of the prawn especially growth and pigmentation was poor, probably due to the purified nature of the ingredients. Indication of poor growth in purified diet has been reported by Briggs *et al.* (1994) in *Penaeus monodon.* In the earlier work of Hilton *et al.* (1984) and Briggs *et al.* (1988) with *Macrobrachium rosenbergii* also no abnormal symptoms like exuvia entrapment disease or moult death syndrome was noticed in the prawns fed with the diets deficient or containing excess lecithin.

6. SUMMARY

1. The study was aimed to evaluate the effect of different dietary levels of lecithin on growth, survival, moulting frequency, FCR and body phospholipid levels of postlarvae of the freshwater prawn *Macrobrachium rosenbergii* and also to characterise the deficiency and excess symptoms brought about by lecithin in the semi-purified diet.

2. Five casein - egg albumin based test diets differing in lecithin content at, 0, 2.5, 5, 7.5 and 10% were prepared and fed to the early postlarvae of *M. rosenbergii* having an average size of 0.05386 g for a period of 56 days. The influence of lecithin on different growth stages was evaluated every fourteen days interval.

3. Supplementation of lecithin to a level of 2.5% (dry weight) in the diet was found to promote growth during the first fortnight and no significant influence irrespective of the levels was noticed subsequently .

4. It is inferred that lecithin at lower levels in the diet enhances the growth rate of *M.rosenbergii* during the early stage of postlarval growth when the PL synthesis take place at a slower rate and after this critical stage endogenous PL derived from the basal lipid source is sufficient to support growth.

5. Survival rate of *M. rosenbergii* was not affected by the deficiency or excess inclusion lecithin in the diet. The result suggests that the diet was not deficient in any of the

nutrients and dietary source of phospholipid over the endogenous level is not essential for survival of freshwater prawns.

6. Moulting rate of *M. rosenbergii* postlarvae fed different test diets was consistant irrespective of lecithin content. Average moulting rate indicated that each prawn moulted at eight day intervals. It is concluded that in freshwater prawn moulting is a process which takes place beyond the influence of lecithin.

7. Moult related abnormalities like exuvia entrapment disease or moult death syndrome were not seen in any of the individuals and the study indicated that deficiency or excess addition of lecithin in the diet was not responsible for these phenomena.

8. Diet provided with 2.5% of lecithin produced significantly lower FCR in the first and second biweekly intervals whereas higher percentages produced high FCR during this period. In the subsequent fortnights all the diets produced similar FCR and it is suggested that addition of lecithin in the diet is beneficial to FCR only during early postlarval growth.

9. Increase in the whole body phospholipid with increase of lecithin in the diet suggests a possible replacement of body phospholipid pool with dietary phospholipids.

10. No other abnormal symptoms like deficiency or excess was noticed in any of the prawns.

11. The study recommends supplementation of lecithin at a lower concentration of 2.5% in the diet as it is essential to accelerate growth during the early PL phase of *M. rosenbergii*.

7. REFERENCES

Ackefors, H., Castell, J.D.Boston, L.D., Racty,P., Sevensson, H.(1992) Standard experimental diets for crustacean nutrition research: growth and survival of juvenile cray fish *Astacus astacus* Linne). fed diets containing various amounts of protein, carbohydrate and lipids. *Aquaculture,* **104**: 341-356.

Aiken, D.E. (1969) Photoperiod endocrinology and the crustacean moult cycle. *Science, N.Y.,* **164:** 49-155.

Alava, V.R. and Pascual, F.P. (1987) Carbohydrate requirements of *Penaeus monodon* (Fabricius) junveniles. *Aquaculture*, **61**: 211-217.

Alava, V.R., Kanazawa, A., Teshima, S.I. and Koshio, S. (1993), Effect of dietary phospholipids and n-3 highly unsaturated fatty acids on ovarian development of kurma prawn. *Bull .Jap. Soc. Sci. Fish.,* **59**: 345-351.

Ali, S.A. (1990) Relative efficiencies of different lipids and lipid levels in the diet of the prawn *Penaeus indicus .Indian.J. Fish.,* **37**: 119-128.

Allen, W.V. (1972) Lipid transport in the dugeness crab *Cancer magister* Dana. *Comp. Biochem. Physiol.,* **43B**: *193-207.*

Andrews, J.W., Sick, L.V., and Baptist, G.L. (1972) Influence of dietary protein and energy levels on growth and survival of penaeid shrimp. *Aquaculture* , **1** : 341-347.

AOAC. (1980) *Official Methods of Analysis of the Association of the Official Chemist.* AOAC 13th edition. Washington., pp. 1018.

AQUACOP. (1978) Study on nutritional requirements and growth of *Penaeus merguiensis* in tanks by means of purified and artificial diets. *Proc. World Maricult. Soc.,* **9**: 225-234.

Baum, N.A., Conklin, D.E. and Chang, E.S. (1990) Effect of dietary lecithin in combination with casein or crab protein in cholesterol uptake and transport in the lobster. *Homarus americanus. J. World Aquacult. Soc.,* **21**: 277-287.

Bianchini, M.L. (1984) Effect of cholesterol in artificial diets for mediteranean prawns. **In**: Y.Taki, J.H. Primavera, J.A. Llobrera (eds.) *Proceedings of the first International Conference on the Culture of Penaeid prawns/shrimps* SEAFDEC, Illoilocity, Philippines. p. 183.

Biddle, G.N. (1977). The nutrition of *Macrobrachium* species. **In:** J.A. Hanson and H.L. Goodwin (editors). *Shrimp and Prawn Farming in the Western Hemisphere.* Hutchinson and Ross Inc.,Pennsylvania. pp. 272-291.

Bilinski, E. (1962) Biosynthesis of trimethylammonium compounds in aquatic animals III: Choline metabolism in marine crustaceans. *J.Fish.Resh.Board.Can.,* **19**: 505-510.

Bliggs, E.G. and Scott, M.A. (1966) Blood lipids of the lobster *Homarus americanus J.Fish.Res.Bd.Can.,* ***23:*** 16z9-1631.

Boghen, A., Castell, J.D. (1980) Consideration of the lecithin and protein requirements of juvenile lobster. *Homarus americanus.* **In:** R.C. Bayer; A.D'Agostino (eds.) *Lobster Nutrition Workshop Proceedings Held at the University of Maime at Orono Jan-15-16* Sea Grant Programme Publ. by Maime Sea. Grant: Walpole M.E. (U.S.A.)*Tech.Rep.Maime.Sea.Grant., **58**: 21-28.*

Bordner, C.E., D'Abramo, L.R., Conklin D.E., Baum, N.A. [1986] Development and evaluation of diets for crstacean aquaculture. *J.World Aquacult. Soc.,* **17:** 44-51.

Bottino, M.R., Gennity, J., Lilly, M.L., Simmoos, E. and Finne, G. (1980) Seasonal and nutritional effect on the fatty acid of three species of shrimps, *Penaeus setiferus, Penaeus aztecus* and *Penaeus duorarum. Aquaculture,* **19:** 139-148.

Bowser, P.R. and Rosemark, R. (1980) Moralities of cultured lobster *Homarus,* associated with a molt death syndrome. *Aquaculture,* **23:** 11-18.

Bray, W.A., Lawrence, A.L. and Lester, L.S. (1990) Reproduction of *Penaeus stylirostris* fed verying levels of total dietary lipids. *J.World Aquacult . Soc,* **21**: 41-52.

Brichon, G.,Chapelle, S., Zwingelstein, G. (1980) Phospholipid composition and metabolism in the haemolymph of *Carcinus maenas* (Crustacea. Decopoda) effect of temperature. *Comp. Biochem. Physiol.,* **67**: 647-652.

Briggs, M.R.P., Jauncey, K. and Brown, J.A. (1988) The cholesterol and lecithin requirement of juvenile prawn *(Macrobrachium rosenbergii)* fed semipurified diet. *Aquaculture,* **70**: 121-129.

Briggs, M.R.P., Brown, J.H., Fox, C.J. (1994) The effect of dietary lipid and lecithin levels on the growth, survival, feeding efficiency, production and carcass composition of post larval *Penaeus monodon* (Fabricius) *Aquaculture and Fisheries Management,* **23**: 279-294.

Brock, J.A. [1983] Diseases [infectious and non-infectious], metazoan parasites, predators and public health consideration in *Macrobrachium* culture and fisheries. **In:** J.P., Mcvey (ed.) C.R.C. Handbook of Mariculture-I, *Crustacean Aquaculture,* CRC Press Inc., Florida p.p. 329-370.

* Cahu, C., Guillaume, J.C., Stephan, G. and Lim, L. (1987) Essentiality of phospholipid and highly unsaturated fatty acid in *Penaeus vannamei* fed purified diets. *World. Aquaculture., Soc., 18th Ann. Con.,* p.22.

Cahu, C., Guillaume, J.C., Stephen, G., Chim, L. (1994) Influence of phospholipid and highly unsaturated fatty acid on spawning rate and egg, and tissue composition on *Panaeus vannamei* fed samipurified diets *Aquaculture,* **126:** 159-170.

Camara, M.R., Tackaert, W., Sorgeloos, P. (1993) Phosphatidylcholine on the growth survival and stress resistance of postlarval *Penaeus japonicus.* **In:** M.Carrillo, L.Dahle, J.Morales, P.Sorgeloos, N.Svennevig, J.Wyban (eds.) *From - Discovery to Commercialization.* Oostende - Beligum, *European. Aquaculture. Soc.,* **19:** p.118.

Castell, J.D. (1982) Fatty acid metabolism in crustaceans. **In**:G.D. Pruder, C.J.Langoon, D.E.Conklin (eds) *Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and physiological Approaches to Shelfish Nutrition.* Lousiana State University. pp.87-105.

Castell, J.D. and Covey, J.F. (1976) Dietary lipid requirements of adult lobsters, *Homarus americanus* (M.E) *J.Nutri.,* **106**: 1159-1165.

Castell, J.D., Mason, E., Covey, J.F. (1975) Cholesterol requirement of juvenile american lobster *Homarus americanus* . *J.Fish. Res. Board. Canada.,* **32**: 1431-1435.

Castell, J.D., Boghen, A.D. (1979) Fatty acid metabolism in juvenile lobster *Homarus americanus* fed a diet low in methionine and istidene. In : J.W., Avarelt, Jr (ed) *Procceedings of 10th Annual Meeting of World Moriculture Society.* p. 720-727.

Castell, J.D., Kean, J.C., D'Abramo, L.R., Lonklin, D.E., (1989) A standard reference diet for crustacean nutrition research I: evaluation of two formulations. *J. World Aquaculture. Soc.,* **20**: 93-99.

* Catacuttan, M.R. (1991) Growth and fatty acid compostion of *Penaeus monodon.* juvenile fed various lipids. *J. Aquaculture. Bamidesh., **43**: 47-56.*

Catacuttan, M.R., and Kanazawa, A. (1985) Effect of dietary fatty acid composition of *Penaeus monodon* juveniles. **In** : Y.Taki, M.Primavera, and Llobrera (eds.) *Proc. First. Int. Con. Culture. of Penaeid prawns/shrimps.* SEAFDEC. Illoilocity, Philippines., p. 185.

Chandge, M.S. (1987) Studies on lipid nutrition in larvae and juveniles of the Indian white prawn, *Penaeus indicus* H.Milne Edwards. *Ph.D.Thesis. Cochin University,* pp: 179.

Chandge, M.S., Raj, R.P. (1993) Studies on dietary lipid requirements of larvae and postlarvae of *Penaeus indicus.* H. Milne Edwards, In N.Natarajan, P.Jayaprakash (eds) *Proceedings of the National Seminar on Aquaculture Development in India, Problems and Prospects* Thiruvananthapuram. pp. 225.

* Chandge, M.S. and Paulraj, R. (1995). Phospholipid: lecithin requirements of larvae of the prawn *Penaeus indicus.* H.Milne. Edwards. Paper presented at National Symposium on Technological Advancement in Fisheries and its Impact on Rural Development Dec. 5-7, Kochi.

Chang, E.S. and O'Conner, J.D. (1983) Metabolism and transport of carbohydrates and lipids. **In:** L.H.Mantel (ed.) *The Biology of Crustacea.* Volume.5. Academic Press, New York. pp. 263-287.

Chapelle, S. (1978) The influence of acclimation temperature on the fatty acid composition of an aquatic crustacean, *Carcinus maenas. J.Exp. Zool.,* **204**: 337-346.

Chapelle, S. (1986) Aspect of phospholipid metabolism in crustacean as related to changes in environmental temperature and salinities. *Comp. Biochem. Physiol.,* **84:** 423-439.

Chapelle, S. and Zwingelstein, G. (1984) Phospholipid composition and metabolism of crustacean gill as related to changes in environmental salinities: relationship between Na^+ K^+ ATPase activity and phospholipids *Comp. Biochem. Phisio.,***78B** *: 363 - 372*

Chapelle, S., Dandrifose, G., and Zwingelstine, G. (1976) Metabolism of phospholipid of anterior or posterior gills of the crab. *Eriocher sinensis.* M.Edw, during the adaptation of the animal to media of various salinities. *Int. J.Biochem.,* **7**: 383-351.

Chapelle, S., Meister, R., Brichon, G. and Zwingelstein, G. (1977) Influence of temperature on the phospholipid metabolism of various tissue from the crab *Carcinus maenas. Comp. Biochem. Physio.,* **58**: 413-417.

Chapelle, S., Zwingelstein, G., Meister, R. and Brichon, G. (1979) The influence of temperature on the phospholipid metabolism of aquatic crustacean *(Carcinus maenas). J.Exp. Zool.,* **210:** 371-381.

Chapelle, S., Brichon, G. and Zwingelstein, G. (1981) Effect of environmental temperature on the phospholipid metabolism of gill mitochondria of *Carcinus maenas. Biochem. System. Ecol.,* **9**: 333-338.

Chapelle, S., Brichon, G. and Zwingelstein, G. (1982) Effect of environmental temperature on the incorporation of H3 elthanolamine in the phospholipids of the tissue of the crab *Carcinus maenas. J.Exp. Zool.,* **229**: 289-297.

* Chapelle, S., Abdul Malak, N and Zwingelstein, G. (1985) Incorporation of w6 polyunsaturated fatty acids into phospholipids of the crab *Carcinus meanas. Biol. Syst. Ecol.* **13**: 459-465.

* Chen, H.Y. (1986) Absorption and retention of dietary fatty acids by marine shrimp *Penaeus monodon.* Paper presented at the Roc - R..S.A. symposium on Aquaculture, Tangkang, Taiwan, September. 3., pp. 231-237.

Chen, H.Y. (1993) Requirement of marine shrimp *Penaeus monodon* juveniles for phosphatidylcholine and cholesterol. *Aquaculture,* **109**: 165-176.

Chen, H.Y. and Jenn, S.S. (1991) Combined effect of dietary phosphatidylcholine and cholesterol on the growth, survival and body lipid composition of marine shrimps, *Penaeus penicillatus. Aquaculture,* **96:** 67-78.

Chen, H.Y. and Jenn, J.S. (1992) Increased pellet water stability by supplementation of phospholipid and its effect on shrimp growth. *J. Asian Fish. Sic.,* **5**: 211-217.

Chiu, Y.N. (1989) Consideration for feeding experiments to quantify dietary requirement of essential nutrients in fish. **In:** S.S. De Silva (ed) *Fish Nutrition Research in Asia. Proceedings of the 3rd Asian Fish Nutrtion Network Meeting.* Asian Fisheries Society

Special Publication. A.F.S., Manila, Philippines, 4: pp. 166.

Clarke, A. and Wickins, J.F. (1980) Lipid content and composition of cultured *Penaeus merguiensis* fed with animal food. *Aquaculture,* **20**: 17-27.

Clifford, H.C. and Brick, R.W. (1978) Protein utilization in freshwater shrimp, *Macrobrachium rosenbergii. Proc. World Maricult. Soc.,* **9**: 195-208.

Colvin, P.M. (1976) The effect of selected seed oils on the fatty acid composition and growth of *Penaeus indicus. Aquaculture,* **8**: 81-89.

Conklin, D.E. (1985) The role of micronutrients in the biosynthesis of the crustacean exoskeleton. *Proc. World Maricult.Soc.* **12**: 125-130.

Conklin, D.E., D'Abramo, L.R., Bordner, C.E. and Baum, N.A. (1980) A successful purified diet for the culture of juvenile lobsters: the effect of lecithin. *Aquaculture,* **21**: 243-249.

Cossins, A.R. (1976) Changes in muscle lipids composition and resistance in the freshwater cray fish. *Austropotamolives pallipes. Lipids,* **11:** 307-316.

D'Abramo, L.R. (1989) Lipid requirement of shrimps. **In :** J.Barret. (ed) *Advances in Topical Aquaculture.* Workshop held in Thati, Frenchpolynesis, Feb-22-March-4, 9: 271-283.

D'Abramo, L.R. and Sheen, H.Y. (1993) Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii. Aquaculture,* **115**: 63-86.

D'Abramo, L.R., Bordner, C.E., Daggett, G.R., Conklin, E.E., Baum, N.A. (1980) Relationship among dietary lipids, tissue lipids and growth in juvenile lobster. *Proc. World Maricult. Soc.,* **11:** 335-345.

D'Abramo, L.R, Bordner, C.E, Conklin, D.E. and Baum, N.A. (1981) Essentiality of deitary phosphatidylcholine for the survival of juvenile lobsters. *Homarus. J. Nutri.,* **111**: 245-431.

D'Abramo, L.R., Bordner,C.E. and Conklin, D.E.(1982) Relationship between dietary phosphatidylcholine and serum cholesterol in lobster *Homarus sp. Marine Biology,* **67***: 231-235.*

D'Abramo, L.R., Bordner, C.E., Conklin, D.E. and Baum, N.A. (1984) Sterol requirement of juvenile lobster *Homarus sp. Aquaculture,* **42:** 13-25.

D'Abramo, L.R., Baum, N.A., Bordner, C.E., Conklin, D.E., Chang, E.S. (1985a) Diet dependent cholesterol transport in American lobster .*J.Exp. Marine Biology Ecol.,* **87**: 83-96.

D'Abramo, L.R., Wright, J.S. Wright, K.H., Bordner, C.E. and Conklin, D.E. (1985b) Sterol requirement of cultured juvenile crayfish *Pacifastacus leniusculus Aquaculture,* **49:** 245-255.

Dall, W. (1981) Lipid absorption and utilization in the Norwegian lobster *Nephros norwegicus. L. J.Exp. Mar. Biol. Ecol.,* **50**: 33-45.

Davis, D.A. and Robinson, E.H. (1986) Estimation of the dietary lipid requirement level of the white crayfish *Procambarus acutus acutus J. World Aquacult. Soc.,* **17:** 37-43.

Deshimaru, O. and Shigueno, K. (1972) Introduction to artificial diet for prawn, *Penaeus japonicus. Aquaculture,* 1: 115-133.

Deshimaru, O. and Kuroki, K. (1974) Studies on purified diet for prawn 1: basal composition of diet. *Bull. Jap. Soc. Sci. Fish.,* **40:** 413-419.

Deshimaru, O. and Yone, Y. (1978) Studies on a purified diet for prawn XII: optimum level of dietary protein for prawn. *Bull.Jap.Soc.Sci.Fish.,* **44**: 1395-1397.

Deshimaru, O., Kurokin, K., Yone, K. (1979) The composition and level of dietary lipid appropriate for growth of nprawn. *Bull. Jap. Soc. Sci. Fish.,* **45**: 591-594.

* Devresse, B., Romdhane, M.S., Bazzi, M., Rasowo,J., Leger,P., Brown,J. and Sorgeloos.P. (1990) Improved larviculture output in the giant freshwater prawn *Macrobrachium rosenbergii* fed a diet of Artemia enriched with n-3 HUFA and phospholipids. *World. Aquacuture,* **21**: 123-125.

Doken, Q.R. and Lawrence, A.L. (1987) Effect of dietary lipid content in particulate diet on the growth, survival and whole body tissue of *Penaeus vennamei* and *Panaeus setiferus.*

J.World Aquaculture. Soc., **18:** 18A.

Douglass, J.S., Conner, W.F. and Lin, D.S. (1981) The biosynthesis, absorption and origin of cholesterol and plant sterols in the Florida land crab *J.Lipid. Res.,* **22:** 961-970.

Durairaj, S., Chandrasekharan, G. and Umamaheswary, R. (1992) Experimental culture of giant freshwater prawn in Tamil Nadu. **In**: E.G. Silas (ed:) *Freshwater Prawns. Proc. Nat. Symp. Freshwater Prawns. Macrobrachium. spp.* Dec. 12-14, Kochi, pp.176-179.

Farkas, T. and Nevenzel, J.C. (1981) Temperature acclimation in the crayfish: effects on phospholipid fatty acids. *Lipids*, **16**: 341-346.

Farkas, T., Nevenzel, G.Y. and Csengeri, I. (1984) Differential response of lipid metabolism and .membrane physical state by an actively and possively overwintering planktonic crustacean., *Lipids,* **6**: 418-422.

Fenucci, L.R., Lawrence, A.L.and Zein - Eldin, Z.P.(1981) The effect of fatty acid and shrimp meal composition of prepared diets on growth of juvenile shrimp, *Penaeus stylirostris. J. World Maricult. Soc.,* **12**: 315 - 324.

Foster, J.R.M. (1976) Studies on the development of compounded diets for prawns. **In**: K.S. Price, W.N.Shaw and K.S. Danberg (eds.) *Proc. First. Int. Cont. Aquaculture, Nutr.,* NOAA (Sea Grant) pp. 229-248.

* Foster, J.R.M. and Beard, T.W. (1973) Growth experiments with the prawn, *Palaemon serratus* Pennant fed with fresh and compounded foods. *Fish. Invest. Minist. Agric. Fish. Food.* (G.B), Ser.II Salmon Freshwater Fish., **27**: 1-16.

Geurden,I., Neto, J.R. and Bergot, P.(1995) Essentiality of dietary phosphalipids for carps (*Cyprinus carpio.* L.) larvae. *Aquaculture,* **131**: 303 - 314.

Gilbert, L.I. and O'Conner, J.D. (1970) Lipid metabolism and transport in arthropods. **In**: M.Florkin and B.T. Sheer (eds.) *Chemical Zoology,* Academic press, New York, **5**: 229-253.

Gomez, G.D., Nakagawa, H. and Kasahara, S. (1988) Effect of dietary protein/starch ratio and energy levels on growth of the gaint freshwater prawn *Macrobrachium rosenbergii. Nippon. Suisan, Gakkaishi,* **54:** 1401-1407.

Gonzalez - Baro, M.D.R. and Pollero, R.J.(1988) Lipid characterization and distribution among tissues of the freshwater crustacean, *Macrobrachium boriellii* during an annual cycle. *Comp. Biochem. Physiol.,* **91 B** : 711 - 715.

Gopakumar, K. and Nair, M.R. (1975) Lipid composition of five species of Indian prawns. *J.Sci.Food. Agric.* **26**: 319 - 325.

Guray, J.C., Kayama, M., Murakami, Y, Ceecaldi, H.J. (1976) The effect of fat-free diet and compound diets supplemented with various oils on moult, growth and fatty acid composition of prawn *Penaeus japanicus Bate. Aquaculture, 7: 245 - 254.*

Hansting, W.H. (1976) Nutrition and fish feed manufacture. **In**: T.V.R. Pillai and A.Gill (eds), *Aquaculture Principles and Practice.* Fishing News Books, London, pp.568-574.

Hepher, B. (1988) *Nutrition of Pond Fishes* Cambridge University Press, Cambridge, pp.388.

Hilton, J.W., Harrison, K.E. and Stanley, J.S. (1984) A, semipurified test diet for *Macrobrachium rosenbergii* and the lack of need for supplemental lecithin. *Aquaculture,* **37**: 209-215.

Jayachandran, K.V. and Jeseph, N.I. (1992) A key for the field identification of commercially important *Macrobrachium* spp. of India with a review of their bionomics. **In**: E.G.Silas (eds) *Freshwater Prawns. Proc. Nat. Symp., Freshwater Prawns. Macrobrachium spp.* Dec -12-14, Kochi, pp. 72-77.

Jones, D.A., Kanazawa, A., Don.K. (1979) Studies on the nutritional requirements of the larval stages of *Penaeus japonicus* using microencapsulated diet. *Mar. Bio.,* **54**: 261-267.

Joseph, J.D. and William, J.E. (1975) Shrimp head oil: a potential feed additive for mariculture. *Proc. World Maricult. Soc.,* **6** : 375 - 381.

Kanazawa, A. (1982) Penaeid nutrition **In**: G.D. Pruder, C.J. Langdon., D.E. Conklin (eds) *Proceedings of the second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition,* Lousiana State University. U.S.A. pp. 87-105.

Kanazawa, A. (1985) Nutrition of penaeid prawns and shrimps. **In**: Y. Taki, J.H.Primavera, J.A.Llobrera (eds.) *Proc. Ist. Int. Con. Cult. Penaeid prawns/shrimps.* SEAFDC, Illoilocity, Philippines, pp.123-130.

Kanazawa, A.(1993) Essential phospholipids of fishes and crustaceans. **In:** S.J. Kanshk, Luguet, P.(eds.) *Fish. Nutrition in Practice*, pp. 519-530.

Kanazawa, A. and Teshima, S. (1971) Conversion of cholesterol to steroid hormones in spiny lobster, *Panulirus japonicus. Bull. Jap. Soc. Sci. Fish.*, **37**: 891-898.

Kanazawa, A. and Teshima, S. (1977) Biosynthesis of fatty acids from acetate in the prawn *Penaeus japonicus. Mem. Fac. Fish. Kagoshima. University.*, **26:** 49-53.

Kanazawa, A. and Koshio, S. (1993) Lipid nutrition of the spiny lobster *Panulirur japonicus* (Decapoda Palinuridae) a review. *Crustaceana.*, **67**: 226-232.

Kanawawa, A., Shimaya, M., Kawasaki, M. and Keshiwada, K. (1970) Nutritional requirements of prawn I : feeding on artificial diet. *Bull. Jap. Soc. Sci. Fish.*, **36**: 949-954.

Kanazawa, A., Tanaka, N., Teshima, S. and Kashiwada, K. (1971) Nutritional requirements of prawn II: requirements for sterols. *Bull. Jap. Soc. Sci. Fish.*, **37**: 211-215.

Kanazawa, A., Teshima, S.I., Sokomoto, Y. and Guray, J.C.B. (1976) The variation of lipids and cholesterol contents in the tissue of prawn *Penaeus japonicus* during the moulting cycle. *Bull. Jap. Soc. Sci. Fish.*, **42**: 1003-1007.

Kanazawa, A., Teshima, S.I. and Tokiwa, S. (1977a) Nutritional requirement of prawn VII: effect of dietary lipid on growth. *Bull. Jap. Soc. Sci. Fish.*, **43**: 849-856.

Kanazawa, A., Tokiwa, S., Kayama, M., Hirata, M. (1977b) Essential fatty acids in the diet of prawn I: effect of linoleic and linolenic acid on growth. *Bull. Jap. Soc. Sci. Fish.*, **43**: 111-114.

Kanazawa, A., Teshima, S., Endo, H., Kayama, M. (1978) Effect of eicosapentaenoic acid on growth and fatty acid composition of the prawn, *Penaeus japonicus. Mem. Fac. Fish. Kagoshima University.*, **27**: 35-40.

Kanazawa, A., Teshima, S.I., Tokiwa, S., Endo, M. and Razek, F.A. (1979a) Effect of short necked clam phospholipid on the growth of prawn. *Bull. Jap. Soc. Sci. Fish.*, **45**: 961-965.

Kanazawa, A., Teshima, S., Tokiwa, S. (1979b) Biosynthesis of fatty acid form palmetic acid in the prawn *Penaeus japonicus. Mem. Fac. Fish. Kagoshima University.*, **28:** 17-20.

Kanazawa, A., Teshima, S., Endo, M. (1979c) Requirement of prawn *Penaeus japonicus* for essential faccy acid. *Mem. Fac. Fish. Kagoshima University.*, **28**: 27-33.

Kanazawa, A., Teshima, S.I., Ono. K., Chalayondya, K. (1979d) Biosynthesis of fatty acids from acetate in the prawns, *Penaeus monodon* and *Penaeus merguiensis. Mem. Fac. Fish. Kagoshima University.*, **28:** 21-26.

Kanazawa, A., Teshima, S., Tokiwa, S., Ceccaldi, H.J. (1979e) Effect of dietary linoleic and linolenic acid on growth of prawn. *Ocenol Acta.*, **2**: 41-47.

Kanazawa, A., Teshima, S., Tokiwa, S., Kayama, H. and Hirata, M. (1979f) Essential fatty acids in the diet of prawn II :effect of docosahexaenoic acid on growth, *Bull. Jap. Soc. Sci. Fish.*, **45**: 1151-1153.

Kanazawa, A., Teshima, S. and Ono. K. (1979g) Relationship between essential fatty acid requirement of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids, *Comp. Biochem. Physiol*, **63:** 295-298.

Kanazawa, A.; Teshima, S.I., Sasada, H and Abdel-Rehman, S. (1982a) Culture of the prawn larvae with micro particulate diet. *Bull. Jap. Soc. Sci. Fish.*, ***42**: 195-199.*

Kanazawa, A., Paulraj, R. Ali, A.S. (1982b) Preparation of artificial diets for nutritional studies. **In**: *Mannual of Research Methods for Fish and Shellfish Nutrition.* CMFRI Special Publication, No:**8**. pp. 90-94.

Kanazawa, A. , Teshima, S.I. and Sakamoto, M. (1985) Effect of dietary lipids, fatty acids and phospholipids on growth and survival of prawn, *(Penaeus japonicus)* larvae. *Aquaculture., **50**: 39-49.*

* Kanazawa,A., Chim,L., Laubier ,A. (1988) Tissue uptake of radioactive cholesterol in the prawn *Penaeus japonicus* Bate. during induced ovarian maturation. *Aquat. Living Resour ., **1** : 27 - 37.*

Kayama ,M. Tsuchiya,Y., Nevenzel, J.C: Fulco,A. and Mead, J.F. (1963) Incorporation of linolenic- 1C14 acid into eicosapentaenoic and docosahexaenoic acid in the fish. *J. American Oil . Chemi. Soc., **40:** 499-502.*

Kayama M., Hirata, M. , Kanazawa,A.,Tokiwa,S., Saito,M (1980) Essential fatty acid in the diet of prawn III. lipid metabolism and fatty acid composition. *Bull. Jap. Soc. Sci. Fish.* **46**: 483-488.

Kean, J.D., Castell, J.D., Boghen, A.G., D'Abramo, L.R. and Conklin, D.E. (1985) A re-evaluation of the lecithin and cholesterol requirement of juvenile lobster, *(Homarus americanus)* using crab protein based diet. *Acquaculture,* **47**: 143-149.

* Kimelberg, H.K. and Papahadjopolus, D. (1972) Phospholipid requirement for Na^{+}+ K^{+} ATPase activity: head group specificity and fatty acid fluidity. *Biochem. Biophys. Acta.,* **282**: 277-292.

Kleinholz, L.H. (1985) Biochemistry of crustacean hormones. **In**: D.Bliss (ed.) *The Biology of Crustacea.* Academic Press, N.Y., pp. 464-522.

* Kontara, E.K. (1986) Preliminary result in larval rearing of tiger shrimps *Penaeus monodon* that receive *Artemia* nauplii fed with lipid containing omega - 3 HUFA. *Bull. Brackishwat. Aquaculture. Dev. Cent.*, Jepara. **8:** 120-126.Kontara, E.K., Nurdjana, M.L. (1992) Grwoth and survival of *Penaeus monodon* postlarvae fed with *Artemia* nauplii enriched with n3-HUFA. *Bull Braklishwat. Aquaculture. Dev. Cent. Jepara.*, **9**: 48-55.

* Kontara, E.K., Nurdjana, M.L. (1992) Grwoth and survival of *Penaeus monodon* postlarvae fed with *Artemia* nauplii enriched with n3-HUFA. *Bull Braklishwat. Aquaculture. Dev. Cent. Jepara.*, **9**: 48-55.

Kompiang, P. (1992) Shrimp feed: phospholipid requirement. **In**: S.A.A. Thobiti, H.M., Al-Ninty, A.Q. Siddiqui, G. Nussain (eds) *Aquaculture Technology and Investment Opportunities. Proc. Ist. Int. Sym. Aquaculture Technology and Investment Opportunities* 11-14 April. Riyadh, Saudi Arabia, pp. 375-378.

* Koshio, S., Kanazawa, A. and Teshima, S. (1992) The effect of protein source and rearing method on the lecithin requirement of the freshwater prawn. *Macrobrachium rosenbergii.* **In**: Abstracts of the 3rd Asian Fisheries Forum. Oct. 26-30, 1992, Singapore, AFS, Manila, Philippiens.

Krzynowek, J., Wiggin, K., Donahue, P. (1982) Cholesterol and fatty acid content in three species of crab found in North East Atlantic. *J.Food. Sci.*, **47:** 147-156.

* Lanchaise, F., Le-Roux, A., Hubert, M., Lafont, R. (1993) The moulting gland of crustaceans: localization, activity and endocrine control. (a review). *J.Crustacean, Biol.*, **13**: 198-243.

Lee, D.J. and Putnam, G.B. (1973) The response of rainbow trout to varying protein/energy ratio in a test diet. *J.Nutr.*, **103**: 916-922.

Liao, I.C. and Liu, F.G. (1989) A breef review of nutritional studies for *Penaeus monodon.* **In**: J. Bareej (ed.) *Advances in Tropical Aquaculture.* IFREMER, Tahiti, French polynesia, pp.355-380.

* Lui, C.W., Sage, B.A. and O'Connor, J.D. (1974) Biosynthesis of lipovitellin by crustacean ovary

J.Exp. Zool., **188**: 289-296.

Lytle, J.S., Lytle, F. and Ogle, J.T. (1990) Polyunsaturated fatty acid as a comparative tool for assessing maturation diets of *Penaeus vannamei. Aquaculture,* **89**: 287-299.

Martin, B.J. (1980) Growth and fattyacid, of *Palaemon serratus* (Crustacea, Decapoda) fed with componded diets containing different proportions of linoleic an linolenic acids. Aquaculture **19**: 325-337.

* Mendoza, E.C. (1982) Quantative dietary lipid requirement of *Penaeus monodon* in a controlled environment. *M.S.Thesis* University of Philippines. pp.43.

Meusy, J.J., (1980) Vitellogenisis, the extraovarian precursor of the protein yolk in crustacea: a review. *Reprod. Nutri. Develop.,* **20** : 2:22.

Meyers, P.S. (1989) Squid protein and soylecithin as beneficial additives in shrimp diets. *INFOFISH - INT.*, **2** : 22 - 24.

Middleditch, B.S., Missler, S.R., Ward, D.G., Mc Vey, J.P., Brocon, A. and Lawrence, A.L. (1979) Maturation of penaeid shrimps : dietary fatty acids. *Proc : World.Maricult.Soc.*,10:472-476.

Middleditch, B.S., Missler, S.R., Hines, H.H., Mc Vey, J.D., Brown, A., Ward, D.G. and Lawrence, A.L. (1980) Metabolic profiles of penaeid shrimp. Dietary lipids and ovarian maturation.,. J. *Chromatogr.* ,**195** : 359-368.

Millamena, O.M and Quinitio, E.T., (1985) Lipid and essential fatty acid in the nutrition of *Penaeus monodon* larvae. **In**: Y.Taki., J.H.Primavera, J.A.Llobera (eds.) *Proc. Ist Int.*

Con Culture of Penaeid praws/shrimps . Illoilocity, Philippines pp:181.

Millamena, O.M., Primavera, J.H., Pudadera, R.A., Caballero, R.V., (1986). The effect of diet on the reproductive performance of pond reared *Penaeus mondon.* (Fabricius) broodstock. **In**: J.L. Maclean, L.B.Dizon, L.V. Hosillos (eds) *Proceedings of the First Asian Fisheries Forum,* Manila, Philippines. 593-596.

Millamena, S.M. (1989) Effect of fatty acid composition of broodstock diet on tissue fatty acid pattern and egg fertilization and hatching on pond reared *Penaeus monodon. Asi. Fish. Sci.,* **2:**127-134.

Morris, R.J. and Sargent. J.R (1973) Studies on the lipid metabolism of some oceanic crustaceans. *Mar. Biol.,* **22 :** 77-83.

Nair, C.M. and Thampy, D.M. (1987) Hatchery production of the seed and culture of *Macrobrachium rosenbergii. Proceeding of the Seminar on Problems and Prospects of Freshwater Aquaculture in Kerala*, Kerala Agricultural University. Kochi.pp 78.

Nair, C.M., Thampy, D.M., Sebastian, M.J. and Syamalal, P. (1989) Mass production of the seed of giant freshwater prawn *Macrobrachium rosenbergii* (De Man). *Proceeding of the National Seminar on Forty years of Freshwater Aquaculture in India.* Association of aquaculturist and CIFA Nov. 7-9 Kausalyaganga, Bhubeneswar.

New, M.B. (1976) A review of dietary studies with shrimps and prawns. *Aquaculture,* **9** : 101-104.

New, M.B. (1987) *Feed and Feeding of Fish and Shrimp. FAO* , Rome, ADCP/REP/87/26 pp. 275.

New, M.B. (1990) Freshwater prawn culture : A review. *Aquaculture,* **88** : 99-143.

New, M.B. (1995) Status of freshwater prawn farming : a review. *Aquaculture Research,* **26** : 1 - 60.

Nicol, J.A.C (1975) The tapetum lucidum in the eyes of cusk-eels (Ophiidae). *Can. J. Zool.*, **53** : 1063-1079.

O' Connor, J.D and Gilbert, L.I (1968) Aspect of lipid metabolism in crustaceans *Am. Zool.* **8**: 529-539.

O' Connor, J.D. and Gilbert, L.I (1969) Alterations in lipid metabolism associated with premolt activity in a land crab and freshwater cray fish. *Comp. Biochem. Physiol.* **29** : 889-904.

Pascual, F.P., Colaso, R.M and Tamse, C.T. (1983) Survival and some histological changes in *Penaeus monodon* (Fabricius) juveniles fed various carbolydrates. *Aquaculture,* **31** : 169-180.

Pascual, F.P. (1985) Lecithin requirement of *Penaeus monodon* juveniles. **In** : Y. Taki., J.H.Primavera., J.A.Llobrera (eds.) *Proc. Ist Int. Con. Cult Penaeid prawns/shrimp* SEAFDC, Illoilo city, Philippines pp. 181.

Pascual, F. (1986) Effect of supplemental lecithin and lipid sources on the growth and survival of *Penacus monodon* juveniles. In :J.L. Mallean., L.B. Dizon., L.V. Hosillos (eds) *Proc. Ist Asian Fisheries Forum.* Manila, Philippines. pp. 615-618.

Paulraj. R (1987) Lipid and essential fattyacid requirements of fish and shellfish. **In**: *Summer Institiute of Recent Advances in Finfish and Shellfish Nutrition* 11-30 may, CMFRI, Kochi. pp. 1-30.

Pearson, P. (1976) *The Chemical Analysis of Food.* Churchil, London. pp. 575.

Pe'queux, A and Gilles, R. (1981) Na^+ fluxes across isolated prefused gill of the chinese crab. *Eriocheir sinensis. J. Exp. Biol.,* **92**: 173-186.

Pe'queux, A and Chapelle, S. (1982) (Na^+ + K^+) - ATPase activity and phospholipids in two euryhaline crabs as related to changes in the environmental salinity *Mar. Biol Lett.*, **3** : 43-52.

Pe' queux, A., Chappel,S., Wanson, S., Coffinet, G. and Francois, C. (1983) Na^{+}_{+} K^{+}) ATP ase activity and phospholpids content of various fractions of *Carcinus maenas* and *Eriocheir sinensis* posterior gills. *Mar. Biol.,* **4**: 267 - 279.

Petriella, A.M. (1990) Study of the moulting cycle of the Argentine prawn. *Artemesia longinaris* Bate.III. Influence of cholesterol. *J.Aquacult. Trop.*, **6** : 77-86.

Primavera, J.H. (1985) A revew of maturation and reproduction in closed thelycum penaeids **In**: Y.Taki, J.H. Primavera and J.A. Llobera (eds.) *Proc. Ist Int. Conf. Cult Penaed prowns/ shrimps* SEAFDEC, Illoilocity, Philippines., 37-57.

Ponat, A. and Adelung, D. (1983) Studies to establish an optimal diet for *Carcinus maenas.* vitamin and lipid requirements. Mar. Biol., **74** : 275-279.

Ra'anan, Z., Cohen, D., Rapport, U. and Zohar, G. (1984) The production of freshwater prawn *Macrobrachium rosenbergii* in Israel : the effect of added substrate on yield in a monoculture system. *Bamidegh.,* **36** : 35-40.

Ramesh, M.X., Kathiresan, K. (1992) Mangrove cholesterol in the diet of penacid prawn, *Penaeus indicus. Indian. J. Mar. Sci.*, **21** : 164-166.

Read, G.H.L. (1981) The response of *Penaeus indicus* (Crustacea : Penaeidae) to purified and compounded diet of varying fatty acid composition. *Aquaculture,* **24** : 245-256.

Rees, J.F., Cure, K., Piyatiratitudrakul, S., Sorgeloos, P., Menasveta, P. (1994) Highly unsaturated fatty acid requirements of *Penaeus monodon* postlarvae : an experimental approach based on Artemia enrichment. *Aquaculture*, **122** : 193-207.

Reigh, R.C and Stickney, R.R. (1989) Effect of purified dietary fatty acids on the fatty acid composition of freshwater shrimp *Macrobrachium rosenbergii., Aquaculture.*, **77** : 157-174.

* Romdhane, M.S., Devresse, B., Leger, P. and Sorgeloos, P. (1994) Effect of feeding (n-3) HUFA enriched *Artemia* during a progressively increased period on the larviculture of

the freshwater prawn *Macrobrachium rosenbergii.J.World Aquacult . Soc.*, **23** : 40 - 46.

Rudolph, P.H. and Spaziani , E. (1992) Formation of ecdysteroids by Y organ of the crab. *Menippe mercernaria:* incorporation of cholesterol in to 7 dehydrocholesterol and secreation products *in vitro. Gen. Comp. Endocrinol.* **88** : 235-242.

Rudolph, P.H., Spaziani, E., Wang, W.L (1992) Formation of ecdysteroids by Y organs of the crab, *Menippe mercennaria :* Biosynthesis of 7 dehydrocholesterol *in vivo. Gen. Comp. Endocrinol.*, **88** : 224-234.

Sandifer, P.A and Joseph, J.D (1976) Growth responses and fatty acid composition of juvenile prawns *(Marobrachium rosenbergii)* fed a prepared ration augmental with shrimp head oil. *Aquaculture* , **8** : 129-138.

* Sarac, Z., Thaggard, H., Saunders,J., Gravel, M., Neill, A., Cowan, R.T. (1993) Observation on the chemical composition of some commercial prawn feeds and associated growth response in *Penaeus monodon. Aquaculture,* **115** : 97 - 110.

Sargent, J.R. (1976) The structure, metabolism and function of lipids in marine organism. **In** : D.C. Molins and J.R.Sargent (Eds.) *Biochemical and Biophysical Perspectives in Marine Biology.* Aecademic Press, London. **3** : 150 - 212.

Sebastian, M.J. (editor) (1990) *The Giant Freshwater Prawn Macrobrachium rosenbergii* (De Man). Kerala Agricultural University, College of Fisheries, Kochi, India. pp.43.

Sheen.S. and D'Abramo, L.R. (1991) Response of juvenile freshwater prawn. *Macrobrachium rosenbergii* to different levels of cod liver oil/corn oil mixture in semi-purified diet. *Aquaculture.*, **93** : 121-134.

Sheen.S.S. and Chen,S.J. (1992) Optimum dietary lipid requirement level of the tiger prawn, *Penaeus monodon. Aquaculture* , 92 : *Growing - Towards the 21st Centuary.* Keelung .,Taiwan. pp. 203.

Sheen.S.S., Liv. P.C., Chen.S.N., Chen.J.C.(1994) Cholesterol requirement of juvenile tiger shrimp *Penaeus monodon. Aquaculture,* **125** : 131-137.

Sherief, P.M., Nair,C.M. and Malika, V. (1992) The cholesterol requirement of larval and post-larval prawn. *(Macrobrachium rosenbergii).* **In** : E.G.Silas (ed) *Freshwater prawns. Proc. Nat. Symp. Freshwater Prawn (Macrobrachium spp.)* 12-14 Kochi. pp. 213-217.

Shewbart,K.L. and Mies.W.L. (1973) Studies on nutritional requirement of brown shrimp. The effect of linolenic acid on growth of *Penaeus aztecus. Proc. World. Maricult. Soc.* **4** : 277-287.

Shiek,H.S. (1969) The biosynthesis of phospholipid in the lobster *Homarus americanus. Comp. Biochem. Physiol.,* **30** : 69-684.

Sick,L.V., and Andrews,J.W. (1973) Effect of selected dietary lipids, carbohydrates and protein on the growth, survival and body composition of *Penaeus duorarum. Proc. World. Moricult. Soc.,* **4** : 263-276.

Smith,I.J. and Sandifer,P.A. (1975) Increased production of tank-reared *Macrobrachium rosenbergii* through the use of artifical substrate. *Proc. World. Maricult. Soc.,* **6** : 55-56.

Snedecor,G.W. and Cochran,G. (1968) *Statistical Methods* Oxford and IBH publishing Co. New Delhi. pp. 593.

Stahl,M.S. and Aheran,G.A. (1978) Amino acid studies with juvenile *Macrobrachium rosenbergii. Proc. World. Maricult. Soc.,* **9** : 209-216.

Tackaert,W., Camara,M.R., Sorgeloos,P. (1991a) The effect of dietary phosphatidylcholine in postlarval penaeid shrimp, diet preparation. **In**: P.Lavens., P.Sorgeloos, E.Jaspers, and F.Ollevier (eds) *Larvi-91, Spec. Pub. Eur. Aquacult. Soc.,* **15** : 76-79.

Tackaert,W., Camara,M.R., Sorgeloos,P. (1991b). The effect of dietary phosphatidycholine in postlarval penaeid shrimp, preliminary culture results. **In**: P.Lavens, P.Sorgeloos, F.Jaspers and F.Ollevier (eds) *Larvi-91 Spec. Pub. Eur. Aqaacult. Soc.,* **15** : 80-83.

Takeda, M., Shimeno,S., Hosokawa,H., Kajiyama,H., and Kaisyo,T. (1975). The effect of dietary caloric to protein ratio on the growth, feed conversion and body composition of young yellow tail. *Bull. Jap. Soc. Sci. Fish.*, **41** : 443-447.

Takeuchi,T., Watanabe,T., and Ogino,C. (1978 a) Studies on nutritive value of dietary lipids in fish. XII : optimum ratio of protein to lipid in diets of rainbow trout. *Bull. Jap. Soc. Sci. Fish* **44** : 683-688.

Takeuchi,T., Watanabe,T., and Ogino,C., (1978 b) Availability of carbohydrate and lipid on dietary energy sources for carp. *Bull. Jap. Soc. Sci. Fish.*, **45** : 977-982.

Teshima,S. (1982) Sterol metabolism **In**: G.D.Pruder., C.J.Langolan and D.E.Conklin (eds) *Proc. IInd Int. Conf. Aquacult. Nutri : Biochemical and Physiological Approaches to Shell-fish Nutrition.* Lousiana State Univ., Benton. Rovge. p. 205-216.

Teshima,S. and Kanazawa,A. (1971a) Biosynthesis of sterol in the lobster *Panulirus japonicus,* the prawn *Penaeus japonicus* and the crab *Protunus trituberculateus. Comp. Biochem. Physiol.* **38** B : 597-602.

Teshima,S. and Kanazawa,A.(1971 b) Bioconversion of the dietary ergosterol to cholesterol in *Artemia salinla Comp. Biochem. Physiol.*, **37** B : 603-607.

Teshima,S. and Kanazawa,A. (1971 c) Bioconvesion of the dietary brassicasterol to cholesterol in *Antemia salina. Bull. Jap. Soc. Sci. Fish.*, **38** : 1305-1310.

Teshima,S. and Kanazawa,A. (1973) Metabolism of demosterol in the prawn, *Panaeus japonicus Mem. Fac. Fish. Kagoshima. Univ.*, **2** : 15-19.

Teshma,S. and Kanazawa,A. (1978 a) Haemolymph lipids of the prawn. *Bull. Jap. Soc. Sci. Fish.*, **44** : 925-932.

Teshima,S. and Kanazawa,A. (1978 b) Release and transport of lipids in the prawn. *Bull. Jap. Soc. Sci. Fish.*, **44** : 1269-1274.

Teshima,S.I. and Kanazawa,A. (1979) Lipid transport mechanism in the prawn. *Bull. Jap. Soc. Sci. Fish.*, ***45*** : 1341-1346.

Teshima, S.I. and Kanazawa,A. (1980) Transport of dietary lipids and role of serum lipoproteins in the prawn. *Bull. Jap. Soc. Sci. Fish.*, **46** : 51-55.

Teshima,S. and Kanazawa,A. (1983) Variation in lipid composition during the ovarian maturation of the prawn. *Bull. Jap. Soc. Sci. Fish.*, **49** : 957-962.

Teshima,S. and Kanazawa,A. (1984) Effect of protein, lipid and carbohydrate levels in purified diet on growth and survival rate of the prawn larvae. *Bull. Jap. Soc. Sci. Fish.* **50** : 1709-1715.

Teshima,S. and Kanazawa,A. (1986) Nutritive value of sterols for the juvenile prawn. *Bull. Jap. Soc. Sci. Fish.*, **52** : 1417

Teshima,S., and Kanazawa,A., (1987) Turnover of dietary cholesterol and beta sistosterol in the prawn. *Bull. Jap. Soc. Sci. Fish.*, **53** : 601-607.

Teshima,S., Kanazawa,A., Sasada,H. and Kawasaki,M. (1982) Requirements of the larval prawn *Penaeus japonicus* for cholesterol and soyabean phospholipid. *Mem. Fac. Fish. Kogoshima. Univ.*, **31** : 193-199.

Teshima,S.I., Kanazawa,A., Sasada,H. (1983) Nutritional value of dietary cholesterol and other sterols to larval prawn, *Penaeus japonicus* Bate. *Aquaculture*, **31** : 159-167.

Teshima,S.I., Kanazawa,A., and Kakuta,Y. (1986 a) Effect of dietary phospholipids on growth and body composition of juvenile prawn. *Bull. Jap. Soc. Sci. Fish.*, **52** : 155-158.

Teshima,S.I., Kanazawa,A., and Kakuta,Y. (1986 b) Effect of dietary phospholipids on lipid transport in the juvenile prawn. *Bull. Jap. Soc. Sci. Fish.*, **52** : 159-163.

Teshima,S.I., Kanazawa,A., and Kakuta,Y., (1986 c) Growth, Survival and body lipid composition of the prawn larvae receving several dietary phospholipids. *Mem. Fac. Fish. Kagoshima. Univ.*, **35** : 17-27.

Teshima, S., Kanazawa, A. and Kakuta,Y. (1986 d.) Role of dietary phospholipid in the transport of (super 14C) cholestorol in the prawn. Bull. Jap. Soc. Sci. Fish., **52**: 719 - 723.

Teshima,S., Kanazawa.A., Hurinouchi,K., Koshid,S. (1988 a) Lipid metabolism in destalked prawn. *Penaeus japonicus:*induced maturation and transfer of lipid reserves to the ovaries. *Bull. Jap. Soc. Scil Fish.,* **54** : 1123-1129.

Teshima, S., Kanazawa, A. Koshio, S. and Horinouchi, K.(1988 b) Lipid metabalism in destalked prawn *Penaeus japonicus* : induced maturation and accumulation of lipid in the ovaries. *Bull. Jap. Soc. Sci. Fish.,* **54** : 1115 - 1122.

Teshima,S., Kanazawa,A., Koshio,S., Kondo,N. (1989) Nutritive value of sistosterol for the prawn, *Penaeus japonicus Bull. Jap. Sco. Sci. Fish.,* **55** : 153-157.

Teshima,S.I., Kanazawa,A., Hitottsumatsu,K.I., Kim,K.S., Oshida,K., Koshio,S. (1992 a) Tissue uptake and bioconversion of eicosapentaenoic acid and phosphotidycholine in prawns *Penaeus and Macrobrachium. Comp. Biochem. Physiol.,* **102 B** : 885-890.

* Teshima,S., Kanazawa.A., and Koshio,S., (1992 b) Ability for bioconversion of n-3 fatty acids in fish and crustaceans. *Oceanis* .**18** : 67-75.

Teshima,S.J., Kanazawa,A., Koshio,S. (1993) Recent developments in nutrition and microparticulte diets of larval prawns. *J.Aquacult - Bamidgel.,* **45** : 175-184.

Tridel,D.J., and Castell,J.D. (1980) Some current findings of the lobster nutrition. **In** : R.C.Bayer., and D'Agostino (eds.) *1980 Lobster Nutrition Workshop, Proceedings Technical Report* 58 Maine Sea Grant Publication., pp. 36-40.

Van Derhost,D.J. (1973) Fatty acids of the female horseshoe erab. *Xiphosura polyphemus (Limulus). Mar. Biol.,* **20** : 291-296.

Wang,Y., Liv,J., Sun,W. (1992) The requirement of fatty acid, cholesterol and phospholipids for fishes and prawns. *Can. Transl. Fish. x Aquat. Sci.,* **5570** : pp. 15.

* Watanabe, T. (1991) Importance of docosahexaenoic acid in marine larval fish. **In** : P.Lavens, P.Sorgeloos, E. Jaspers, F.Ollevier (eds.) *Larvi - 91, Spec. Pub. Eur. Aquacult. Soc.*, **15**: 19.

Whitney,J.O. (1974) The effect of external salinity upon lipid synthesis in the blue crab *Callinectes sapidus* Rathbun and in the spider crab *Libnia emerginate* Leech. *Comp. Biochem. Physiol.*, **49** : 433-440.

Xu,X.L., Ji,W.J., Castell,J.D., O'Dor,R. (1993a) The nutritional value of dietary n-3 and n-6 fatty acids for the chinese prawn. *(Penaeus chinensis) Aquaculture.*, **118** : 277-286.

* Xu,X.L., Ji,W.J., Castell,J.D., O'Dor,R.K., (1993b) Essential fatty acid requirement of chinese prawn *(Penaeus chinesis).* In:M.Carrilo., L.Dahee., J.Morales., P.Sorgeloos., N,Svennevig., J,Wyban (eds) *From Discovery to Commercialization, Euro . Aquacult. Soc.*, **19** : 182.

Xu,X.L., Ji,W.J., Castell,J.D., and O'Dor,R.K., (1994) Influence of dietary lipid sources on fecundity , egg hatchability and fatty acid component of chinese prawn *Penaeus chinensis* broodstock *Aquaculture.*, **199** : 359-370.

Yates,A., Michael,E., Chanmberlain,G.W., and Miget,R.J. (1987) Effect of long chain highly unsaturated fatty acids on penaeid larval nutrition. *Proc. 18th Ann. Mer. World. Aquacult. Soc.*, **18** : 14 A.

Zandee,D.E. (1966) Metabolism of the crayfish *Astacus astacus.L.* The fatty acid composition and the biosynthesis of the fatty acids. *Achive of International Physiology and Biochemistry,* 74 : 614-626.

Zandee,D.I. (1967) Absence of cholesterol synthesis as contrasted with the presence of fattyacid synthesis of some arthropods. *Comp. Biochem. Physio.*, **20** : 811-822.

Zhou,H., Wang,Y. (1992) Nutritional requirement in cholesterol and phospholipid for the chinese prawn. *(Penaeus orientalis) Can. Transl. Fish. Aquat. Sci.*, 5568 pp 14.

Zilversmith, D.B. and Davis, A.K.(1950) Microdetermination of phospholipid. *J.lab. Clin. Med.* **35**: 155 - 161.

* Not consulted in orginal.

EFFECT OF DIFFERENT DIETARY LEVELS OF LECITHIN ON GROWTH, SURVIVAL, MOULTING AND BODY PHOSPHOLIPID LEVELS IN *MACROBRACHIUM ROSENBERGII* POST LARVAE

By

MAHESH.S, B.F.Sc.

ABSTRACT OF THE THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE

MASTER OF FISHERIES SCIENCE

FACULTY OF FISHERIES

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

PANANGAD, COCHIN

1996

8. ABSTRACT

The effect of dietary levels of lecithin on growth, survival, moulting, FCR and body phospholipid levels of early postlarval freshwater prawn *Macrobrachium rosenbergii* was examined using semi-purified diets in a 56 day feeding experiment.

Five casein-egg albumin based semi-purified diets incorporated with 0,2.5,5, 7.5 and 10% of purified soy lecithin (60% phosphatidylcholine) were formulated and fed to prawns (ten numbers per treatment) with an initial mean weight of 0.05386g. In order to examine the influence of lecithin at different growth stages of postlarvae the assessment was made every fourteen days during the study period.

The result showed that although the overall growth performance was not affected by dietary inclusion of lecithin, SGR% of the prawns fed with the diet containing 2.5% of lecithin showed a significant difference at $P<0.01$ level during the first fourteen days of growth. Similarly the FCR also showed significant variation ($P<0.01$) between the diets tested in the first and second fortnights (14th and 28th day). In the first fortnight the diet with 2.5% of lecithin (D_2) produced lower FCR, whereas in the second fortnight the first three diets (D_1, D_2 and D_3) produced consistent FCR, while the diets supplemented with higher levels produced higher values.

No significant differences ($P<0.01$) between treatments were detected with regard to survival rate, moulting frequency and body phospholipid levels of the prawns with the

termination of the experiment . The prawns moulted once in every eight days and no abnormalities like exuvia entrapment were noticed in any of the experimental animals. Increment of body phospholipids with respect to levels of dietary lecithin suggests a possible replacement of body PL pool with that of dietary PL.

The result of the study suggests that supplementation of lecithin at a level of 2.5% in the diet can accelerate growth and improve FCR during the early postlarval phase of *Macrobrachium rosenbergii* (ie., upto 4 weeks after larval settlement); beyond this, supplementation of lecithin is not needed in the diet . No other deficiency or excess symptoms was detected in the experimental prawns and it is found that lecithin is not responsible for Exuvia Entrapment Disease in *Macrobrachiam rosenbergii* postlarvae.