

**Proceedings of the Summer Institute in  
Recent Advances in Finfish and Shellfish Nutrition**

**11 TO 30 MAY 1987**



**CENTRAL MARINE FISHERIES RESEARCH INSTITUTE  
Dr. SALIM ALI ROAD  
COCHIN-682 031**

SUMMER INSTITUTE IN  
RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

11-30 May, 1987

---

LIPIDS AND ESSENTIAL FATTY ACID REQUIREMENTS  
OF FISH AND SHELLFISH

R. PAUL RAJ

Nutrition Section  
Central Marine Fisheries Research Institute  
Cochin-682 031.

INTRODUCTION

Lipids, the water insoluble biomolecules were considered as mere sources of energy for animals, until Burr and Burr (1930) for the first time demonstrated the need for a essential fatty acid in the diets of rates. Researches carried out during the past three decades revealed the significance of lipids in the nutrition of fish and shellfish as important sources of energy (8 to 9 Kcal/g ME) and fatty acids essential for normal growth and survival of both finfish and shellfish. Besides these functions, they do have important dietary roles as carriers of certain non-fat nutrients, notably the fat-soluble Vitamins A, D and K. The unsaturated fatty acids play an important role in the transportation of other lipids.

Recent studies indicate that sterols and phospholipids are essential dietary nutrients for crustaceans. Phospholipids and sterol esters, play a vital role in the structure of biomembranes at both the cellular and sub-cellular levels. Lipids are also important in the flavour and textural properties of the feed consumed by finfish and crustaceans.

Lipids are involved in many other aspects of metabolism; for example many of the hormones are steroids. Besides, the long-chain polyunsaturated fatty acids (PUFA) are precursors for prostaglandins in fish and crustaceans. Tanaka and Strickland (1965) have shown that the  $\text{Na}^+ \text{K}^+$  - activated ATPase require a phospholipid for its normal function; either phosphatidylserine or phosphatidylglycerol are effective in activating this enzyme (Kimelberg and Papahadjopolus, 1972). Further the work of Nicol et al. (1975) showed the possible role of PUFA of the w3 series in brain and nerve activity. In the case of crustaceans Lester et al. (1975) have shown by in vitro experiments that N- (N-dodecanoyl sarcosyl taurine (DST), the crustacean emulsifier require cis-dococ - 5 enoic acid as a constituent.

#### ENERGY VALUE OF DIETARY LIPIDS

Dietary lipids serve as sources of metabolic energy. In most ingredients they are 85-90% digestible by fish. Experiments with cultured fish have shown that the optimal lipid intake is essentially similar to that for wild fish (Cowey and Sargent, 1979). Lee and Putnam (1973) raised rainbow trout at 12°C on diets containing up to 24% dry weight as herring oil, with excellent feed and energy conversions as well as growth rates. Adron et al. (1976) fed diets containing up to 9% lipid and found that the weight gain and protein utilization of the fish to increase up to the maximum level of lipid used. Channel catfish have been successfully grown on diets containing 10-12% lipid (Stickney and Andrews, 1972). Increasing the energy level of the diet using lipid at constant protein always resulted in improved protein utilization. According to Cowey and Sargent (1979) lipids not less than 10% and not more than approximately 20% can be added to fish diets with excellent results. However, recent

studies conducted at CMFRI, Cochin indicates that the fry and fingerlings of mullets, Liza macrolepis and Liza parsia, as well as the milk fish, Chanos chanos require relatively low lipid levels in the diet for maximum growth. Lipid levels above 6% in these species did not significantly enhance growth or improve the food conversion ratio. In all the species lipid levels above 9% in the diet resulted in growth retardation. The differences observed can be mainly be attributed to the feeding habits of the fish species.

The main protein sparing effect of dietary lipids is to replace protein which could otherwise have been catabolized and used both for energy and to synthesize lipid. The sparing of dietary protein by lipids has been extensively investigated in various species of fish (Lee and Putnam, 1973; Bromely, 1980). Takeuchi et al. (1978) have reported an optimum ratio of protein to lipid in diets of rainbow trout as 35:15-20%. The protein concentration can be decreased by approximately 15% in rainbow trout diets if high quality lipids, capable of satisfying the EFA requirements of the fish, are added at the level of approximately 18%. Takeda et al. (1975) have reported that the protein level of yellow tail diets can be reduced from 70 to 55% without retardation of growth, if the caloric content was maintained at a high level with pollock liver oil (Takeda et al., 1975). On the other hand, omnivorous fish such as the carp can utilize effectively both carbohydrate and lipids as dietary energy sources. Addition of lipid at levels of 5-15% to diets resulted in no improvement in growth, feed conversion, or the value of NPU, when the dietary protein level remained around 32% (Takeuchi et al., 1979a). Protein to energy ratio requirements of channel catfish fingerlings as a function of protein deposition were found to be 88 mg protein/kcal, between dietary energy concentrations of 275

to 341 kcal/100g (Garling and Wilson, 1976). Striped bass fingerlings reared at 20.5°C and fed 37, 47 or 57% protein with 7, 12 or 17% lipid in a 3 X 3 factorial design showed maximal protein sparing action of lipid for growth when fed 12% lipid combined with 47% dietary protein or 17% lipid combined with 57% protein (Millikin, 1982).

As far as crustaceans are concerned the quantitative dietary lipid requirements have been worked out for very few species. However, several workers used lipids derived from plant products, animal products and their mixtures, according to the availability in local areas. While reviewing the nutritional requirements, Forster (1976) reported that penaeid prawns do not require high levels of dietary lipids and suggested that optimum level may fall between 5 and 10% in the various species. Kanazawa et al. (1970) reported better growth of P. japonicus when fed a purified diet with 8% Soybean oil as the lipid source. In P. merguensis better growth was observed with 7% codliver oil (Aquacop, 1978). Studies on P. indicus showed that larvae and post-larvae require almost 10% lipid, whereas advanced postlarvae and juveniles require about 10-12% lipid for promoting maximum growth, food and protein conversion, and protein retention (Chandge, MS). Studies with P. indicus (Chandge, MS) and Macrobrachium rosenbergii (Clifford and Brick, 1978) have shown that protein utilization is improved by sufficient amount of fat and carbohydrate.

#### ESSENTIAL FATTY ACIDS

Fatty acids occur in very large amounts as building block components of saponifiable lipids and only traces occur in free form in cells and tissues. About 100 different kinds of fatty acids have been isolated from lipids of various animals and plants. All possess a long hydrocarbon chain

and a terminal carboxyl group. The hydrocarbon chain may be saturated without any double bond as in palmitic acid or it may have one double bond as in oleic acid then it is called as monounsaturated or monoenic fatty acid. When two or more double bonds are present in the hydrocarbon chain, it is known as polyunsaturated fatty acid (PUFA), such as linoleic (18:2 w6) and linolenic acid (18:3 w3). Sometimes unsaturated hydrocarbon chain may have 20 or more carbon atoms then it is called as highly unsaturated fatty acid (HUFA) such as eicosapentaenoic (20:5 w3) and docosahexaenoic acid (22:6 w3). Unsaturated fatty acids have lower melting points than saturated fatty acids of the same chain. So they are abundant in marine animals and plants (Sargent, 1976). Certain fatty acids have specific nutritional importance which are not biosynthesized de novo are called as "Essential Fatty Acids" (EFA). These fatty acids have to be included in the diets for normal survival, growth, maintenance and proper functioning of physiological processes (Alfin-Slater and Aftergood, 1968).

In both fresh water and marine fish the w3 acids predominate although substantial amounts of w6 acids are also present. In marine fish, however, the level of w6 PUFA are significantly low, so that the ratio w3/w6 is substantially higher in marine fish than in freshwater fish (Ackman, 1967). Most of the marine penaeid prawns also have high contents of w3 HUFA.

Mead and Kayama (1967) reviewed the fatty acid metabolism in fish. Fish are able to synthesise, de novo from acetate, the even-chain saturated fatty acids, as shown in Fig. Radio tracer studies have shown that fish can convert 16:0 to the w7 monoene and 18:0 to the w9 monoene. The w5, w11 and w13 monoenes are proposed based on the identification

of these isomers in the monoenes of herring oil (Ackman and Castell, 1966). Fish are unable to synthesize any fatty acids of the w6 or w3 series unless a precursor with this 'w' structure is present in the diet. Fish are able to desaturate and elongate fatty acids of the w9, w6 or w3 series as outlined in Fig. 1. The ability to elongate and desaturate fatty acids is not the same in all species of fish. The turbot was able to desaturate and elongate only 1-15% of 18:1w9, 18:2w6 or 18:3w3, while in the rainbow trout, 70% of 18:3 w3 was converted to 22:6w3. Crustaceans also, in general, are unable to synthesize 18:2w6 and 18:3w3 and the rate of conversion of these fatty acids to higher fatty acids seems to be very low (Kanazawa, 1985).

The demonstrated or suggested requirements of a number of fishes and crustaceans of present or possible future aquaculture interest are presented in Table.

#### Essential fatty acids for finfish:

Rainbow trout, a cold-water fish, requires w3 fatty acids as EPA in the diet and the EPA requirement was met by 1% 18:3w3 in the diet, and no combination of 18:3w3 with 18:2w6 resulted in as fast a growth rate or efficient feed conversion ratio as 1% of 18:3w3 alone in the diet (Castell et al., 1972); Watanabe et al., 1974). Though inclusion of 18:2w6 in the diet did result in some improvement in growth and feed conversion compared with EPA deficient diets, the w6 fatty acids did not prevent some EPA deficiency symptoms such as the "Shock Syndrome" (Castell et al., 1972; Yu and Sinnhuber, 1975). In rainbow trout, dietary 18:2 w6 or 18:3 w3 were readily converted to C-20 and C-22 PUFA of the same series. Takeuchi and Watanabe (1977) found that either 20:5w3 or 22:6w3 or their mixture was superior to 18:3w3 in EPA value for rainbow trout.

In coho salmon (Oncorhynchus kisutch) the optimum level of dietary w3 fatty acids ranged from 1% to 2.5%, and dietary w6 fatty acids higher than 1% depressed the growth of the same salmonid. Chum salmon (Oncorhynchus keta) showed best weight gain and feed efficiency when offered a diet with 3% methyl laurate plus simultaneous supplements of 1% 18:2w6 and 1% 18:3w3 (Takeuchi et al., 1979).

Studies with carp (Cyprinus carpio) have shown that it requires 18:2w6 and 18:3w3 with best weight gain and feed conversion in fish receiving a diet with both 1% 18:2w6 and 1% 18:3w3 (Watanabe et al., 1975b; Takeuchi and Watanabe, 1977a).

The eel Anguilla japonica has also a requirement for both 18:2w6 and 18:3w3. Arai et al. (1971) found that a mixture of corn oil and codliver oil in the ratio 2:1 containing both 18:2w6 and 18:3w3 was the most favourable for growth of eels. Subsequently, Takeuchi et al. (1980) found that the eel required 18:2w6 and 18:3w3 in the same proportion as the carp, but at a level of 0.5% of each.

Takeuchi et al. (1979c and 1980b) investigated the need for EFA of chum salmon (Oncorhynchus keta) held in both freshwater and seawater and found that the requirement of chum salmon for EFA did not change according to their living environment. The best weight gain and feed efficiency were obtained in fish receiving the diet supplemented with both 1% 18:2w6 and 1% 18:3w3.

Kanazawa et al. (1980) examined the EFA requirement of Tilapia zilli, a herbivorous fish, which is able to live in both freshwater and seawater. In this species, the growth promoting effects of 18:2w6 and 20:4w6 were found to be superior to those of 18:3w3 and 20:5w3 indicating that this



fish requires w6 fatty acids rather than w3 fatty acids. The dietary requirement was 1% of either 18:2w6 or 20:4w6.

Significant variations exist among various fish species in their ability to elongate and desaturate dietary 18 carbon fatty acids to 20 or 22 carbon fatty acids. Several marine species appear to have lower enzymatic elongation-desaturation capabilities than freshwater fishes. Yamada et al. (1980) examined the possibility of bio-conversion of 18:3w3 into higher fatty acids by administering 18:3w3 (1-14C) to individuals of red sea-bream, black sea-bream (Mylio macrocephalus) opaleye - (Girella nigricans), striped mullet (Mugil cephalus) and rainbow trout, and found that only rainbow trout exhibited appreciable radioactivity in 22:6w3 of body lipids. Therefore, it was concluded that marine species have limited ability to elongate and desaturate 18:3w3, resulting in dietary essentiality of eicosapentaenoic (20:5w3) or docosahexaenoic fatty acids (22:6w3). Kanazawa et al. (1979) showed that injections of 1-14C) 18:3w3 into rainbow trout resulted in bioconversion to 20:5w3 and 22:6w3, demonstrating the non-essentiality of 20:5w3 and 22:5w3; whereas very low percent bio-conversion of 18:3w3 to 20:5w3 and 22:6w3 occurred in marine fish such as globefish (Fugu rubripes), Japanese eel, red sea bream, rockfish (Sebasticus marmoralis) and ayu (Plecoglossus altivelis).

Cowey et al. (1976) concluded that turbot (Scophthalmus maximus) lack the necessary microsomal desaturases to effectively convert 18:1w9, 18:2w6 or 18:3w3 into polyunsaturated fatty acids for deposition in neutral fats or phospholipids based upon growth and body composition. Yone and Fujii (1975) demonstrated that 18:3w3 is not of much importance per se for the marine fish such as red sea bream, black

sea bream, opal eye and yellow tail (Seriola quinqueradiata). Stickney and Andrews (1971) found that 18:2w6 has a repressive effect in the catfish, Ictalurus punctalis but inclusion of menhaden oil containing 20:5w3 and 22:6w3 had no detrimental effects.

Essential fatty acids for crustaceans:

Crustacean lipids have both saturated and unsaturated fatty acids, particularly greater percentage of w3 HUFA such as 20:5w3 and 22:6w3 (Gopakumar and Nair, 1975; Guary et al., 1976). Although essential fatty acids content of crustaceans is high, they are unable to synthesize these fatty acids from other saturated fatty acids (Kanazawa, 1985). Nutritional studies have demonstrated that crustaceans require essential fatty acids in their diets for normal survival and growth (Kanazawa et al., 1979). Kanazawa and Coworkers through radio active tracer experiments reported the absence of de novo synthesis of linoleic (18:2w6), linolenic acid (18:3w3), eicosapentaenic acid (20:5w3) and decosahexaenoic acid (22:6w3) from acetate or palmitic acid in the prawns, P. japonicus, P. monodon and P. merguensis (Kanazawa et al., 1979). Similarly, Zandee (1967) reported the inability of the cray fish, Astacus astacus and the lobster, Homarus gammarus to synthesize PUFA. Recent studies have further shown that 18:2w6 and 18:3w3 are poorly converted into HUFA of the same series (Kanazawa et al., 1979). Bottino et al. (1980) reported that P. setiferus, P. aztecus and P. duorarum were unable to biosynthesize C20 and C22 PUFA from C18 fatty acid precursors in adequate levels.

Studies conducted at the Central Marine Fisheries Research Institute also clearly indicated the inability of Penaeus indicus larvae, post-larvae and juveniles to synthe-

size 18:2w6 or 18:3w3. However, slow rate of conversion of 18:2w6 and 18:3w3 to higher fatty acids was observed. Similarly Colvin (1976) suggested limited capacity for bioconversion of EFA to longer chain PUFA in P. indicus juveniles. In Penaeus indicus inclusion of 1% linoleic as well as 1% linolenic acids in the diets resulted in growth improvement over that of the diets deficient in these fatty acids. While more than 1% of these fatty acids had no beneficial effect, fatty acids levels above 3% were detrimental to prawns. Besides, linoleic acid is found to be inferior to that of linolenic acid in efficacy. Among diets tested with various types of lipids, the lipids containing a mixture of 18:2w6, 18:3w3, 20:5w3 and 22:6w3 along provided the best weight gain and food conversion in P. indicus, clearly indicating the essentiality of a blend of unsaturated fatty acids of the w3 and w6 series for proper survival growth, FCR, PER and retention of protein and lipids. Exclusion of polyunsaturated fatty acids from the diets of P. indicus juveniles severely affected the utilization of ingested food and protein, and protein deposition in the body. Besides, diets containing linoleic and linolenic acids alone were poorly accepted by P. indicus larvae and these diets when fed to larvae induced complete mortality. In P. japonicus also similar results have been reported by Kanazawa et al. (1985). In both the above species, it is well proved that lipids containing 20:5w3 and 22:6w3 fatty acids are essential for the normal growth and metamorphosis of larvae.

Hill and Holman (1980) suggested that in chronic malnutrition in which protein intake is usually low, the protein deficiency may increase the EFA requirement and precipitate marginal EFA deficiency. On the other hand Steffens and Albrecht (1973) found that some fatty acids when added to the food accelerated the growth and at the same time reduced the protein amount required for production of unit weight.

TABLE : ESSENTIAL FATTY ACID REQUIREMENTS OF  
FINFISH AND CRUSTACEANS

Species	Suggested EFA requirement	Reference
Chinook salmon	18:2w6 and 18:3w3	Nicolaides and Woodall (1962)
Rainbow trout	18:3w3- 1% in the diet	Castell <u>et al.</u> (1972)
Rainbow trout	18:3w3- 0.8 to 1.6%	Watanabe <u>et al.</u> (1974)
Rainbow trout	18:3w3 - 1% at a lipid level of 4% and 2% at a lipid level of 14%	Takeuchi and Watanabe (1977b)
Channel catfish ( <u>Ictalurus punctatus</u> )	NO. EFA requirement demonstrated. High levels of 18:2w6 or 18:3w3 (10% safflower oil or linseed oil respectively) reduced growth	Stickney and Andrews (1971,72)
Common carp ( <u>Cyprinus carpio</u> )	Mixture of 1% 18:2w6 and 1% 18:3w3 superior to either fatty acid fed separately. PUFA 20:5w3 and 22:6w3 more efficient than 18:2w6 and 18:3w3	Takeuchi and Watanabe (1977)
Chum salmon ( <u>Oncorhynchus keta</u> )	18:2w6 and 18:3w3 at 1% each in the diet	
Eel ( <u>Anguilla japonica</u> )	Mixture of 0.5% 18:2w6 plus 0.5% 18:3w3 or 0.5% 20:5w3 and 22:6w3 PUFA	Takeuchi <u>et al.</u> (1980)
<u>Tilapia zilli</u>	1% of 18:2w6 or 20:4w6; both superior to 18:3w3	Kanazawa <u>et al.</u> (1980)

Species	Suggested EFA requirement	Reference
<u>Marine Fish</u>		
Yellow tail ( <u>Seriola</u> <u>quinqueradiata</u> )	Fish oils superior to corn oil	Tsukahara <u>et al.</u> (1967)
Ayu ( <u>Plecoglossus</u> <u>altivelis</u> )	PUFA - 20:5w3 and 22:6w3 superior to 18:3w3 superior to 18:2w6	Oka <u>et al.</u> (1980)
Turbot ( <u>Scophthalmus</u> <u>maximus</u> L.)	0.6% w3 HUFA slightly superior to 3.7% 18:3w3. Both superior to 18:2w6	Leger <u>et al.</u> (1979)
Red sea bream ( <u>Chrysophrys</u> <u>major</u> )	1% w3 HUFA required 2% w3 HUFA required	Cowey <u>et al.</u> (1976) Fujii and Yone (1976)
Milkfish fry <u>Chanos chanos</u>	Require a mixture of marine fish oil and vegetable oil containing 18:2w6, 18:3w3, 20:5w3 and 22:6w3	CMFRI (unpublished data)
<u>Crustaceans</u>		
Lobster ( <u>Homarus</u> <u>americanus</u> )	Adults - fish oil high in w3 PUFA superior to corn oil high in 18:2w6	Castell and Covey (1976)
	Juvenile lobsters have similar requirements	D'Abramo <u>et al.</u> (1980)
<u>Shrimp</u>		
<u>Penaeus japonicus</u>	1% w3 PUFA, 20:5w3 or 22:6w3 superior to 18:3w3	Kanazawa <u>et al.</u> (1979a, b)
	Addition of 18:3w3 or 18:2w6 to fish oil high in w3 PUFA resulted in growth enhancement	Kanazawa <u>et al.</u> (1979c)
<u>Penaeus indicus</u> juveniles	Fish oil w3 PUFA superior to 18:2w6 or 20:4w6 supplemented	Read (1981)

Species	Suggested EFA requirement	Reference
	alone. Fish oil plus both 18:3w3 and 18:2w6 superior to fish oil alone	
<u>Penaeus indicus</u> larvae	Incorporation of 18:2w6 or 18:3w3 or their mixture resulted in complete mortality of larvae. Diets containing a mixture of lipids of marine and plant oils containing 18:2w6, 18:3w3, 20:5w3, 22:6w3 essential for survival, growth and metamorphosis	Chandge (MS)
Post-larvae and juveniles	18:3w3 better than 18:2w6. A mixture of both the fatty acids are better than individual fatty acids. Best growth feed efficiency obtained only in diets with a mixture of plant and marine animal lipids	-do-
<u>Penaeus azlicus</u>	Supplementing commercial marine diet with 1% 18:3w3 improved growth	Sheedbart and Mies (1973).
<u>Palaemon serratus</u>	Requirement for both w6 and w3 fatty acids. 18:2w6/18:3w3 in the ratio 2.2 optimal 20:5w3 plus 22:6w3 superior to 18 carbon PUFA	Martin (1980)
<u>Macrobrachium rosenbergii</u>	Diet containing 3% shrimp head oil produced larger shrimp and after 12 weeks the total biomass produced was twice as high as the control. High w3:w6 diets are beneficial to carideans	Sandifer and Joseph (1976)

---

Species	Suggested EFA requirement	Reference
<u>Crab</u>		
<u>Carcinus maenas</u>	Fish oil superior to vegetable oil high in 18:2w6	Ponat and Adelung (1980)
<u>Oyster</u>		
<u>Crassostrea virginica</u>	Codliver oil with w3 highly unsaturated fatty acids promoted maximum growth	Trides and Castell (1979)

---

EFA DEFICIENCY SYMPTOMS RECORDED  
IN FINFISH AND CRUSTACEANS

<u>Species</u>	<u>EFA Deficiency Symptoms</u>	<u>References</u>
<u>Finfish</u>		
Chinook Salmon	Impaired pigmentation	Nicolaides and Woodall (1962)
Rainbow trout	Poor growth and feed conversion; shock syndrome; erosion of caudal fin; heart myopathy, swollen livers; altered permeability of biomembranes as exhibited by increased rate of swelling of isolated liver mitochondria; fatty degeneration of livers; decreased haemoglobin levels and decreased red blood cell volume; increase in <u>de novo</u> synthesis of 20:3w9 by rainbow trout	Castell <u>et al.</u> (1972 a,b,c)
Yellow tail	Growth, red blood cell counts, hematocrit and haemoglobin levels were affected by EFA deficiency	--
Common carp	Reduced growth and feed efficiency; increased production and incorporation of 20:3w9 into polar lipids	Takeuchi and Watanabe (1977)
Japanese eel	Same as in common carp	Takeuchi <u>et al.</u> (1980)
<u>Crustaceans:</u>		
<u>Penaeus japonicus</u>	Poor growth rate, feed efficiency and survival	Kanazawa <u>et al.</u> (1979c)



Species	EFA Deficiency Symptoms	References
<u>Penaeus indicus</u>		
larvae	Delayed larval metamorphosis and mortality	Chandge (MS)
Post-larvae and juveniles	Reduced growth; poor survival; poor food and protein conversion; poor protein retention	Chandge (MS)
American lobster	Reduced growth rate and feed efficiency; reduced blood cell numbers; serum protein levels, and percent edible meat; increased intermolt period; and reduced weight gain per moult	Castell and Covey (1976)

### Effect of EFA Deficiency on spawning

The recent studies by Watanabe et al. (1978,1979) have demonstrated that EFA deficiency greatly affected the spawning of rainbow trout and red sea bream and that EFA play as important a role in reproductive physiology as fopopherols (Watanabe et al., 1977) in fish as in higher animals.

The adult rainbow trout fed on the EFA deficient casein diet containing methyl laurate as the sole dietary lipid for 3 months before spawning matured, but the eggs produced had a low hatching rate. But addition of 1% ethyl lineleate in place of linolenate in the diet improved the egg condition (Yu et al., 1979). The results obtained in red sea bream fed the EFA deficient purified diet for 6 months before spawning indicated that the total egg production, proportion of eyed eggs and hatchability were significantly influenced by the EFA status in the diet and were quite low in the group given the EFA deficient diet. These low quality eggs showed abnormality in the number of oil globules, the average being seven whereas in normal eggs it is usually one. Almost all the fish larvae obtained from the group also showed various kinds of deformations in the body. Supplementation of cuttlefish liver oil high in w3 HUFA effectively prevented these pathologies.

### PHOSPHOLIPIDS

Phospholipids have a transport and structural function in crustaceans (O'Connor and Gilbert, 1968). Phospholipids tend to be more unsaturated than neutral lipids due to their high content of polyunsaturated fatty acids. In view of their importance in the transport of lipids and as structural component of biomembranes, many studies have been

carried out in the phospholipid content and its composition in crustaceans. Gopakumar and Nair (1975) reported that phospholipid constitutes 62% of the total lipids in Penaeus indicus. Several other reports have also shown that phospholipids are the major lipids of crustaceans, such as the lobster Homarus americanus (Bligh and Scott, 1966), the crab Carcinus maenas and the prawn P. japonicus (Teshima and Kanazawa, 1978). In common with other life forms the major phospholipids in crustaceans are phosphatidylcholine and phosphatidylethanolamine which are important from nutritional point of view (Sargent, 1976).

Van Den Oord et al. (1964) and Testima and Kanazawa (1978a and b) suggested that crustacean phospholipids probably play important role in emulsification, absorption and interorgan transport of lipids. Lester et al. (1975) observed that lecithin enhanced cholesterol solubilization when associated with N-(N-dodecanosarcosyl) taurine (DST) a model of the type of detergents synthesized by crustaceans.

Kanazawa et al. (1979) found that inclusion of lecithin from the short-necked clam at 1% level in the purified diet of Penaeus japonicus had a growth promoting effect. Conklin et al. (1980) found that the inclusion of soy lecithin into purified diets fed to juvenile lobsters eliminated mortality associated with a 'moult-death syndrome'. Tridel and Castell (1980) found that survival of juvenile lobsters increased with increasing lecithin level in a casein based diet upto 4-6%.

Studies conducted in Penaeus indicus larvae, post-larvae and juveniles at CMFRI showed that inclusion of phospholipids promote growth, improve and food conversion in juveniles. It was also observed that inclusion of phospholipids at levels greater than 2% in the diet had no

significant beneficial effect. Lecithin deficiency induced high mortality in larvae during the metamorphosis and all the larvae died before reaching the post-larval stage. Inclusion of lecithin in the diet improved the efficiency of protein utilization. It is suggested that lecithin when included in the diet may provide choline, which acts as a methyl donor during trans-methylation reactions, thereby sparing the sulphur amino acid, methionine (another methyl donor) for enhancement of protein synthesis. Thus lecithin inclusion in the diet improves protein retention (Chandge, MS).

#### CHOLESTEROL

The most abundant steroid in animal tissues is cholesterol (Lehninger, 1984). It occurs in the plasma membranes of many animal cells, in the lipoprotein of blood plasma and large quantities occur in the brain and nerve tissues (Lehninger, 1984). Vertebrates including fish are known to biosynthesize cholesterol from precursors such as acetate and mevalonate. But crustaceans do not have the ability to biosynthesize cholesterol (Zandee, 1964, 1966; Kanazawa et al., 1971 a; Teshima et al., 1983; D'Abramo et al., 1984). Therefore cholesterol should be included in their diet.

Functionally cholesterol together with phospholipids helps in transportation of lipids in body of animals. It is also a poor insulator against electrical discharge, especially in the brain, where it acts as insulator against nerve impulses which are electrical in character. Cholesterol is known to decrease the fluidity of artificial lipid biomolecular leaflets (liposomes) by decreasing the surface area of the membrane (Van Deenen et al., 1972). It has also been demonstrated that diets supplemented with cholesterol can

alter the kinetic properties of enzymes in the erythrocyte membranes (Farias et al., 1975). In crustaceans cholesterol is also the precursor for various physiologically important compounds like steroid hormones, brain and moulting hormones and Vitamin D (Kanazawa et al., 1971; New, 1976). Sterols have also been found to be important components in the cellular and sub-cellular membranes particularly in the hypodermis in Arthropoda (Gilbert, 1969; New, 1976). About 0.5% cholesterol has been found to be sufficient in the diet of most crustaceans. In a series of studies Kanazawa et al. (1971 a, b, c) have shown that P. japonicus utilized cholesterol better than ergo-, stigma and B-sitosterol and that these sterols are transported after ingestion with cholesterol, and also that the spiny lobster is able to convert cholesterol into steroid hormones.

#### NEGATIVE ASPECTS OF LIPIDS

##### Rancidity

The requirement by fish for PUFA of the w3 series creates problems with respect to feed storage, since highly unsaturated fatty acids are very labile to oxidation. The products of lipid oxidation may react with other nutrients such as amino acids, vitamins and reduce their biological availability. The effect of oxidized lipids on dietary proteins, enzymes and amino acids have been demonstrated by Andrews et al. (1965) and Crawford et al. (1965) and many others. Fowler and Banks (1969) found that rancid herring and hake meals in fish feeds caused dark coloration, anemia, lethargy brown yellow pigmented livers, abnormal kidneys and some gill clubbing in Chinook Salmon. Addition of alpha-tocopherol rancid fish meals alleviated the symptoms. Sinnhuber et al. (1968) and Watanabe and

Hashimoto (1968) demonstrated the sparing effect of alpha-tocopherol in rancid rainbow trout and carp feeds. Thus, it is clear that when oils with high contents of PUFA are used antioxidants should be added.

#### Cyclopropenoic fatty acids

Cyclopropenoic fatty acids found in cotton seed oil and the oil of other plants of the order Malvales, have been suspected to interfere with the fatty acid desaturase enzyme system as well as with normal lipid and protein metabolism. Fatty acid desaturases of fish are affected in the same way by the inhibitor sterculic acid as are free fatty acid desaturases of mammals. Sterculic acid is a cyclopropenoic fatty acid occurring naturally in triacylglycerols from plants of the order Malvales (Cowey and Sargent, 1979). Roehm et al. (1969) fed rainbow trout diets containing salmon oil supplemented with up to 200 ppm of sterculic acid. The inhibitor caused increased ratios of 16:0/16:1 and 18:0/18:1 as well as a decreased level of 22:6w3 in the trout. These changes were accompanied by liver pathology and a decreased growth rate during the initial stages of feeding.

Synthesis of saturated and Monoenoic fatty acids

Acetate

14:0	14:1w5	16:1w5
16:0	16:1w7	18:1w7
18:0	18:1w9	20:1w9
20:0	20:1w11	22:1w11
20:0	22:1w13	

Polyunsaturated fatty acids

	18:1w9		18:2w6		18:3w3	
20:1w9		18:2w9	20:2w6	18:3w6	20:3w3	18:4w3
	20:2w9			20:3w6		20:4w3
	20:3w9		22:3w6	20:4w6	22:4w3	20:5w3
				22:4w6		22:5w3
				22:5w6		22:6w3

Fig: 1. Flow diagram for fatty acid synthesis mechanism in fish (adapted from Castell, 1979).

REFERENCES

- Ackman, R.G. 1967. Characteristics of the fatty acid composition and biochemistry of some freshwater fish oils and lipids in comparison with marine oils and lipids. Comp. Biochem. Physiol., 22: 907-922.
- Ackman, R.G. and J.D. Castell 1966. Isomeric monoethylenic fatty acids in herring oil. Lipids, 1(2): 341-348.
- Adron, J.W., A. Blair, C.B. Cowey and A.M. Shanks 1976. Effects of dietary energy level and dietary energy source on growth, feed conversion and body composition of turbot (Scophthalmus maximus L.), Aquaculture, 7: 125-132.
- Alfin-Slater, R.B. and L. Aftergood 1968. Essential fatty acids reinvestigated. Physiol. Rev., 48: 758-784.
- Andrews, F., J. Bjorksten and F.B. Trenk 1965. The reaction of an autooxidized lipid with proteins. J. Am. Oil Chem. Soc., 42: 779-781.
- Arai, S., T. Nose and Y. Hashimote 1971. A purified test diet for the eel, Anguilla japonica. Bull. Freshwater Fish. Res. Lab., 22(2): 161-173.
- Aquacop 1987. Study of nutrition requirements and growth of Penaeus merguensis in tanks by means of purified and artificial diets. Proc. World Maricult. Soc., 9: 225-234.
- Bligh, E.G. and M.A. Scott 1966. Blood lipids of lobster Homarus americanus. J. Fish. Res. Bd. Can., 23: 1629-1637.
- Bottino, N.R., J. Gennity, M.L. Lilly, E. Simmons and G. Finne 1980. Seasonal and nutritional effects on the fatty acids of three species of shrimp, Penaeus setiferus, P. aztecus and P. duorarum. Aquaculture, 19: 139-148.
- Bromely, P.J. 1980. Effect of dietary protein, lipid and energy content on the growth of turbot (Scophthalmus maximus). Aquaculture, 19: 359-369.
- Burr, G.O. and M.M. Burr, 1930. On the nature and role of the fatty acids essential in nutrition. J. Biol. Chem., 86: 587-621.



- Castell, J.D. and J.F. Covey 1976. Dietary lipid requirements of adult lobsters, Homarus americanus. J. Nutr., 106: 1159-1165.
- Castell, J.D., R.O. Sinnhuber, J.H. Wales and J.D. Lee 1972. Essential fatty acids in the diet of rainbow trout (Salmo gairdnerii). Growth, feed conversion and some gross deficiency symptoms. J. Nutr., 102: 77-86.
- Chandge, M.S. (MS). Studies on lipid nutrition in larvae and juveniles of the Indian white prawn, Penaeus indicus H. Milne Edwards. Ph.D. thesis (MS).
- Clifford, H.C. and R.W. Brick 1978. Protein utilization in the 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., L.R. D'Abramo, C.E. Bordner and N.A. Baum 1980. A successful purified diet for the culture of juvenile lobsters: the effect of lecithin. Aquaculture, 21: 243-249.
- Cowey, C.B. and J.R. Sargent 1979. Nutrition. In W.S. Hoar, D.J. Randall and J.R. Brett (eds), Fish Physiology, volume VIII: Bioenergetics and growth, p. 1-69, Academic Press, New York.
- Cowey, C.B., J.M. Ower, J.W. Adron and C. Middleton 1976. Studies on the nutrition of marine flat fish. The effect of different dietary fatty acids on the growth and fatty acid composition of turbot (Scophthalmus maximus). Br. J. Nutr., 36: 479-486.
- Crawford, D.L., T.C. Yu and R.O. Sinnhuber 1966. Reaction of malonaldehyde with glycine. Agric. Food. Chem., 14: 182-184.
- D'Abramo, L.R., C.E. Bordner, G.R. Daggett, D.E. Conklin and N.A. Baum 1980. Relationship among dietary lipids, tissue lipid and growth in juvenile lobster. Proc. World Maricult. Soc., 11: 335-345.
- D'Abramo, L.R., C.E. Bordner, D.E. Conklin and N.A. Baum 1984. Stérol requirement of juvenile lobster Homarus sp. Aquaculture, 42(1): 13-25.

- Forster, J.R.M. 1976. Studies on the development of compounded diets for prawns. Proc. First Intl. Conf. on Aquaculture Nutr., Delaware, NOAA (Sea Grant), 14-15 October 1975, pp. 229-248.
- Fowler, L.G. and J.L. Banks 1969. Tests of vitamin supplements and formula changes in the Abernathy Salmon Diet, 1966-67. U.S. Bur. Sport. Fish. Wildl. Tech. Rep., No. 26: 3-19.
- Fujii, M. and Y. Yone 1976. Studies on nutrition of red sea bream. XIII. Effect of dietary linolenic acid and W3 polyunsaturated fatty acids on growth and feed efficiency. Bull. Jap. Soc. Sci. Fish., 42(5): 583-588.
- Gopakumar, K. and M.R. Nair 1975. Lipid composition of five species of Indian prawns. J. Sci. Food Agric., 26: 319-325.
- Garling, D.L. Jr., and R.P. Wilson 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, Ictalurus punctatus. J. Nutr., 106: 1368-1375.
- Guary, J.C., M. Kayama, Y. Murakami and H.J. Ceccaldi 1976. The effect of a fat-free diet and compounded diets supplemented with various oils on moults, growth and fatty acid composition of prawn, Penaeus japonicus. Aquaculture, 7: 245-254.
- Hill, E.G. and R.T. Holman 1980. Effect of dietary protein level upon essential fatty acid (EFA) deficiency. J. Nutr., 110: 1057-1060.
- Kanazawa, A. 1985. Nutrition of penaeid prawns and shrimps. Proc. of the First Intl. Conf. on the culture of penaeid prawns/shrimps. Illoilo city, Philippines, 1984, pp. 123-130.
- Kanazawa, A., M. Shimaya, M. Kawasaki and K. Kashiwada 1970. Nutritional requirements of prawn. 1 - Feeding on artificial diet. Bull. Jap. Soc. Sci. Fish., 36: 949-954.
- Kanazawa, A., N. Tanaka, S. Teshima and K. Kashiwada 1971. Nutritional requirements of prawn - II. Requirement for sterol. Bull. Jap. Soc. Sci. Fish., 37(3): 211-215.
- Kanazawa, A., S. Teshima, M. Ando and M. Mayama 1979 a. Requirements of prawn, Penaeus japonicus for essential fatty acids. Mem. Fac. Fish., Kagoshima Univ., 28: 27-33.

- Kanazawa, A., S. Teshima, M. Ando and M. Kayama 1979 b. Biosynthesis of fatty acids from palmitic acid in the prawn, Penaeus japonicus. Mem. Fac. Fish., Kagoshima Univ., 28: 17-20.
- Kanazawa, A., S. Teshima, K. Ono and K. Chalayondeja 1979 c. Biosynthesis of fatty acids from acetate in the prawns, Penaeus monodon and Penaeus merguensis. Mem. Fac. Fish., Kagoshima Univ., 28: 21-26.
- Kanazawa, A., S. Teshima, M. Sakamoto and M.A. Awal 1980. Requirements of Tilapia zillii for essential fatty acids. Bull. Jap. Soc. Sci. Fish., 46: 1353-1356.
- Kimelberg, H.K. and D. Papahadjopolus 1972. Phospholipid requirement for (Na<sup>+</sup> + K<sup>+</sup>)-ATPase activity: Head group specificity and fatty acid fluidity. Biochem. Biophys. Acta., 282: 277-292.
- Lee, D.J. and G.B. Putnam 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr., 103: 916-922.
- Leger, C., F.J. Gatesoupe, R. Metailler, P. Luquet and L. Fremont 1979. Effect of dietary fatty acids differing by chain lengths and w series on the growth and lipid composition of turbot, Scophthalmus maximus L. Comp. Biochem. Physiol., B64: 345-350.
- Lehninger, A.L. 1984. Biochemistry. 4th edition. Kalyani Publishers, Ludhiana, India, 1104 p.
- Lester, R., M.C. Cary, L.A. Cooperstein and S.R. Dowd 1975. Crustacean intestinal detergent promoter sterol solubilization. Science, New York, 189: 1018-1100.
- Markin, B.J. 1980. Croissance et acides gras de la Crevette Palaemon serratus (Crustacea, Decapoda) nourrie avec des aliments composes contenant differentes proportions d'acide linoleique et linolenique. Aquaculture, 19: 325-337.
- Mead, J.F. and M. Kayama 1967. Lipid metabolism in fish. Fish oils. Westport, Conn., Avi Publishing Co. Ltd., pp. 289-299.
- Millikin, M.R. 1982. Qualitative and quantitative nutrient requirements of fishes: A review. Fish. Bull., 80: 655-686.

- New, M.R. 1976. A review of dietary studies with shrimps and prawns. Aquaculture, 9: 101-144.
- Nicol, J.A.C. et al., 1975. The tapetum lucidum in the eyes of cusk-eels (Ophiidae). Can. J. Zool., 53: 1063-1079.
- Nicolaides, N. and A.H. Woodall 1962. Impaired pigmentation in chinook salmon fed diets deficient in essential fatty acids. J. Nutr., 78: 431-437.
- Oka, A., N. Zuzuki and T. Watanabe 1980. Effect of fatty acids in rotifers on growth and fatty acid composition of larval ayu Plecoglossus altivelis. Bull. Jap. Soc. Sci. Fish., 46: 1413-1418.
- O'Connor, J.D. and L.I. Gilbert 1968. Aspects of lipid metabolism in crustaceans. Am. Zool., 8: 529-539.
- Ponat, A. and D. Adelung 1980. Studies to establish an optimal diet for Carcinus maenas. II - Protein and lipid requirements. Mar. Biol., 60: 115-122.
- Read, G.H.L. 1981. Response of Penaeus indicus (Crustacea, Penaeidea) to purified and compounded diets of varying fatty acid composition. Aquaculture, 24: 245-256.
- Roehm, J.M., D.J. Lee, J.H. Wales, S.D. Polityka, and R.O. Sinnhuber 1970. The effect of dietary sterculic acid on the hepatic lipids of rainbow trout. Lipids, 5: 80-84.
- Sandifer, P.A. and J.D. Joseph 1976. Growth response and fatty acid composition of juvenile prawn (Macrobrachium rosenbergii) fed a prepared ration augmented with shrimp - head oil. Aquaculture, 8: 129-139.
- Sargent, J.R. 1976. The structure, metabolism and function of lipids in marine organisms. In: Biochemical and Biophysical perspectives in Marine Biology, D.C. Malins and J.R. Sargent (Eds.) Academic Press, London, 3: 150-212.
- Sinnhuber, R.O., J.H. Wales and D.J. Lee 1968. Dietary factors and hepatoma in rainbow trout (Salmo gairdnerii). II. Cocarcinogens by cyclopropenoic fatty acids and the effects of gossypol and altered lipids in aflatoxin induced liver cancer. J. Nat. Cancer Inst., 41(6): 1293-1301.

- Shewhart, K.L. and W.L. Mies 1973. Studies on nutritional requirement of brown shrimp. The effects of linolenic acid on growth of P. aztecus. Proc. World. Maricult. Soc., 4: 277-287.
- Stickney, R.R. and J.W. Andrews 1971. Combined effects of dietary lipids and environmental temperature on growth, metabolism and body composition of channel catfish (Ictalurus punctatus). J. Nutr., 101: 1703-1710.
- Stickney, R.R. and J.W. Andrews 1972. Effects of dietary lipids on growth, food conversion, lipid and fatty acid composition of channel catfish. J. Nutr., 102: 249-258.
- Tanaka, R. and K.P. Strickland 1965. Role of phospholipid in the activation of  $\text{Na}^+$ ,  $\text{K}^+$  activated adenosine triphosphate of beef brain. Arch. Biochem. Biophys. 111: 583-592.
- Takeda, M., S. Shimeno, H. Hosokawa, H. Kajiyama and T. Kaisyo 1975. The effect of dietary calorie 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. and T. Watanabe 1977 b. Effect of eicosapentaenoic and docosahexaenoic acid in pollack liver oil on growth and fatty acid composition of rainbow trout. Bull. Jap. Soc. Sci. Fish., 43: 947-953.
- Takeuchi, T. and T. Watanabe 1977 a. Requirement of carp for essential fatty acids. Bull. Jap. Soc. Sci. Fish., 43(5): 541-551.
- Takeuchi, T., T. Watanabe and C. Ogino 1978. 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., T. Watanabe and C. Ogino 1979a. Availability of dietary energy sources

- Takeuchi, T., S. Arai, T. Watanabe and Y. Shimma 1980. Requirement of eel, Anquilla japonica for essential fatty acids. Bull. Jap. Soc. Sci. Fish., 46: 345-353.
- Teshima, S. and A. Kanazawa 1978. Release and transport of lipid in the prawn. Bull. Jap. Soc. Sci. Fish., 44(11): 1269-1274.
- Teshima, S. and A. Kanazawa 1983. Digestibility of dietary lipids in the prawn. Bull. Jap. Soc. Sci. Fish., 49: 963-966.
- Trider, D.J. and J.D. Castell 1980. Some current findings of the lobster nutrition group. In: 1980 lobster nutrition workshop, Proceedings. Bayer R.C. and D'Agostino (eds). Technical Report 58 Maine Sea Grant Publication, pp. 36-40.
- Tsukahara, H., A. Furukawa and K. Funae 1967. Studies on feed for fish. VIII. The effects of dietary fat on the growth of yellow tail (Seriola quinqueradiata Temminak et Schegel). Bull. Nakai Reg. Fish. Res. Lab., 24: 29-50.
- Van Den Oord, A., H. Danielson and R. Ryhage 1964. Absence of bile salts in the gastric juice of crab. Nature, 203, 301.
- Watanabe, T., F. Takashima and C. Ogino 1974 a. Effect of dietary methyl linolenate on growth of rainbow trout. Bull. Jap. Soc. Sci. Fish., 40: 181-188.
- Watanabe, T., C. Ogino, Y. Koshishi and T. Matsunaga 1974b. Requirement of rainbow trout for essential fatty acids. Bull. Jap. Soc. Sci. Fish., 40: 493-497.
- Yamada, K., K. Kobayashi and Y. Yone 1980. Conversion of linolenic acid to W3 highly unsaturated fatty acids in marine fishes and rainbow trout. Bull. Jap. Soc. Sci. Fish., 46: 1231-1233.
- Yone, Y. and M. Fujii 1975. Studies on nutrition of red sea bream. XI. Effect of W3 fatty acid supplement in a corn oil diet on growth rate and feed efficiency. Bull. Jap. Soc. Sci. Fish., 41: 73-77.
- Zandee, D.I. 1964. Absence of sterol synthesis in some arthropods. Nature (London), 20: 1315-1336.

Zandee, D.I. 1966. Metabolism in cray fish Astacus astacus IV. The fatty acid composition and biosynthesis of the fatty acids. Arch. Inst. Physiol. Biochem., 74: 614-626.

Zandee, D.I. 1967. Absence of cholesterol synthesis as contrasted with the presence of fatty acid synthesis in some arthropods. Comp. Biochem. Physiol. 200: 811-822.