CHAPTER II

LITERATURE REVIEW

P. monodon

The gaint tiger prawn, *P. monodon* Fabricius, is one of the largest penaeid prawns in the world, reaching some 270 mm in body length, and is of commercial importance in markets.

Recently in Southeast Asian Countries, enthusiasm for natural and artificial propagation of both fry and adult giant tiger prawn have been growing rapidly among government and private aquaculturists due to strong demand with higher prices in national and international markets (Motoh, 1984).

Motoh (1984) reviews that the gaint tiger prawn is widely distributed throughout the greater part of the Indo-West Pacific region, South Africa, Tanzania, Kenya, Somalia, Madagascar, Saudi Arabia, Oman, Pakistan, India, Bangladesh, Sri Lanka, Hongkong, Taiwan, Korea, Japan, Australia and Papua New Guinea. In general, *P. monodon* is distributed from longitude 30°E to 155°E and from latitude 35°N to 35°S. However, the main fishing grounds are mostly located in tropical countries, particularly in Indonesia, Malaysia and the Philippines. The fry, juveniles and adolescents inhabit inshore waters such as

shore areas and mangrove estuaries, while most of the adults inhabit offshore waters to the depth of 160 m.

Lipids

Lipids are substances of biological origin that are soluble in organic solvents such as chloroform and methanol but are only sparingly soluble, if at all, in water. Hence, they are easily separated from other biological materials by extraction into organic solvents and may be further fractionated by such techniques as adsorption chromatography, thin layer chromatography, and reverse-phase chromatography. Fat, oils, certain vitamins and hormones, and most nonprotein membrane components are lipids (Voet and Voet, 1995).

Lipids have four major biological functions; 1) in all cells, the major biological structural elements of membranes are composed of lipids; 2) certain lipids, the triacylglycerols, serve as efficient reserves for storage of energy; 3) many vitamins and hormones found in animals are lipids or derivatives of lipids; and 4) the bile acids help to solubilize the other lipid classes during digestion (Zubay, 1993).

Fatty Acids

The common biological fatty acids is shown in Table 1. Compounds with the structural formula $CH_3(CH_2)_nCOOH$ that contain no carbon-carbon double bonds are known as *saturated fatty acids*. The two most abundant

Table 1. The common biological fatty acids (Voet and Voet, 1995).

Symbol[a]	Common Name	Systematic Name	Structure	mp (°C)
Saturated Fatty Acids				
12:0	Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$	44.2
14:0	Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$	52
16:0	Palmitic acid	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$	63.1
18:0	Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	69.6
20:0	Arachidic acid	Eicosanoic acid	$CH_3(CH_2)_{18}COOH$	75.4
22:0	Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$	81
24:0	Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$	84.2
Unsaturated fatty acids (all double bonds are cis)				
16:1	Palmitoleic acid	9-Hexadecenoic acid	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	-0.5
18:1	Oleic acid	9-Octadecenoic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	13.4
18:2	Linoleic acid	9,12-Octadecadienoic acid	$CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_6COOH$	-9
18:3	α-Linolenic acid	9,12,15-Octadecatrienoic acid	$CH_3CH_2(CH=CHCH_2)_3(CH_2)_6COOH$	-17
18:3	γ-Linolenic acid	6,9,12-Octadecatrienoic acid	$CH_3(CH_2)_4(CH=CHCH_2)_3(CH_2)_3COOH$	
20:4	Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	$CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COOH$	-49.5
20:5	EPA	5,8,11,14,17-Eicosapenta-enoic acid	$CH_3CH_2(CH=CHCH_2)_5(CH_2)_2COOH$	-54
24:1	Nervonic acid	15-Tetracosenoic acid	$CH_3(CH_2)_7CH=CH(CH_2)_{13}COOH$	39

[a] Number of carbon atoms: Number of double bonds.

saturated fatty acids are palmitic and stearic acids. All saturated fatty acids belong to high melting point (mp).

Fatty acids with one or more double bonds in the aliphatic chain are called *unsaturated fatty acids*. Monounsaturated fatty acids have one double bond, while polyunsaturated fatty acids (PUFAs) contain more than one double bond. The double bonds in naturally occurring fatty acids are *cis*. The double bonds in PUFAs are always separated by one methylene group (Zubay, 1993).

Physical property of fatty acids

Large numbers of individual fatty acid have been isolated from many animal tissues and identified by gas-liquid chromatography of their methyl ester derivatives. The commonset ones found in fish tissues are two fatty acids of the linoleic acid family; linoleic and arachidonic acids, and three of the linolenic acid family; eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid. Other PUFAs found as minor components include 16:4n-3 and 18:4n-3 both present in phytoplankton and transferred through the food web to shrimp, and a variety of 18C and 20C fatty acids of both the n-3 and n-6 families (Bell, Henderson and Sargent, 1986).

X-ray crystallographic data have shown that, below the phase transition temperature, only phospholipids containing fully saturated acyl chains adopt a configuration in which the acyl chain carbons are fully extended. Introduction of *cis* double bond interrupts the fully extended (anti) conformation in at least one

position of the chain, preventing the close packing which was possible with the all *anti* configuration. Thus the presence of even a single double bond profoundly influences the physical properties. For example, the melting points of stearic acid (18:0) and oleic acid (18:1n-9) are 70.1°C and 16.3°C, respectively. The melting points of 18:1, octadecamonoenoic acid, isomers are highly dependent on the position of the double bond. A double bond in the centre of the chain reduces the melting point some 40°C below that of the isomers with the double bond at either end of the chain. The situation with 18:2, octadecadienoic acid, is similar, with the Δ 8,11 isomer melting 52.5°C lower than the Δ 14,17 isomer. The nearer the double bonds are to the 7 centre of the chain the lower the melting point (Bell et al., 1986).

In some cases the melting points depend more on the position of the double bonds than on the number of double bonds. Thus, the melting point 18:3 Δ 9, 12, 15 (-10°C) is similar to that of 18:2Δ 9, 12 but is about 28°C lower than that of 18:2Δ 12, 15. The melting points of arachidonic acid 20:4n-6 (-49.5°C), eicosapentaenoic acid 20:5n-3 (-54.4°C) and docosahexaenoic acid 22:6n-3 (-44.5°C) are quite similar, although it is noteworthy that 22:6n-3 melts some 10°C higher than 20:5n-3. All PUFAs are therefore fluid at temperatures well below those ever encountered by biological systems and in particular there is no real physio-chemical basis for believing that membrane fluidity is enhanced either by replacing 20:4n-6 with 20:5n-3 or by replacing the latter by 22:6n-3. In fact the major PUFAs in biomembranes are fluid under standard conditions of storage in a deep-freeze (-20°C), a situation that accounts for the well known deterioration of phospholipids in this situation. PUFAs longer and more

unsaturated than 20:4n-6 inclusive are able to exist in a spiral conformation (Bell et al., 1986).

The physical properties of fatty acids vary with their degree of unsaturation

The first double bond of an unsaturated fatty acid commonly occurs between its C9 and C10 atoms counting from the carboxyl C atom (a Δ^9-or 9-double bond) as shown in Table 1. Among polyunsaturated fatty acids, the double bonds tend to occur at every thrid carbon atom towards the methyl terminus of the molecule (such as -CH=CH-CH_2-CH=CH-). Double bonds in PUFAs are almost never conjugated (as in -CH=CH-CH=CH-). Triple bonds rarely occur in fatty acids or any other compound of biological origin (Voet and Voet, 1995).

Saturated fatty acids are highly flexible molecules that can assume a wide range of conformations because there are relatively free rotation about each of their C-C bonds. Nevertheless, their fully extended conformation is that of minimun energy because this conformation has the least amount of steric interference between neighboring methylene groups. The melting points of saturated fatty acids, like those of most substances, increase with molecular mass (Voet and Voet, 1995).

Fatty acid double bonds almost always have the cis configuration. This puts a rigid 30^{o}C bend in the hydrocarbon chain of unsaturated fatty acids that

interferes with their efficient packing to fill space. The consequent reduced Van der Waals interactions cause fatty acid melting points to decrease with their degree of unsaturation. Lipid fluidity likewise increases with the degree of unsaturation of their component fatty acid residues (Voet and Voet, 1995).

Families of fatty acids and their metabolisms

Relationships between fatty acids in pathways of metabolic conversions can be evaluated by considering groups or families of fatty acids based on the primary parent or initial unsaturated acid in the sequence (Figure 1). The predominant fatty acid families are the n-6 acids derived from 18:2n-6, the n-3 acids derived from 18:3n-3, the n-9 acids derived from 18:1n-9, and the n-7 acids derived from 16:1n-7 (Vance and Vance, 1985).

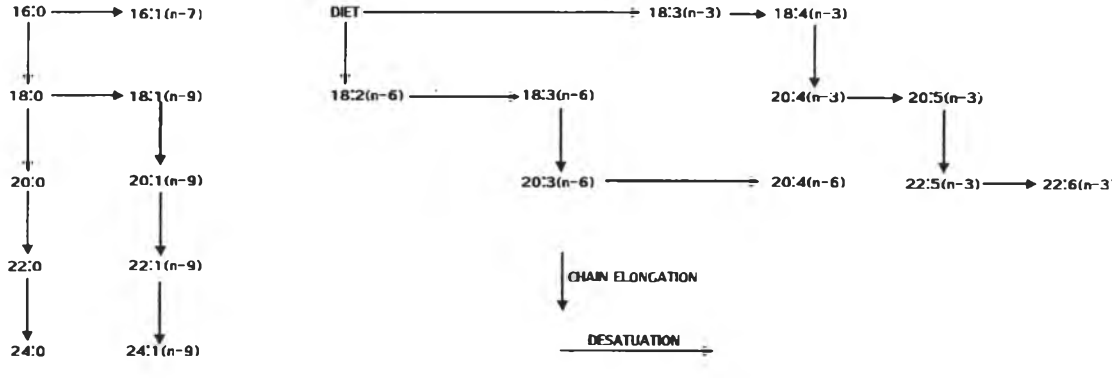

Figure 1. Major parthways of fatty acid biosynthesis by desaturation and chain elongation in animal tissues (Modified from Vance and Vance, 1985)

Biosynthesis & marine species

The biosynthesis of fatty acids from precursor is different from families of fatty acids based on precursor in sequence. The precursor fatty acids of n-3, n-6, n-9 families are linolenic (18:3n-3), linoleic (18:2n-6), oleic (18:1n-9) acids, respectively. The primary precursor will synthesize essential fatty acid by elongation and/or desaturation in each family. Exception n-9 families of fatty acids, they can synthesize from saturated fatty acids (Thongrod, 1992).

The biosynthesis of fatty acids from acetate-1-^{14}C is examined on the prawns, *P. monodon* and *P. merguiensis*. After injection of acetate-1-^{14}C in both *P. monodon* and *P. merguiensis*, radioactivity is mainly associated with palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0) and oleic acid (18:1n-9), but scarcely or slightly with linoleic acid (18:2n-6), linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3). These results suggest that 18:2n-6, 18:3n-3, 20:5n-3 and 22:6n-3 may be essential for *P. monodon* and *P. merguiensis* (Kanazawa et al., 1979c). It can be concluded that *P. monodon* and *P. merguiensis* lack the ability for de novo synthesis of 18:2n-6 and 18:3n-3 from acetate (Kanazawa et al., 1979c; Kanazawa and Teshima, 1977).

Previous studies, the prawn, *P. japonicus* has been to be incapable of synthesizing 18:2n-6, 18:3n-3, and HUFA such as 20:5n-3 and 22:6n-3 from acetic acid-^{14}C and palmitic acid (16:0)-^{14}C, but capable of converting exogenous 18:3n-3-^{14}C to 20:5n-3 and 22:6n-3 (Kanazawa et al., 1977b; Kanazawa,

Teshima and Tokiwa, 1979e). It assumes that the radioactive n-3 HUFAs might be formed by the addition of radioactive C_2-unit, which is produced during β-oxidation, to preexisting C_{18} and C_{20} acids with double bonds at the n-6 and n-3 positions by the same mechanism as demonstrated in fish and crustaceans (Kanazawa et al., 1979c).

Kanazawa and Koshio (1994) examined the ability for bioconversion of 18:3n-3 to other fatty acids, especially HUFAs by crustaceans. It was found that *P. japonicus, P. chinensis, Macrobrachium rosenbergii* and *Palaemon paucidens* had a limited ability for bioconversion of 18:3n-3 to 20:5n-3 and 22:6n-3 and larvae *P. japonicus* had a greater ability to bioconvert 18:3n-3 to n-3 HUFAs than juveniles, suggesting changes of fatty acid metabolism before and after metamorphosis. The larvae also showed active turnover of dietary 18:3n-3 to lower fatty acids such as 16:0.

Although, prawns are able to metabolize parent acids such as linoleic and linolenic acids to n-6 and n-3 HUFA in the same way as demonstrated in fish, it is probable that their conversion ability is less active (Kayama et al., 1980) and unable to elongate and desaturate linolenic acid to n-3 HUFA. This ability is very limited and cannot satisfy the quantitative needs of the shrimp which must be provided with a sufficient dietary n-3 HUFA supply (Kanazawa, Teshima and Endo, 1979a). The conversion ability of prawns are not so strong as in the rainbow trout (Castell, Lee and Sinnhuber, 1972a; Watanabe et al., 1974; Watanabe and Takeuchi, 1976).

The conversion of n-3 and n-6 type essential fatty acid to longer chain PUFAs has been found to depend on the relative amount of each in the diet (Colvin, 1976). The high content of PUFAs in lipids of all the tissue in the cultured herring is observed that it is possible to inhibit the elongation and/or desaturation of fatty acids (Owen and Middleton, 1977).

Essential fatty acid (EFA)

Generally, animals are not able to synthesize EFA in body or have limited ability to synthesize EFA for growth. EFA must therefore be supplied in diet (Castell et al.,1972b; Watanabe et al.,1974). The n-3 and n-6 series fatty acids are essential fatty acid in marine animals. The n-3 series fatty acids such as 18:3n-3, 20:5n-3 and 22:6n-3 are generally found in marine fish oils but the n-6 series fatty acids were almost abundant in terrestrial plant oils.

The principle functions of essential fatty acid

The principle functions of EFA (n-6 and n-3 PUFAs) are related to skin-, membrane-, and eicosanoid-related phenomena (Table 2). Essential fatty acid deficiency (EFAD) animals, particularly rats, lose considerable amounts of water through the skin. This limits growth rates because of the need for thermogenesis to compensate for evaporative heat loss.

Table 2. Major functions of essential fatty acids (Kinsella, 1991)

Parameters	Functions	Specific fatty acids
Skin	Reduce transepidermal water loss Component of acylglycoceramides	Linoleic acid columbinic acid
Membrane integrity, fluidity, thickness	Transport, receptors, enzymes	Long-chain polyunsaturated fatty acid (C_{20},C_{22})?n-3 PUFA, DHA
Eicosanoid precursor	Prostanoids (smoth muscle, secretory, etc.) Leukotrienes (cell:cell interactions, immune functions)	Arachidonic acid and eicosapentaenoic acid
Modulation	Eicosanoid synthesis	n-3 HUFA: n-9 HUFA

Essential fatty acids are important in membrane structure as integral components of phospholipids which are required for integrity and fluidity of intracellular and plasma membrane (Table 3). In this regard, long-chain HUFAs with 20 and 22 carbon atoms are apparently preferred. The long-chain n-3 HUFA EPA (20:5n-3) and DHA (22:6n-3) are readily incorporated into membranes and replace arachidonic acid (AA). Eicosatrienoic (ETA, 20:3n-9) is synthesized when dietary PUFAs are limited (Kinsella, 1991).

Table 3. Possible roles of polyunsaturated fatty acids in membranes (Kinsella, 1991)

-Influence membrane hydrocarbon chain order and thickness
-Modulate physical fluidity and flexibility
-Influence nonspecific permeability of bilayers to small molecules
-Influence lateral pressure and compressibility
-Influence rates of motion of molecular components
-Interact specifically with membrane protein to facilitate and receptor functions
-Modulate specific interactions of head groups of polar lipids with ion and acyl chains with cholesterol
-Modulate activity of membrane-bound enzymes and receptors
-Function as selective membrane barrier component

Essential fatty acid & marine species

EFAs which are important for normal growth in marine shrimp are n-3 and n-6 fatty acids. The n-6 and n-3 PUFAs have different metabolic functions and the same distinct differences in the PUFAs nutrition exist between the freshwater prawn and marine shrimp species (D'Abramo and Shen, 1993). It is also known that EFA requirements differ not only between species but also between growth stages (Rodriguez et al., 1994).

Overall, n-6 fatty acids are well assimilated by *P. monodon*. However, marine fish tend to utilize n-3 better than n-6 fatty acids (Merican and Shim, 1994). Sandifer and Joseph (1976) suggested that n-3 fatty acids are used for the biosynthesis of longer chain PUFAs for tissue incorporation, whereas n-6 fatty acids are utilized as energy source.

Kanazawa, Teshima and Ono (1979b) have investigated the capacity for conversion of 18:3n-3 to HUFAs (20:5n-3 and 22:6n-3) in various species of aquatic animals, and find that marine fish and prawn have a lower ability for such a bioconversion than freshwater fish and biosynthesize adequate amounts of this HUFA from dietary precursors (Colvin, 1976; Kanazawa et al., 1979b; Bottino et al., 1980; Kayama et al., 1980). Clearly, these fatty acids must be present in the diet to ensure successful growth and survival (Xu et al., 1993).

The influence of one type of fatty acids on the efficiency of utilization of the others has been ascribed to competitive inhibition of active enzyme sites, although there is no conclusive evidence that this inhibitory relationship exists for fish or other aquatic animals (Colvin, 1976).

The degree of fatty acids essentiality and roles of HUFAs in marine species are summarized in Table 4 and Table 5, respectively.

Table 4. Summary of degree of essentiality for fatty acids of crustaceans examined (Kanazawa and Koshio, 1994).

Lipid	Crustacean speices				
	Penaeus chinensis	*Penaeus japonicus*	*Penaeus monodon*	*Penaeus vannamei*	*Homarus spp.*
Fatty acids					
18:2n-6	-	+	nd	nd	nd
18:3n-3	++	++	nd	nd	nd
20:5n-3	++	+++	nd	nd	nd
22:6n-3	+++	+++	nd	nd	nd
Lipid Sources					
Soy bean oil	-	+	+++	+	+
Pollack liver oil	++	+++	++	+++	+++
Squid liver oil	+++	+++	nd	nd	nd

Notes: +++: most effective fatty acid or oil ++: middly effective fatty acid or oil
+: slightly effective fatty acid or oil -: not effective fatty acid or oil
nd: not determined

Table 5. Roles of HUFAs in marine species

Fatty acids	Functions	Animals
Liniolenic acid	Enhance growth and	rainbow trout
(18:3n-3)	health	(Castell et al.,1972 a,b)
	Play a crucial and specific role	trout
	in embryo development	(Leray and Pelletier, 1985)
n-3 HUFA	Growth and development of	fish (Watanabe, 1982)
	larva	
	Play a critical role in the phos-	marine organism
	pholipid fraction of bio-	(Izqierdo et al., 1989;
	membrane	Takeuchi et al., 1990)
	Vitellogenesis	penaeid shrimp
		(Xu et al., 1994b)
Eicopentaenoic	Play a role in the development	red sea bream
acid	of the swim bladder	(Kanazawa et al., 1982)
(EPA,20:5n-3)	Play some specific role in the	*P. chinesis*
	ovarian development process	(Xu et al., 1994a)
	relating to fecundity	
Docosahexaenoic	Play some specific roles in	*P. chinesis, P. setiferus*
acid	brain and neurological	(Xu et al., 1994a)
(DHA,22:6n-3)	development during early	
	embryogenesis related to egg	
	hatchability	
	Stress resistance	fish (Ako et al., 1994)

Essential fatty acid requirement

Although studies on lipid metabolism in crustaceans are conducted earlier, significant studies on EFA requirements of marine crustaceans are not initiated until the mid-to late 1970'S. Guary et al. (1976) demonstrated that 18:2 n-6, 18:3n-3, 20:5n-3 and 22:6n-3 were EFA supplied in the diet (Kanazawa, Teshima and Tokiwa, 1977a; Kanazawa et al., 1978; 1979b). In studies with *P. japonicus* (Kanazawa et al.,1977b), *P. indicus* (Read, 1981) and *P. serratus* (Martin, 1980), it has been shown that 18:3n-3 had greater EFA value than 18:2 n-6, with *P. japonicus*, where longer chain n-3 fatty acids like 20:5n-3 (Kanazawa et al.,1978) and 22:6n-3 (Kanazawa et al.,1979d) have greater EFA value than 18:2n-6 or 18:3n-3. The n-3 and n-6 series fatty acids provide normal growth and survival of juvenile Chinese prawn (*P. chinesis*). The n-3 family of fatty acids have greater EFA value than the n-6 in the diet of prawn. Long-chain fatty acids of either family have greater EFA value than shorter-chain fatty acids of the same series (Xu et al.,1993).

Enviromental influences of fatty acids in animals

1. Salinity

The proportions of saturated to unsaturated fatty acids in gills and kidney are lower in animals reared in seawater than those reared in freshwater. Likewise, the ratio of n-3 to n-6 fatty acids and total PUFAs of gills and kidney are higher in animals reared in seawater than reared in freshwater. The fatty acid

patterns of the phospholipid fractions show that seawater-reared milkfish have higher total PUFAs, especially n-3 fatty acids, than the freshwater-reared one. The differences in lipids and fatty acid compositions reflect a physiological response to the salinity in which milkfish were reared (Borlongan and Benitez, 1992).

2. Temperature

The n-3 fatty acid requirement would be greater for fish raised at low water temperatures. One explanation is that the structure of n-3 fatty acids with methylene interrupted double bonds permit a greater degree of unsaturation, which is necessary for membrane phospholipids to maintain flexibility and permeability characteristics at low water temperatures (Thongrod et al.,1990).

The quantity of n-3 HUFAs in diet

The n-3 HUFAs are important in metabolism of marine orgranisms to improve weight gain of the prawn. But animals can not sufficiently synthesize n-3 HUFAs from the precursor in the diet to promote good for growth. It impiles that n-3 HUFAs must be supplied in diet. However, suppressed growth of fish fed fatty acids, such as EFA has been observed in rainbow trout (Takeuchi and Watanabe, 1979), coho salmon (Yu and Sinnhuber, 1979) and channel catfish (Satoh, Poe and Wilson, 1989). The increase of 20:5n-3 or 22:6 n-3 levels from 1.0 to 2.0 % gives no further improvement of weight in prawn

(Kanazawa et al.,1979d). It is clear that marine animals need an optimum level of n-3 HUFAs in the diet for growth promotion.

The optimum level of dietary 20:5n-3 or 22:6n-3 for the prawn, *P. japonicus,* is about 1 % respectively. *P. monodon* postlarvae (PL) grew well on an *Artemia* with between HUFAs-content 2.65 mg/g and 12.55 mg/g, however, for increase osmotic stress resistant, feeding Artemia enriched with n-3 HUFAs 12.55 mg/g provides a better result (Ree et al.,1994). In freshwater shrimp, *M. rosenbergii,* requires n-3 HUFAs at 0.075% for growth and survival (D'Abramo and Shen, 1993).

Essential fatty acid deficiency (EFAD)

In the absence of dietary unsaturated fatty acids, growth is impaired. Structural and metabolic pertubations evident is characterized by reduced growth rates, keratosis, increased water loss via skin ,increased susceptibility to bacterial infection, male and female sterility, decreased eicosanoid synthesis, reduced contraction of myocardial tissue, abnormal platelet aggregation, impaired monocyte and macrophage function, and defective immune responses (Kinsella, 1991).

Essential fatty acid index

The EFA index (ratio of 18:1n-9/ n-3 HUFA in the phospholipid fraction) has been suggested as criterion for EFA status in marine fish. The ratio

less than 1.0 were considered indicative of sufficient levels of phospholipid n-3 HUFA in red seabream (Koven et al.,1992).

According toWatanabe (1982), the ratio of 20:3n-9/20:4n-6 or 20:3n-9/ 22:6n-3 are suggested for EFA index, however, these only an index for evaluation of EFAD condition of fish.