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Bertajuk

THE ESSENTIAL FATTY ACIDS- Revisited

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He has received several awards to date including the University Excellent Service Award consecutively for 1996-2002, MSAP Golden Service Award (2000), Melbourne University Simpson Scholarship Award (1984), Tory Foundation Award (2000) and the Kesatria Mangku Negara medal by the Malaysian Government (2000).

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THE ESSENTIAL FATTY ACIDS - REVISITED

SUMMARY

Since the discovery of the essential fatty acids (EFA) namely linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) in 1929 there has been a tremendous amount of research carried out on their possible sources, metabolism, physiological functions, deficiency symptoms, status assessments and dietary requirements for most animal species including man. They are termed essential fatty acids as they cannot be synthesised by the mammalian cellular system and must be derived from dietary sources. They are also essential functionally as they are required for the well being and health of the animals, including man.

Linoleic and α-linolenic acid are widely distributed in nature, where they are found in plants, and together with their polyunsaturated fatty acid (PUFA) metabolites are also found in many animal products. The EFA occur in a variety of vegetable oils such as corn, cottonseed, safflower, soybean, sunflower, olive, linseed and palm oil. Grass represents a rich source of EFA providing herbivores with adequate supplies of these fatty acids.

In the body, linoleic and α-linolenic acid are metabolised to their polyunsaturated fatty acid (PUFA) metabolites by a common enzymic sequence involving desaturation and chain elongation of the fatty acid molecule. They give rise to two separate families of essential PUFA namely the n-6 family derived from linoleic acid and the n-3 family derived from α-linolenic acid with no interconversion between members of the two families. These PUFA, now generally known as the omega-6 and omega-3 fatty acids are more biologically active than their precursors. They have structural functions in the maintenance of proper membrane function and integrity. They also have physiological and regulatory roles which are attributed to the conversion of the essential PUFA metabolites to biologically active prostaglandins, thromboxanes and leukotrienes.

The dietary requirement for the EFA has been established for most animal species studied so far including man. Generally the minimum requirement for linoleic acid to prevent EFA deficiency in most mammals is about 1-2.5% of the total dietary energy. It seems reasonable to assume that the requirement for α-linolenic acid is less than that for linoleic acid. Currently, ratios of between 2-5:1 of n-6:n-3 PUFA have been recommended for healthy human populations.

This lecture also covers the author's close research encounters with the EFA including the assessment of the EFA status of the fetal and neonatal lamb, dietary manipulations carried out to successfully produce more "healthy" unsaturated mutton and the dietary

manipulations to increase the essential PUFA in popular, local freshwater fishes which is currently being carried out.

Reassessment of the EFA status of the fetal and newborn lamb revealed that these animals were normal, refuting earlier suggestions that they were EFA-deficient. The ewe's milk was confirmed to be an important source of EFA during the early postnatal period. The low levels of the EFA and high levels of their PUFA metabolites in fetal and newborn lambs are due to the combined effects of a significant placental transfer of EFA from the ewe to the fetus and their extensive metabolism in the feto-placental tissues.

The dietary manipulations employing various combinations of a commercial concentrate and oil palm (*Elaeis guineensis*) frond pellets demonstrated the feasibility of increasing the unsaturated fatty acid (UFA) content of mutton to a level that would promote healthy changes in the consumer's blood fatty acid profiles.

Early experimental trials showed that local, popular freshwater fishes may represent rich sources of EFA and PUFA in the human diet. Certain species such as the catfish contain low levels of the more desirable omega-3 fatty acids demonstrating a potential of dietary manipulations to increase these PUFA and decrease the n-6:n-3 fatty acid ratios to fall within the recommended values.

The validity of the *Lipid hypothesis* presented some decades earlier which had suggested that the saturated fatty acids (SFA) and cholesterol are the main causes for cardiovascular and cancer problems in man is also reviewed. The trans-fatty acids (TFA) produced during chemical processing for example in the making of margarines may also be a contributing factor. While there is an obligatory need for the EFA and their PUFA metabolites, there is also a danger arising from their excessive intake and possible conversions through oxidation to undesirable, health threatening products. There is also a need for accurate, quantitative determinations of the concentrations of the EFA and essential PUFA in foods which will assist in the selection of the type and quantity of these materials to be recommended and included in the diet.

INTRODUCTION

Historically, the essential fatty acids (EFA) namely linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) were discovered and reported in 1929 by George and Mildred Burr who described deficiency symptoms such as retarded growth and scaly skin in young rats reared for several months on a fat-free diet (Burr and Burr, 1929). The EFA are naturally-occurring unsaturated fatty acids of chain length 18, 20 or 22 carbon atoms and contain two to six methylene interrupted cis, cis double bonds, the latter being a requirement for EFA activity (Holman, 1970). Both EFA cannot be manufactured in the body because the mammalian enzyme system is unable to insert a double bond beyond the ninth carbon in the fatty acid chain. Hence, they must be obtained from dietary sources. They are also termed essential as they have specific physiological functions which promote good health and well being of animals including man. It is now well recognised that the EFA are required by most, if not all animal species including man for normal growth and maintenance and for other physiological processes.

As the term EFA includes both the parent members linoleic and (-linolenic acid and their derived polyunsaturated fatty acid (PUFA) metabolites, the term EFA will be used for only linoleic and α-linolenic acid while the term PUFA will refer to their derived metabolites.

DIETARY SOURCES OF EFA

Linoleic and α-linolenic acid are widely distributed in nature, where they are found in plants, and together with their PUFA metabolites are also found in many animal products. The EFA occur in a variety of vegetable oils such as corn, cottonseed, safflower, soybean and sunflower oil which contain about 50% linoleic acid. Groundnut, olive and palm oil contain about 10-30% linoleic acid. Linseed oil contains over 50% of α-linolenic acid. Grass contains about 60% α-linolenic acid and 13% linoleic acid which provide herbivores with adequate supplies of these fatty acids.

Linoleic, and to a lesser extent, α-linolenic acid are components of membrane phospholipids in the tissues of the body. Their PUFA metabolites namely arachidonic acid (AA, 20:4n-6) from linoleic acid is a major component of membrane phospholipid in most tissues while docosahexaenoic acid (DHA, 22:6n-3) derived from (-linolenic acid is abundant in the retina and brain. The AA may also be obtained in the diet from animal meats and poultry, while DHA is abundant in fish and seafood.

Human milk contain 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3 (EPA) and 22:6n-3 (Crawford *et al*, 1973) and their inclusion in infant formula was recommended. Cow milk on the other hand is devoid of DHA. Breast-fed infants had higher EPA and DHA in their erythrocytes than those that were bottle-fed (Sanders and Naismith, 1976).

METABOLISM OF EFA

After the discovery of the EFA in 1929, little was found about their metabolism and conversion to PUFA because investigations in this area were restricted due to the inadequacy of analytical methods. However, when gas-liquid chromatography (GLC) was developed in the mid-1950"s this resulted in more specific determinations of fatty acid structure and rapid advances in the knowledge of the metabolism of EFA were made.

It is now established that there are two separate important families of essential PUFA namely the (n-6) family derived from linoleic acid and the (n-3) family derived from α-linolenic acid. There is no interconversion of fatty acids from one family into fatty acids of another. The parent EFA are metabolised to their PUFA metabolites by a common enzymic sequence involving alternate desaturation and chain elongation of the fatty acid molecule, as shown in Figure 1. Incidentally, oleic acid (18:1n-9) which is a non-essential fatty acid is also metabolised by the same enzymic system.

It is generally assumed that only three specific desaturases, which are exclusively microsomal, are used in PUFA biosynthesis. These enzymes introduce double bonds, hence making the fatty acid more unsaturated, at the 6-, 5- and 4- positions (Figure 2). Much of the basic knowledge about these desaturases are based on inferred information that is based on elaborate studies of the Δ9-desaturase, which converts the non-essential palmitic acid (16:0) to palmitoleic acid (16:1n-9) and stearic acid (18:0) to oleic acid (18:1n-9), and is not used in the n-6 or n-3 PUFA biosynthesis. The desaturase activities are not evenly distributed among the body organs and they decrease with age (Brenner, 1971) suggesting that the high desaturase activities present in the very young animals provide essential PUFA for the synthesis of phospholipids, which together with protein form major constituents of the membranes of new tissues.

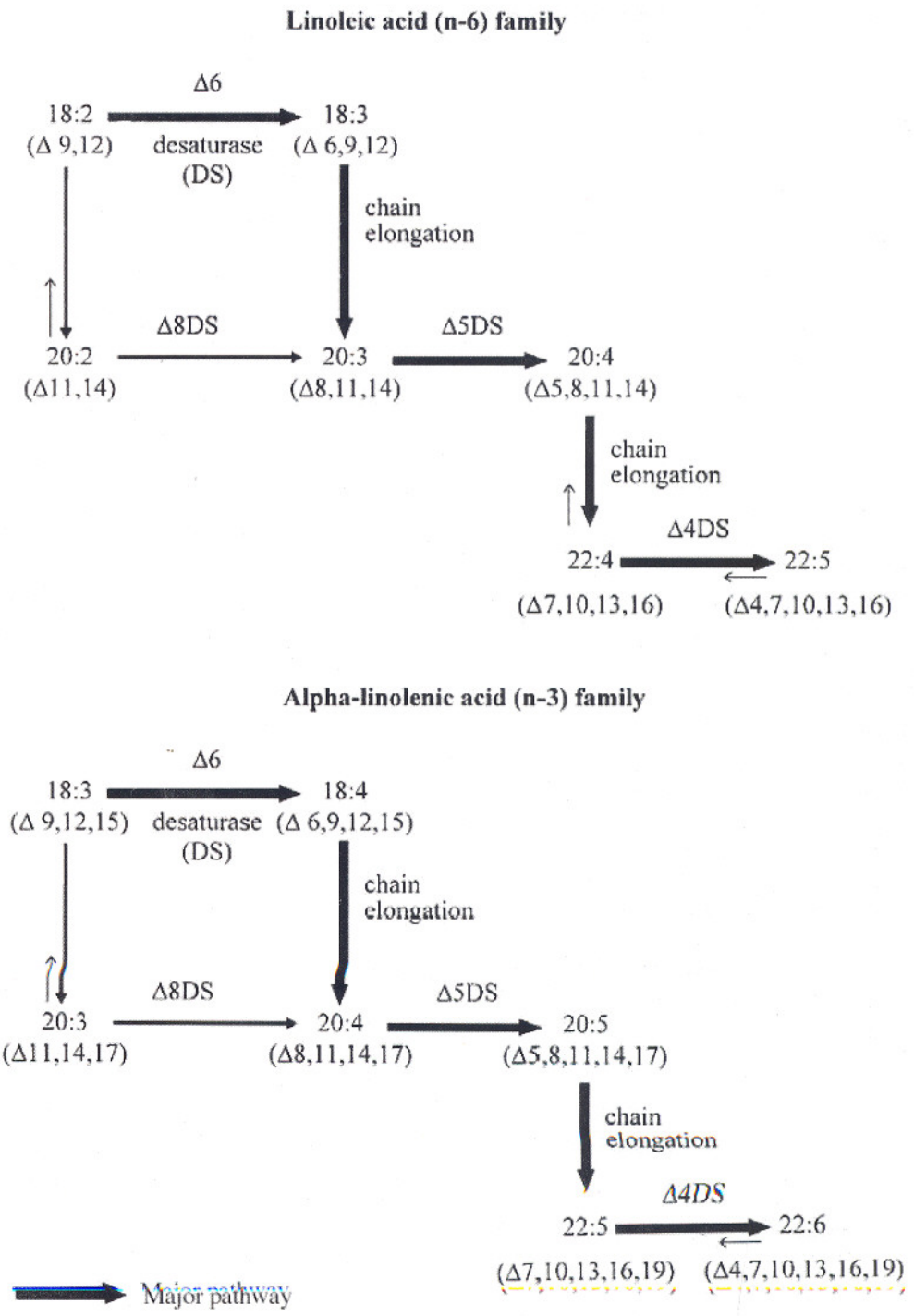

Figure 1: Biosynthetic pathways of PUFA n-6 and PUFA n-3

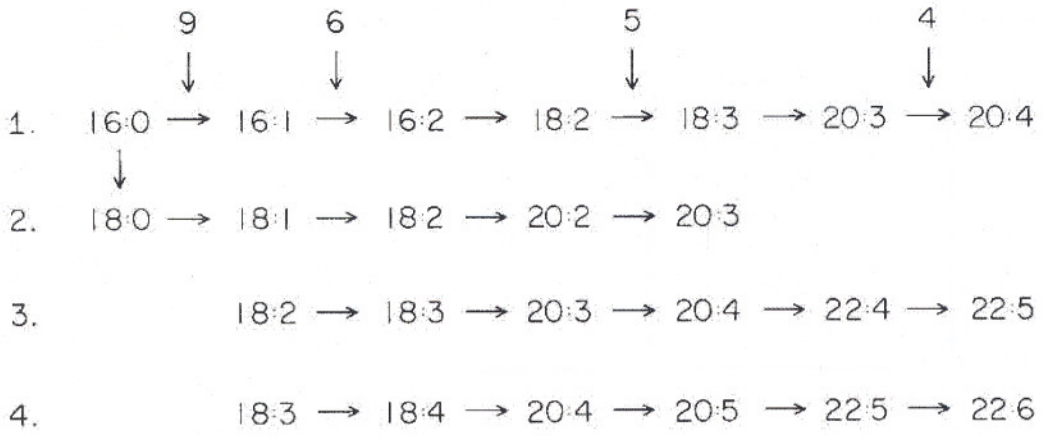

Figure 2: Pathways for the biosynthesis of the four families of polyunsaturated fatty acids

Chain elongation reactions which are also mainly microsomal occur at the carboxyl end of the fatty acid molecule where malonyl CoA is the obligatory source of the two-carbon increment (Sprecher,1990) (Figure 3). For example, the conversion of 18:2n-6 (linoleic acid) to 22:5n-6 requires two chain elongation steps - i.e. 18:3n-6 to 20:3n-6 and 20:4n-6 (arachidonic acid) to 22:4n-6. Similarly, 18:4n-3 and 20:5n-3 (eicosapentaenoic acid) are substrates for chain elongation in the synthesis of 22:6n-3 (docosahexaenoic acid).

Another process in the metabolic pathways of PUFA in animal systems is called retroconversion, which is the chain shortening of PUFA. It occurs by the loss of either a two-carbon fragment or a four-carbon fragment containing a double bond from PUFA containing 20 or 22 carbons. Retroconversion or partial degradation of the PUFA is really partial β-oxidation which occurs in the mitochondria and peroxisomes.

Generally, the type and amount of essential EFA and PUFA incorporated into tissue lipids are determined ultimately by an interaction of regulated processes which include the substrate competition for the desaturases and chain elongases, feedback inhibition, enzymic competition for substrates and hormonal and dietary modifications of desaturase activities. As an example, linoleic, α-linolenic and oleic acid compete as substrates for the Δ6-desaturase with α-linolenic having the greatest affinity for the enzyme, followed in order by linoleic and then oleic acid. The desaturase activities involved in the biosynthesis of both essential and non-essential PUFA have been shown to respond to hormonal stimuli including insulin and glucagon suggesting a possible interaction between glucose and PUFA metabolism. The enzyme activities can also be modified by dietary components including carbohydrates, fatty acids and proteins. Clearly, there are different mechanisms *in vivo* which are involved in dictating what regulates the conversions of PUFA which may not be explained by enzymatic studies.

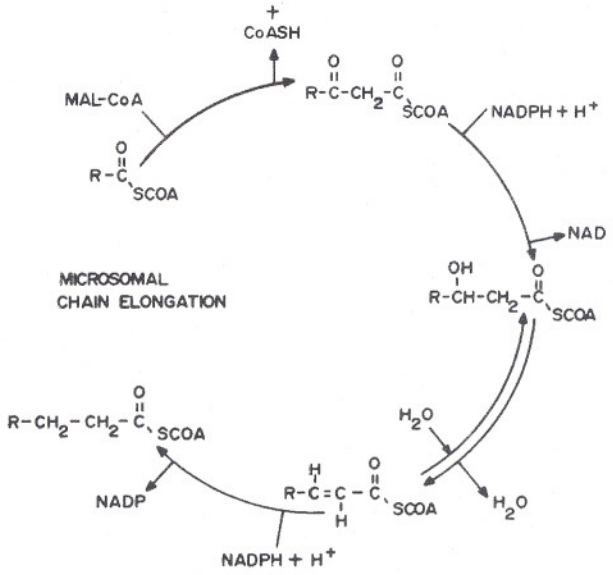

Figure 3: The reaction pathway for the microsomal malonyl-CoA dependent chain elongation of fatty acids

PHYSIOLOGICAL FUNCTIONS OF EFA

The EFA have structural functions in the maintenance of proper membrane function and integrity (Gurr and Harwood, 1991). They have physiological and regulatory roles which are attributed to the conversion of some of their PUFA metabolites to biologically active eicosanoids. The EFA also have a role in lipid transport.

Membrane function and integrity

The EFA form an integral part of the phospholipids which are one of the components of all biomembranes. In EFA deficiency, the partial replacement of EFA in the 2-position of phospholipids by the fatty acid 20:3n-9 derived from oleic acid may result in deleterious effects on biomembrane function and integrity. Which may include mitochondrial swelling, increased cellular fragility and increased permeability of the skin to water. The variation in chain length and unsaturation of component fatty acids in the phospholipids largely determine the membrane fluidity. Generally, the organs or tissues involved in storage (adipose tissues), chemical processing (liver), mechanical work (muscle) and excretion (kidney) tend to have membranes in which the n-6 fatty acids predominate, while the n-3 fatty acids predominate in the membranes of tissues for nervous, reproduction and retinal functions (Gurr and Harwood, 1991). For example, the high concentration and specific

incorporation of docosahexaenoic acid (22:6n-3) in the cell membrane of the brain and retina suggests a functional role of the n-3 fatty acid, probably in imparting the required degree of membrane fluidity to these neuronal tissues, since fluidity is necessary for the biochemical processes involved in the initiation and propagation of electrical impulses. Retinal atrophy and virtual loss in eyesight have been observed in n-3 deficient animals.

Precursors for the synthesis of eicosanoids

The EFA and their PUFA are precursors for the synthesis of the biologically active eicosanoids (prostaglandins, thromboxanes, leukotrienes) which are synthesised in various tissues when required, are rapidly metabolised and have numerous physiologic effects. The prostaglandins play important roles in reproduction, parturition, regulating the release of fat from adipose tissue, stimulation of smooth muscle and other physiological functions. When required, arachidonic acid is released from membrane phospholipids by phospholipase A_2 and converted to eicosanoids (Thomas and Holub, 1994) (Figure 4). Cyclo-oxygenase converts arachidonic acid to the various prostaglandins (PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2) and thromboxanes (TxA_2), while lipoxygenase converts arachidonic acid to various leukotrienes (LTA_4, LTB_4, LTC_4, LTD_4, LTE_4). The PGE_2 is an important physiological component in the process of LHRH (Luteinising Hormone Releasing Hormone) secretion from the hypothalamus and ovulation (Smith *et al*, 1989). The role of $PGF_{2\alpha}$ in reproductive processes particularly during parturition has long been established. The TxA_2, a vasoconstrictor and an activator of platelet aggregation, and PGI_2 which inhibits platelet aggregation and relaxes blood vessels, contribute to the regulation of vascular tone and haemodynamics. The leukotriene, LTB_4 is a chemotactic agent produced by neutrophils which function in the inflammatory response by aiding in the removal of foreign bodies such as bacteria.

Lipid Transport

The plasma very low density lipoproteins which are normally responsible for lipid transport from the liver contain high levels of linoleic and arachidonic acids. This lipid transporting ability was found to be reduced in EFA deficiency (Sinclair, 1968) resulting in an accumulation of cholesterol, triglycerides and cholesteryl esters in the liver (Holman, 1968). The EFA are involved in the transport of cholesterol in the blood circulation and helps reduce the workload of the heart.

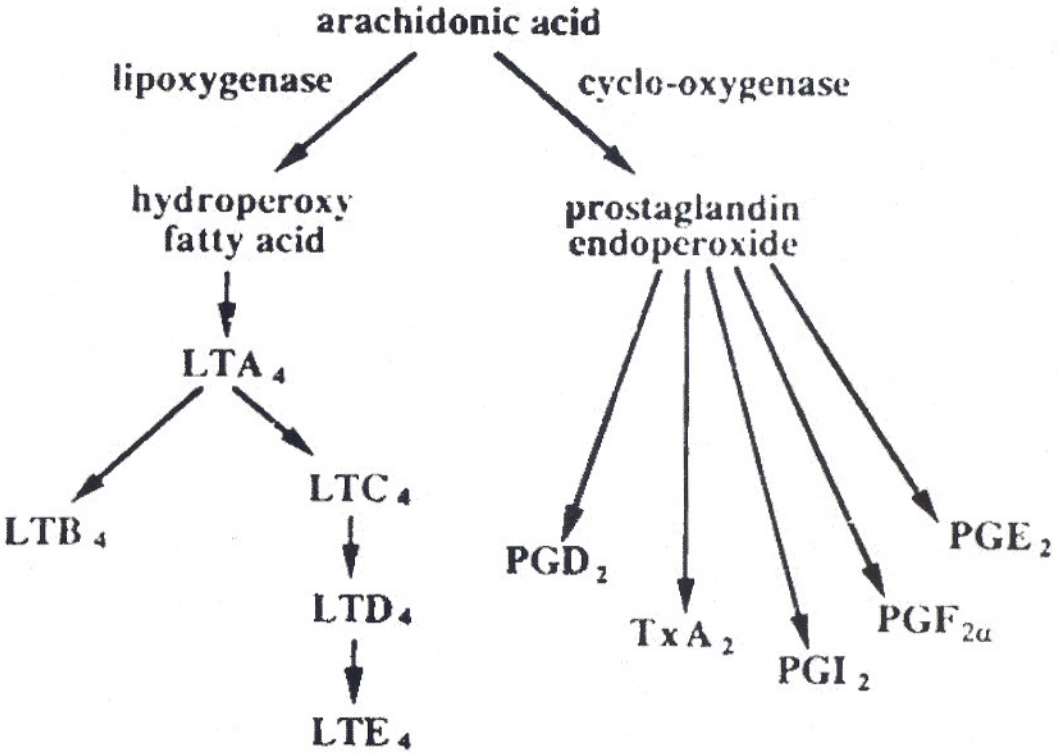

Figure 4: Pathway for the formation of prostaglandins (PG), thromboxanes (Tx) and leukotrieness

ROLES OF EFA IN HUMAN NUTRITION AND HEALTH

The eicosanoids produced by arachidonic acid are involved in the development of various diseases including cardiovascular diseases, arthritis, hypertension, asthma and allergies and cancer (Thomas and Holub, 1994). Research has demonstrated the potential beneficial effects of n-3 fatty acid-rich diets on these disease processes. The favourable effects of these diets could be due to the production of eicosanoids from 20:5n-3 (EPA) which are less potent than the eicosanoids derived from arachidonic acid. The action of cyclo-oxygenase on EPA forms prostaglandins and thromboxanes which are designated with a subscript 3 (e.g. TxA_3) while the action of lipoxygenase on EPA produce leukotrienes with a subscript 5 (e.g. LTB_5).

Cardiovascular diseases

Arterial thrombosis or excessive platelet aggregation is a significant factor in the development of cardiovascular disease. The relative balance of TxA_2 (potent stimulator) and PGI_2 (inhibitor) determines the tendency for platelet aggregation. An increase in the TxA_2/ PGI_2 ratio will promote platelet aggregation and thrombosis. Consumption of diets high in n-3 fatty acids through eating fish or fish oil supplementation will result in a reduction in platelet aggregation and increase in bleeding times, which are effects due to altered eicosanoid production.

Consumption of fish or fish oil supplementation also reduce plasma triglycerides associated with reduced plasma very low density lipoproteins (VLDL) as the latter represents the major carrier of triglycerides in the blood. The effect on plasma total cholesterol levels have been variable but LDL-cholesterol and HDL-cholesterol levels have been increased by fish oil consumption.

Rheumatoid Arthritis

Rheumatoid arthritis is characterised by an inflammation of the joints, usually of the hands, feet and knees. Fish oil supplementation has been successful in alleviating some of the symptoms of this inflammatory disease, probably due to reduced LTB_4 production by neutrophils whose arachidonic acid content is reduced by 20:5n-3 (EPA). Less arachidonic acid would then be available for LTB_4 synthesis.

Hypertension

An increase in vasoconstrictor over vasodilatory prostaglandins would give rise to elevated blood pressures (hypertension). Both systolic and diastolic blood pressure are reduced by fish or fish oil supplementation where the effect may be related to the balance of vasoconstrictor (TxA_2 from arachidonic acid)) and non-vasocontrictor (TxA_3 from eicosapentaenoic acid) prostaglandins produced. In addition, PGI_3 (from EPA) is a vasodilator with similar potency to PGI_2.

Asthma/Allergies

Asthma or an allergic reaction results in difficult breathing due to constriction of the airway passages. An allergic response involves the stimulation of mast cells to release leukotrienes, LTC_4, LTD_4, LTE_4 which are potent constrictors of the airways.

Cancer

The type of dietary fat has been shown to influence the carcinogenic process. Diets rich in n-6 PUFA appear to stimulate the growth of tumors of the breast to a greater extent than diets rich in either saturated, mono-unsaturated or n-3 PUFA, where the enhancing effect of the n-6 PUFA could be due to the presence of linoleic acid at levels far exceeding the requirement for growth. Generally, the n-6 fatty acids in the diet appear to stimulate the growth of tumors whereas fish oil diets containing n-3 PUFA arrest tumor growth.

EFA DEFICIENCY

Essential fatty acid deficiency will develop if the metabolic requirements for n-3 and n-6 EFA and PUFA are not met (Lands, 1992). The signs of EFA deficiency have been described in various animal species and the effects include gross symptoms like reduced growth, scaly skin, loss of hair or fur; pathological effects which include fatty liver, reproductive failure, testicular damage, kidney damage, increased susceptibility to infections and biochemical and/or physiological effects including increased skin water loss, increased 20:3n-9, decreased 18:2n-6 and 20:4n-6 and increased serum cholesterol and triglycerides. Some differing characteristics of n-3 and n-6 EFA deficiencies in man are shown in Table 1.

Table 1. The differing characteristics of n-3 and n-6 essential fatty acid deficiencies

	n-3	n-6
Clinical features	Normal skin, growth and reproduction Reduced learning Abnormal electroretinogram Impaired vision Polydipsia	Growth retardation Skin lesions Reproductive failure Fatty liver Polydipsia
Biochemical markers	Decreased 18:3n-3 and 22:6n-3 Increased 22:4n-6 and 22:5n-6 Increased 20:3n-9 (only if n-3 are also low)	Decreased 18:2n-6 and 20:4n-6 Increased 20:3n-9 (only if n-3 are also low)

(Adapted from Connor, W.E., Neuringer, M. and Reisbick, S., *World Rev. Nutr. Diet*, 66: 118, 1991)

DIETARY REQUIREMENT FOR EFA

A requirement for EFA has been established for most animals and is modified by factors such as the species and age of the animal, the diet and prevailing environmental conditions. Different biological criteria may require different amounts of EFA. For example, while 100 mg/d of linoleic acid are required for optimal growth in the rat, only 30 mg/d were required to cure skin lesions (Houtsmuller, 1973).

The minimum dietary requirement for linoleic acid to prevent EFA-deficiency has been established at 1-2% of the total dietary energy for most mammals (Mohrhauer and Holman, 1963) and higher levels of 3-4% have been recommended for the human infant (Hassam et al, 1977). Obligate carnivores such as the cat lack a functional Δ6-desaturase system and therefore has a reduced ability to synthesise PUFA. In this case, dietary linoleic acid at a level of 2.5% of energy with 0.04% of arachidonic acid are necessary to meet the requirement of the cat. Little is known regarding the dietary requirement of EFA in the ruminant species. Although the intake of dietary EFA by the ruminant animal usually exceeds 1% of the dietary energy, biohydrogenation of the ingested fatty acids by the rumen microorganisms (Figure 5) will reduce the amounts of EFA available to the animal. Currently, not only the requirement for the n-3 fatty acids is recommended, the n-6:n-3 fatty acid ratio values also have to be considered as the two families compete for the same desaturase and chain elongase enzyme systems. A n-6:n-3 fatty acid ratio of between 2-5:1 is recommended which is provided by 3% (n-6 fatty acids) and 0.3-0.5% (n-3 fatty acids) of total dietary calories. An intake of α-linolenic acid at 800-1100 mg/d and 300-400 mg/d of 20:5n-3 and 22:6n-3 should prevent deficiencies in human adults (Simopoulos, 1996).

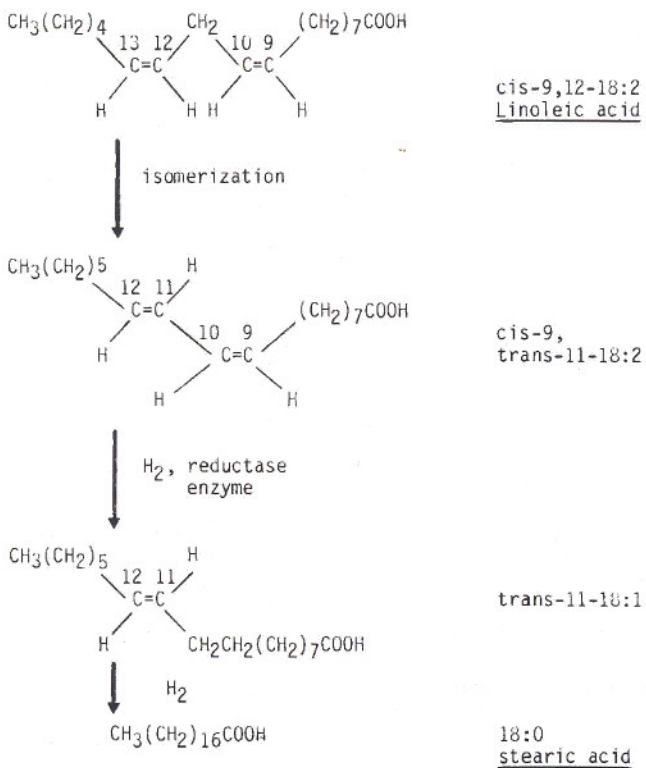

Figure 5: Biohydrogenation of linoleic acid

SOME CLOSE ENCOUNTERS WITH THE EFA'S

Reassessment of the EFA status of fetal and newborn lambs

Previous reports available in the literature had suggested that the fetal and newborn lamb were EFA-deficient based on 20:3n-9/20:n-6 ratios exceeding 0.4 and the occurrence of low levels of linoleic and α-linolenic acid in the plasma and tissues of these animals. In contrast to previously published reports, and with improved analytical techniques particularly through improved extraction procedures and improved resolution by capillary gas liquid chromatography, the 20:3n-9/20:n-6 ratios obtained for fetal lambs and at birth were generally less than 0.4 (Rajion, 1985; Rajion *et al*, 1985).

The occurrence of low levels of linoleic and α-linolenic acid in the plasma and tissues of the fetal and newborn lambs were accompanied by high levels of their essential PUFA metabolites. Radiolabelled linoleic and α-linolenic acid injected into 87d and 125d-old chronically-cannulated fetal lambs were found to be converted to their PUFA metabolites indicating the presence of active Δ6-, Δ5- and Δ4-desaturation and elongation systems. The fetal placental cotyledons and liver were the major organs involved in the synthesis and supply of essential PUFA to the fetal lamb (Figure 6a,b). The n-6 and n-3 PUFA concentrations progressively increased from the maternal liver through the placenta to the fetal liver and brain. The rate of metabolism of n-3 fatty acids was greater than that of the n-6 fatty acids.

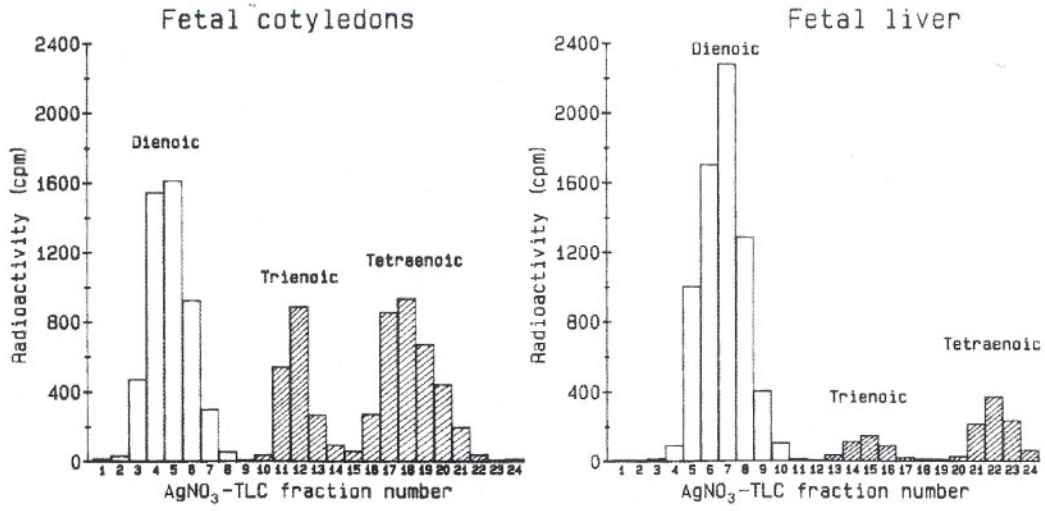

Figure 6a: Radioactivity in 18-2 n-6 and PUFA n-6 in fetal tissues

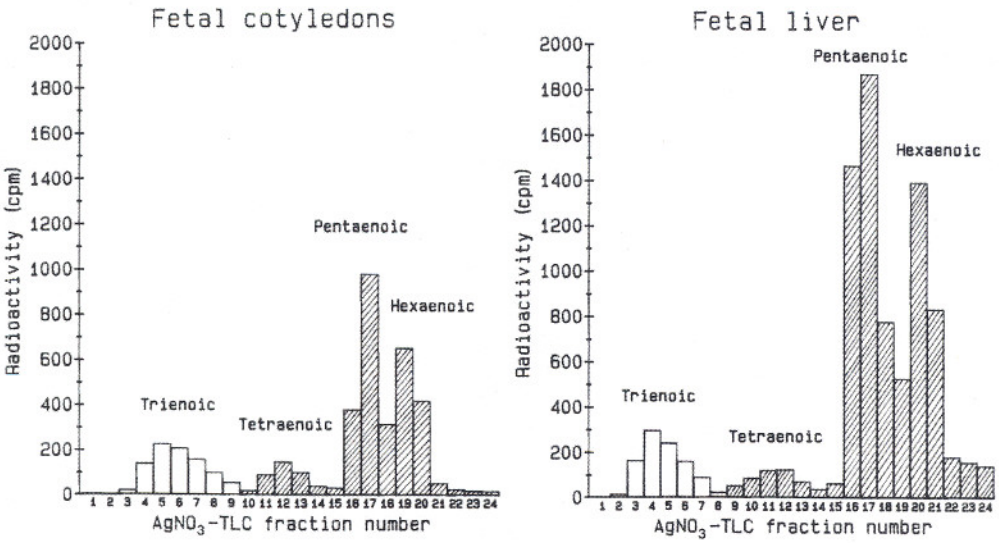

Figure 6b: Radioactivity in 18:3 n-3 and PUFA n-3 in fetal tissues

The sheep placenta was also shown to have the capacity to store large amounts of EFA and metabolise maternally-derived EFA to their PUFA metabolites and also the capacity to hydrolyse maternal plasma cholesteryl esters to release the fatty acids which would then be available for transport to the fetus.

The transfer of [1-14C]-labelled linoleic and α-linolenic acid across the sheep placenta was investigated in chronically-cannulated ewes and fetuses using a continuous infusion technique. The fatty acids were infused into the maternal uterine artery in six single-pregnant ewes ranging between 120-128d of gestation and the incorporation of radioactivity in the maternal and fetal plasma lipids was measured at regular intervals up to 24hr after the start of infusion. The uptake and metabolism of the infused radiolabelled EFA in the maternal, placental and fetal tissues were also assessed. Contrary to previous reports, the results showed a significant placental transfer of both EFA to the fetus. During the infusion period the radioactivity detected in the fetal plasma lipids ranged between three to 33% of the radioactivity found in the maternal plasma lipids. Between one to eight percent of the infused fatty acids were transferred across the placenta and taken up by the fetal tissues. There was an extensive uptake and metabolism of the infused EFA by the maternal liver, fetal placental cotyledons and fetal liver. The metabolism of n-3 fatty acids was greatest in the fetal liver while the metabolism of n-6 fatty acids was greatest in the fetal cotyledons.

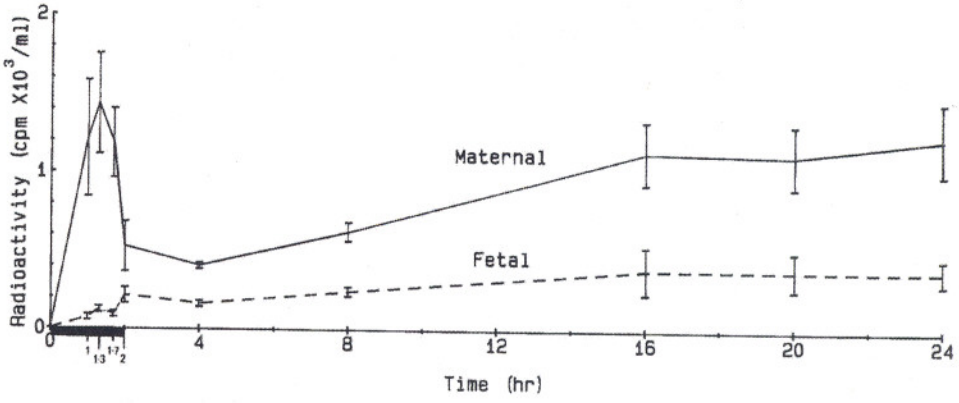

Figure 7a: Radioactivity in maternal and fetal plasma total lipids after infusion of [1-^{14}C] – linoleic acid into the maternal circulation

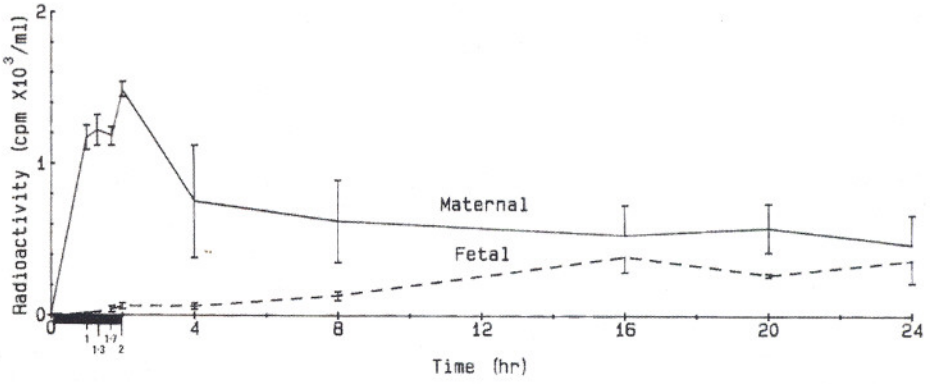

Figure 7b: Radioactivity in maternal and fetal plasma total lipids after infusion of [1-^{14}C] – α–linolenic acid into the maternal circulation

It was clear from this investigation that the traditional parameters such as the 20:3n-9/20:4n-6 ratios and high levels of oleic acid normally associated with EFA deficiency in the monogastric animals are not suitable criteria for assessing the EFA status of the fetal and newborn lambs (Rajion *et al*, 1986). The high tissue and plasma levels of oleic acid found in the lambs were characteristic of this species and, in the presence of low levels of EFA, the production of 20:3n-9 from oleic acid is a normal consequence of the metabolism of oleic acid by active desaturase and chain elongase systems present in the tissues. The total PUFA metabolites of linoleic and α-linolenic acid are more useful criteria for the assessment of the EFA status in the fetal and newborn lambs. This is particularly important for the fetal lamb in which active brain growth occurs prenatally and so the requirement for essential PUFA such as AA and DHA is high and accounts for the fact that the lamb has a well developed brain at birth. A diagrammatic representation of the possible supply of EFA and PUFA to the fetal lamb is shown in Figure 8.

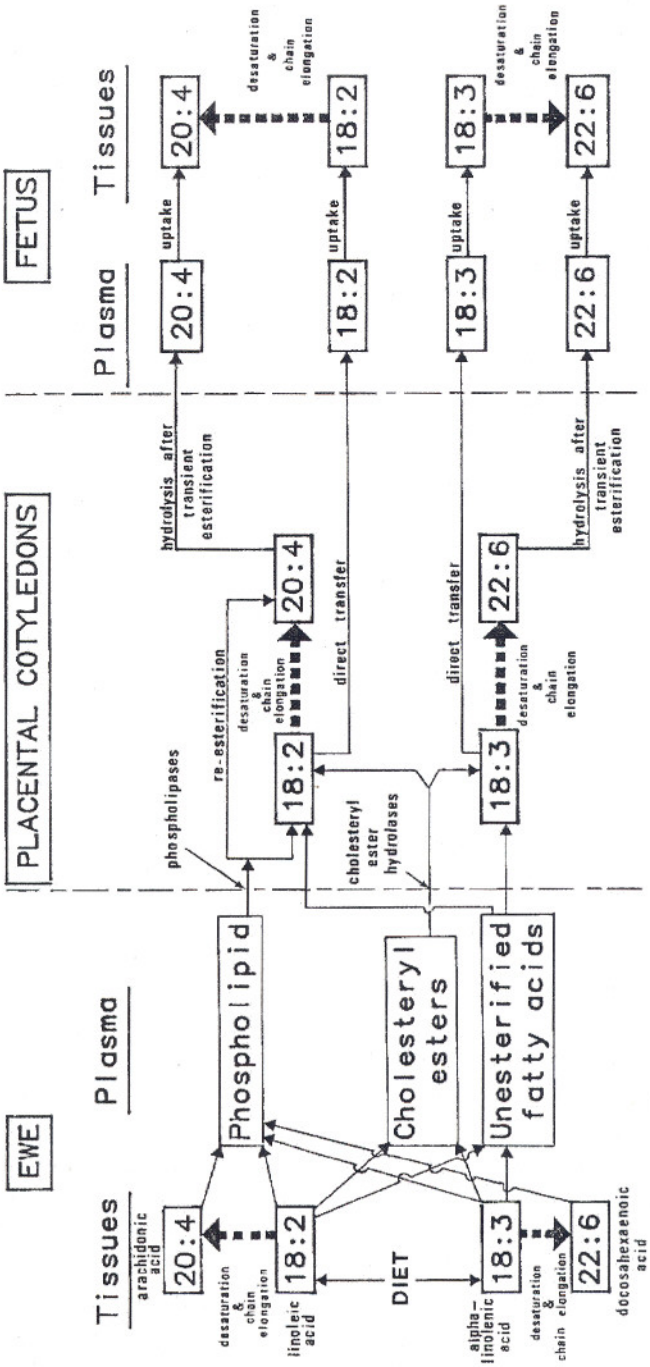

Figure 8: Diagrammatic representation of the source and metabolism of EFA in the maternal, placental and fetal tissues of the sheep

Dietary manipulation to increase unsaturated fatty acids in mutton

An investigation was carried out where 43 seven-month old Black Belly x Malin crossbred rams were fed either 80% commercial pellet + 20% (%w/w) oil palm (*Elaeis guineensis*) frond pellets (CON group), 50% commercial pellet + 50% oil palm frond pellets (HAF group) or 20% commercial pellet + 80% oil palm frond pellets (OPF group) for 14 weeks. The levels of EFA and their PUFA were measured in the plasma during the trial and tissues at slaughter. Sprague-Dawley rats were used as bioindicators and fed either standard rat chow, meat pellets derived from the slaughtered experimental sheep or meat pellets prepared from commercially available mutton. Oesophageal intubations were carried out at zero, two, four, six and eight hours post feeding in nine surviving animals to determine the changes in the rumen conditions due to the treatment diets.

The plasma and tissues of the CON animals had significantly increased levels of unsaturated fatty acids compared to the HAF and OPF groups (Rajion *et al*, 2001; Goh *et al*, 1999). The CON animals had more n-6 PUFA in their tissues whereas the n-3 PUFA were more abundant in the OPF animals. Although the tissue n-6 and n-3 PUFA content and the resultant n-6:n-3 ratios which exceeded 15:1 was far from being desirable for human health as a result of the feed not formulated to contain n-6 and n-3 fatty acids in favourable proportions, there is a vast potential for further stringent manipulations to produce "healthy" mutton. The CON mutton actually increased the rat serum HDL-Cholesterol significantly compared to those fed the commercially available mutton (Goh *et al*, 2000). A significant observation was that there was a low (0.35% of total fatty acids) plasma and tissue content of trans fatty acids in direct contrast to the levels in typical ruminant meat and milk (9% per 100 g fats). The consumption of these fatty acids appear to be strongly correlated with increased cancer and cardiovascular diseases.

Dietary manipulation to increase essential PUFA in local freshwater fishes

Fish can be a rich source of essential n-3 and n-6 PUFA where the former have been shown to have preventive and curative effects on cardiovascular diseases, neurodevelopment in infants, cancers and fat glycemic control as discussed earlier. However, the local freshwater fishes, compared to the marine fishes, generally contain high levels of n-6 PUFA and low levels of the more desirable n-3 PUFA (Rajion *et al*, 2003a; Suriah *et al*, 1994) As most of the earlier work reported the levels of fatty acids in fish tissues expressed as the percentage of total fatty acids, it becomes necessary to determine the absolute amounts of essential PUFA in freshwater fishes to which will help to identify the types which contain high levels of the more desirable n-3 fatty acids when selecting fish for diets.

In a preliminary trial, 14 each of laboratory, tank-reared adult Catfish (*Clarias macrocephalus*) and Red Tilapia (*Oreochromis mossambicus x Oreochromis niloticus*) were filleted, subjected to fatty acid extractions using chloroform:methanol (2:1, v/v), transmethylated using 14% methanolic boron trifluoride, separated and determined by gas liquid chromatograph.

These two species were selected as they represent popular local freshwater fishes in the Malaysian diet. The absolute amounts of the fatty acids were determined by an internal standardization method employing Heneicosanoic acid (C21:0) as the reference standard, added prior to transmethylation. The fatty acid concentrations were expressed as mg fatty acid/g tissue after correcting for moisture loss. This expression is more useful than the commonly used percentage of the total fatty acids as the former is a measure of the actual content of the fatty acids in the fish fillet

The results showed that the Red Tilapia contained significantly higher levels of essential PUFA, inclusive of a higher total of both n-6 and n-3 fatty acid concentrations (Rajion *et al*, 2003b)(Table 2). Although the n-6 fatty acid concentrations in the Red Tilapia were consistently higher than the Catfish, the concentration of the more desirable n-3 PUFA particularly DHA was significantly higher than those in the Catfish. This is an important observation as the DHA has been shown to play a crucial role in the prevention of arteriosclerosis, heart attack, depression and cancer. Expectedly, the n-6:n-3 ratio was very low (2:1) for Red Tilapia compared to 18:1 for Catfish.

The present study showed that the internal standardization method employed which measured the absolute amounts of the fatty acids in the fish tissues is preferred to allow for more quantitative fatty acid determinations in fish tissues and feeds for more accurate interpretations of analytical data. It also showed that these consumer-popular, local freshwater fishes could be significant sources of essential PUFA in the Malaysian diet. It also demonstrated the potential for possible dietary manipulations in freshwater aquaculture to adjust the n-6: n-3 ratios in these fishes, particularly the Catfish, to fall within the recommended range of between 1:1 to 5:1 for healthy human populations.

Currently, similar local freshwater fishes are being fed modified diets to include either cod liver oil (low (-linolenic, high PUFA n-3) or flaxseed oil (high α-linolenic, low PUFA n-3) to investigate changes in the n-6 and n-3 fatty acid profiles of the fish fillet and hypothesise on the desaturase and elongase enzyme systems in these fishes.

Table 2. Essential PUFA concerntrations (mg/g tissue; mean ± SEM, n=14) in the Red Tilapia and Catfish

Fatty acid	Red Tilapia	Catfish
Linoleic (18:2 n-6)**	69.9 ± 2.9 (14.1)[a]	50.7 ± 2.7 (16.2)
Arachidonic (18:2 n-6)**	30.2 ± 1.9 (6.1)	3.9 ± 0.2 (1.2)
Linolenic (18:3 n-3)**	16.4 ± 1.2 (3.3)	2.1 ± 0.1 (0.7)
Eicosapentaenoic (20:5 n-3)**	1.5 ± 0.1 (0.3)	0.1 ± 0.0 (0.03)
Docosapentaenoic (22:5 n-3)**	1.8 ± 0.2 (0.4)	0.2 ± 0.0 (0.1)
Docosahexaenoic (22:6 n-6)**	27.3 ± 3.9 (5.5)	0.6 ± 0.1 (0.2)
Total PUFA n-6**	100.1 ± 3.5 (20.1)	54.6 ± 2.7 (17.4)
Total PUFA n-3**	47.0 ± 4.1 (9.5)	3.0 ± 0.1 (1.0)[a]
n-6 : n-3 ratio	2.1	18.2

a % of total fatty acids
** $p<0.01$

CONCLUSIONS

The *Lipid Hypothesis* which is the current theory stating that saturated fats and cholesterol clog the arteries leading to atherosclerosis, heart disease and notably cancer has been challenged. It appears that the consumption of trans-fatty acids (TFA) that are produced during chemical processing, such as during the preparation of margarine and vegetable oils is strongly correlated with increased cardiovascular diseases and cancer, not the consumption of saturated fat or cholesterol. In fact an increased refined sugar intake is also one of the main causes of heart disease.

In Malaysia, deaths due to circulatory diseases account for 7.5 % of the total mortality, ranking fifth in 1965 (Tee, 1999). A decade later, cardiovascular diseases were rated as the number one killer and account for 20-30 % of the total deaths among Malaysians annually and have remained that way till today. The increased mortality coincided with the increased per capita availability of especially dietary fat and calories which was from 18 % of the total caloric energy in the 1960"s to 31 % three decades later (Gurr, 1999).

The current trend in agricultural and livestock production is to produce omega-3 and omega-6 fortified meat, milk and eggs. While small amounts of the essential PUFA are required by the animal body, excessive consumption is extremely deleterious and yet consumers have been advised for a long time to consume more PUFA. Furthermore, the PUFA are less stable chemically and are prone to oxidation which could produce life threatening products. Cardiovascular disorders and cancer could be related to the person's total fat intake or the type of fat consumed and not simply one's consumption of the EFA.

Previously, much of the research on EFA had largely centred on the n-6 fatty acid family and the eicosanoids. In fact the ten years from 1965 to 1975 were the decade of the n-6 fatty acids in biomedical studies related to lipid metabolism. The next ten years from 1975 to 1985 saw the shift towards research with the n-3 fatty acid family, which was long overdue. The n-3 PUFA are known to be "anti inflammatory" whereas the n-6 PUFA are "pro-inflammatory". Presently the two n-6 and n-3 PUFA are taken into account together when formulating diets as the balance between them, hence the n-6:n-3 fatty acid ratio becomes important. The n-3 fatty acids act as a counterbalancing agent to the n-6 fatty acids in the body (Oomah and Mazza, 1998).

The n-3 fatty acids may also play a critical role in human mental health. It was postulated that depression may be caused by n-3 fatty acid deficiency and a low intake may contribute to aggressive behaviour and an altered mental state that may lead to suicides (Newton, 1997; Gurr, 1999).

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