YOU ARE WHAT YOU EAT: FOLLOWING FATS THROUGH FOOD CHAINS

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The following short verse by the English writer Walter de la Mare (25 April 1873 – 22 June 1956) aptly summarises a truism

It's a very odd thing
As odd as can be
That whatever Miss T. eats
Turns into Miss T.

but when we talk about fats and fatty acids it is probably more correct to paraphrase de la Mare, and change 'turns into' to 'becomes part of'. In other words, the fatty acid composition of Miss T's cells and tissues will reflect what she has eaten. The conservation of fatty acid compositions through food chains is so pronounced that fatty acids can be used as markers to examine predator-prey interactions, and in forensic investigations to reveal fraudulent mislabelling of food products, such as attempts to pass off a farmed fish as a more expensive wild-caught counterpart.

This conservatism of fats and their fatty acids can have profound consequences for human health and disease. Recent decades have seen changes in the diets of Europeans and North Americans to include increasing amounts of 'fast' or 'convenience' foods that contain a lot of terrestrial animal and plant fats. These foods are often both high in fat and rich in saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and n-6 polyunsaturates (PUFAs), but are low in n-3 highly-unsaturated fatty acids (HUFAs) (see Box 1 and http://lipidlibrary.aocs.org for a primer on fats and fatty acids). The main n-3 HUFAs are eicosopentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3). Both n-3 and n-6 fatty acids are essential components of the diet, but it is low consumption of n-3 HUFAs that is a matter of particular concern. This is because the n-3 HUFAs are considered to be very beneficial for human health and development. At present, most Europeans and North Americans are consuming diets that are high in saturated fats and n-6 PUFAs, and are characterised by n-6 to n-3 fatty acid ratios within the range 7-20:1. Rec-

Table 1. Percentages of the main fatty acids (as % of total fatty acids) in some farm animal meats, plant oils, marine fish oils and fish fillets.

Fatty acid	Beef	Pork	Soya oil	Linseed oil	Herring oil	Cod liver oil	Cod fillet	Tilapia fillet	Atlantic salmon fillet
Saturates (SFAs)									
16:0	25	23	10	5.5	14.5	12.5	16.5	15	15
18:0	15	12	4	4	1		3	6.5	3
Monounsaturates (MUFAs)									
16:1			0.5		6	9.5	3	2	6
18:1	39	37	23	20	10	22.5	11	22.5	25
20:1			0.5		15.5	7.5	4	1	7
22:1					22	6	1		10
Polyunsaturates (PUFAs)									
18:2 n-6 (LA)	2.5	15	51	12.5	1.5	1.5	1	20.5	8
18:3 n-3 (ALA)	1	1.5	7	53.5	1.5		0.5	10.5	2
n-3 HUFAs									
20:5 n-3 (EPA)	0.5	0.5			5	13	14.5	0.5	6.5
22:6 n-3 (DHA)		0.5			6.5	12.5	35	5	9.5

ommendations about total fat, n-6 and n-3 fatty acid consumption are regularly re-appraised and modified, with nutritionists currently advising that fat should supply about 30% of dietary energy. In addition, the n-6 PUFAs should provide 5-8% of dietary energy, and the dietary ratio of n-6 to n-3 fatty acids should be below 5:1. The recommendations are to reduce intake of saturated farm animal fats, maintain intake of n-6 PUFAs from plant fats and increase consumption of n-3 HUFA rich seafood and/or dietary supplements to achieve these goals. The fillets of fatty fish, such as herring (*Clupea harengus*) and mackerel (*Scomber scombrus*), contain 5-20% fat that is relatively rich in n-3 HUFAs. Fatty marine fish are currently the main source of n-3 HUFAs in the human diet. Marine fish oils, such as cod liver oil, may be taken as a dietary supplement; n-3 HUFAs can be 20-30% of the fatty acids present in marine fish oils, and a daily dose of 5 g cod liver oil (ca. 5 ml) would provide 1-1.5 g n-3 HUFAs to a human consumer (Table 1). The n-6 to n-3 fatty acid ratio of cod liver oil, at about 0.06:1, is also very favourable from a dietary perspective.

Terrestrial and aquatic fatty acid food chains

Micro-organisms, plants and animals from terrestrial and aquatic ecosystems differ in the types of fatty acids that make up the fats present in their tissues and organs. These fatty acids differ in the numbers of carbon atoms in the molecule, and in the number and positioning of double bonds between carbon atoms (Table 1). Fatty acids found in terrestrial plants and animals generally have carbon chain lengths of 14-18, and are a mix of SFAs, MUFAs and PUFAs. The fats present in the seeds and grains of a number of plants, such as soya, rapeseed (canola), sunflower and corn (maize) contain quite large amounts of PUFAs. The PUFAs found in these fats are 18C fatty acids of the n-6 and n-3 series; proportions vary, with soya being rich in linoleic acid (LA; 18:2 n-6) and linseed rich in alpha-linolenic acid (ALA; 18:3 n-3) (Table 1). The large proportions of PUFAs present in plant fats means that they are fluid oils at room temperature. In contrast to terrestrial plants and animals, fatty acids with up to 22 carbon atoms in their carbon chain are commonly encountered in aquatic organisms. Some of these long-chain fatty acids contain several double bonds, and these are the highly-unsaturated fatty acids (HUFAs)(Table 1).

The fats present in terrestrial and aquatic organisms differ not only in the chain-lengths and degree of saturation of their fatty acids. There are also differences in the types of unsaturated fatty acids that are present, so the fatty acids typical of food chains in terrestrial and aquatic environments show several marked differences. Amongst the PUFAs, n-6 fatty acids are typically found in terrestrial and freshwater environments, whereas in marine systems n-3 fatty acids are much more frequently encountered. Freshwater food chains are characterised by LA, arachidonic acid (ARA; 20:4 n-6), ALA and EPA. On the other hand, the fats of marine phytoplankton and zooplankton tend to have quite high proportions of EPA and DHA, and this pattern is generally retained at higher levels in the food chain. Thus, when the fatty acid compositions of freshwater and marine fish are compared large differences are often found, and these differences reflect the differences in the fatty acid compositions of the food the fish have eaten.

Marine fish oils have traditionally been an important component of the pellet feeds used to farm high-value, carnivorous fish such as salmonids, sea basses and sea breams, and marine flatfishes (soles and flounders). This has been the case because marine fish oils have been readily available on the international market at reasonable price, and the use of these oils in pellet feeds also ensures that the fillet fats of the farmed fish contain n-3 HUFAs. There is, however, now an increase in the use of plant oils to partially replace marine fish oils in the feeds given to farmed fish. This has become common because of a combination of a shortfall in supply and increasing prices of feed quality marine fish oils. The replacement of marine fish oils by plant oils may create some problems for farmed fish production because marine fish oils are good sources of n-3 HUFAs whereas plant oils are not (Table 1). The plant oils contain MUFAs and n-6 PUFAs, and some contain a high percentage of ALA, but they are devoid of n-3 HUFAs. As such, fish species that have limited ability to convert ALA to n-3 HUFAs will not deposit much n-3 HUFA in their tissues when they are fed pellets that contain a lot of plant oil, but very little marine fish oil.

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Something old, something new

Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*) are well-established as food-fishes in western Europe and North America, and they are familiar sights in food stores. These fish are often the species of choice for the preparation of fish dishes served in hotels and restaurants and for meals of fresh fish eaten at home. Although cod farming is on the increase the vast majority of cod on sale in fresh-food outlets is wild-caught, whereas the Atlantic salmon sold in supermarkets and other food stores is of farm origin.

Atlantic cod is a white-fleshed, lean-filleted fish. It has 0.5-1% fillet fat, and n-3 HUFAs usually make up about half of the fatty acids (Table 1). Despite this high proportion of n-3 HUFAs, and very favourable n-6 to n-3 fatty acid ratio (0.04-0.08:1), the low fat content of the fillet means that a meal of fresh cod fillet will provide the consumer with only a small amount of n-3 HUFAs; 0.25-0.5 g n-3 HUFAs in each 100 g fillet. In addition, both fat content and fatty acid composition can be changed during the preparation of the cod fillet for consumption, with frying having the greatest effects. For example, frying a cod fillet in sunflower oil, which has a high LA content, can increase fat content to 3-3.5% and change the n-6 to n-3 fatty acid ratio to 6-7.

In contrast to the cod, the fillet of a farmed Atlantic salmon will usually contain 15-25% fat, and neither fat content nor fatty acid composition will be much affected by frying. The fat content and fatty acid composition of the salmon fillet will be mostly affected by the amount of fat and the fatty acid compositions of the fats (plant, marine fish etc.) in the feed pellets given to the fish during their grow-out phase in seawater. For example, the greater the fat content of the feed pellets the higher will be the percentage fat in the salmon fillets that are produced. Further, assuming that marine fish oils were quite a large proportion of the oil mixture used to make the feed pellets, around 15-20% of the fatty acids present in the fillet would be n-3 HUFAs; in this case 100 g of farmed Atlantic salmon fillet would contain 2-2.5 g n-3 HUFAs, making the fish a good source of these fatty acids. In addition, the n-6 to n-3 fatty acid ratio of the salmon fillet is also favourable; generally about 0.5:1.

In recent years two ’exotic’ farmed fish have become a relatively common sight on supermarket shelves in Europe and North America; these are the tilapias (Fig. 1) and pangasius (*Pangasianodon hypophthalmus*)(Fig. 2). Both are freshwater fish that are mostly farmed in South-east Asian countries. Pangasius farming has undergone explosive growth, driven mostly by expansion in Vietnam and other countries of the Mekong River basin. The development of pangasius farming has played an important role in the socioeconomics of rural communities in these countries, with over 90% of farmed pangasius being processed for export. In contrast to pangasius, the history of tilapia farming is very long; but just like pangasius culture the farming of tilapia has experienced a recent rapid expansion. Tilapia farming is carried out in about 100 countries, with the major producers being China, Egypt, Philippines, Indonesia, Thailand and Brazil. Annual production now rivals or exceeds that of salmonids, and tilapia is now a familiar sight in international markets; within the space of two-to-three decades tilapias graduated from fish of interest only to poor, ’backyard’ farmers in developing countries to a globally-traded, prime white-fish commodity. Tilapias of several species are farmed, but Nile tilapia (*Oreochromis niloticus*) is the most widely cultivated, accounting for about 80% of annual worldwide production.

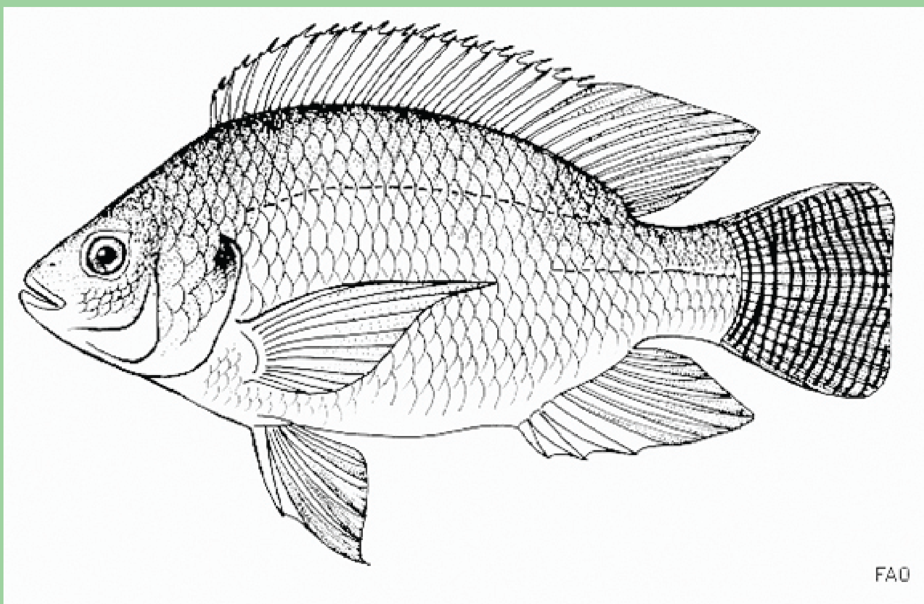

*Figure 1. The Nile tilapia (*Oreochromis niloticus*) is an important fish for warmwater aquaculture wordwide. Tilapias are farmed in South-east Asia, the Middle East, Central and Southern America and in several African countries. Annual production of farmed tilapia is over 2 million tonnes, and rivals or exceeds that of farmed salmonids. (source: FAO).*

Both tilapia and pangasius are white-fleshed, freshwater fish with fillets that contain 1-2% fat. In contrast to their white-fleshed marine counterparts, such as codfishes and marine flatfishes, the fillet fats of these freshwater fish have only relatively small proportions of n-3 HUFAs; 6-20% of fillet fatty acids as n-3 HUFAs in tilapia and pangasius compared to roughly half in the codfishes. This is a clear demonstration of one of the differences in fatty acid profiles between freshwater and marine fish (Table 1). Freshwater and marine fish also differ in their abilities to convert ALA to n-3 HUFAs; in general, freshwater fish have a greater capacity to synthesize EPA and DHA from

ALA. This means that it is possible to increase the fillet n-3 HUFA content of farmed freshwater fish, such as tilapia, by providing them with feeds that contain high proportions of linseed, or other plant oils that are rich in ALA. It must be cautioned, however, that the conversion of ALA to n-3 HUFAs is not very efficient so it is ALA, rather than the n-3 HUFAs, that tends to accumulate in the fillet fat (Fig. 3). All of this means that a meal consisting of a farmed, white-fleshed freshwater fish, such as tilapia or pangasius, will not provide the human consumer with a very large dose of n-3 HUFAs; 0.1-0.4 g n-3 HUFAs in each 100 g fresh fillet eaten. Furthermore, the n-6 to n-3 fatty acid ratios of tilapia and pangasius fillets (usual range 1-5:1) are not as favourable as those of marine fishes, e.g codfishes, flounders and soles, and farmed Atlantic salmon.

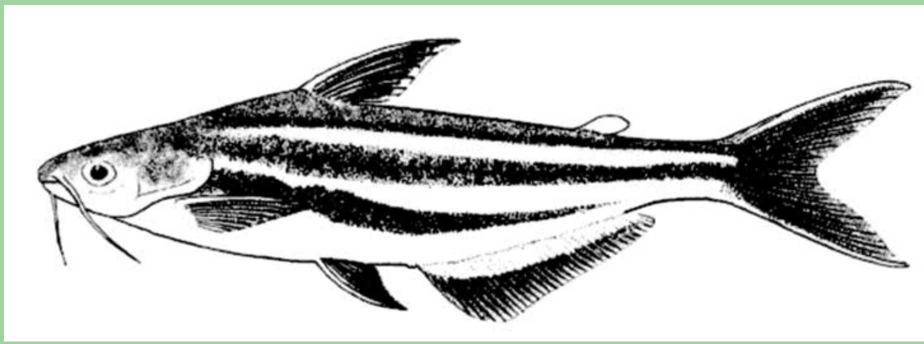

*Figure 2. Pangasius (*Pangasianodon hypophthalmus*) is an increasingly important farmed fish in South-east Asia, particularly in countries of the Mekong River basin (Vietnam, Thailand, Cambodia and Laos) but also in Indonesia, Malaysia, Bangladesh, Myanmer (Burma) and China. Alternative names for the fish include the iridescent shark, striped or sutchi catfish. (source: FAO)*

Value added products: What's in a name?

We are all familiar with convenience fish products such as fish fingers, fishsticks and fish crispies. All have been processed to food products that differ markedly from a fish fillet, and they are called value added products as a result of the processing; but what's in a name? Does the processing increase the nutritional value of these fish products? If fat content and fatty acid compositions are used as the criteria for evaluation the answer to the question must be a definite no. Many processed fish products contain only about 50% fish, the remainder being fillers, batters and coatings made up of wheat or maize flour, potato powder, starches and sugars, plant oils, flavourings and spices. For products made with codfishes [cod, haddock (*Melanogrammus aeglefinus*), saithe (*Pollachius virens*) or Alaska pollock (*Theragra chalcogramma*)], with a fillet fat content of 0.5-1%, the fat content in the final product (6-15%) will often be increased several-fold over that in the fish upon which it is based. There will also be a profound change in the fatty acid profile, from one dominated by n-3 HUFAs to one high in SFAs, MUFAs and n-6 PUFAs. This means that the n-6 to n-3 ratio in convenience fish food products is often far higher (10-30:1) than the recommended maximum (5:1). The frying or cooking of these convenience fish foods in culinary oils, such as corn (maize), palm, rapeseed (canola) or sunflower, will further increase the fat content of the meal and reduce the relative contribution of n-3 HUFAs. We should not, therefore, delude ourselves. We will not markedly increase our n-3 HUFA consumption, and reduce the n-6 to n-3 ratio of our diet, by eating deep-fried convenience fish foods, such as fish crispies and fish fingers.

ALA is usually assigned essential fatty acid status because many animals, including humans, can use it as a precursor for the formation of n-3 HUFAs. The bioconversion of ALA to n-3 HUFAs is, however, often relatively inefficient, and especially so when the diet contains high concentrations of LA. The reduced synthesis of n-3 HUFAs from ALA occurs because the two 18C fatty acids – 18:3 n-3 (ALA) and 18:2 n-6 (LA) - compete for conversion to longer-chained and more unsaturated fatty acids via the same elongase and desaturase enzymes. We have already seen that when fish are given feeds that are rich in ALA they tend to deposit it in fillet fat, and usually do not have a very large increase in fillet n-3 HUFAs (Fig. 3). As such, we can debate the wisdom of trying to boost the n-3 fatty acid contents of farmed fish by providing them with ALA-rich feeds, and then attempting to market them as n-3 enriched, value added products. This has been advocated, but has usually been met with scepticism and resistance from most aquaculture nutritionists.

I don't like fish

Many people are not particularly fond of eating fatty fish, or taking marine fish oils and oil capsules as dietary supplements. To meet the food preferences of these people attempts have been made to enrich several commonly consumed foods with n-3 HUFAs through the addition of marine oils; either fish or microalgal oils. Foods that have been test-marketed include mayonnaise, margarines and fat spreads, breads and biscuits, milk, fruit juices and soft drinks. Several of these products were not well-received and suffered poor sales. Many of the products had a relatively short shelf-life because they rapidly developed a 'peculiar' odour or a strong-fishy or rancid 'off-flavour' during storage, due to fatty acid oxidation. As a result several n-3 HUFA enriched products were withdrawn from the market after a short time, but others persisted and are still available.

There have been several recent novel developments that have had an impact on the enriched food sector. These include the widespread use of antioxidants and improved packaging, leading to an increase in product shelf-life. Techniques that improve the extraction and separation of oils and fatty acids from raw materials have also been developed, making it possible to produce fatty acid concentrates and high n-3 HUFA marine phospholipid extracts. In addition, modern technology allows minute quantities of oil to be encased within microcapsules that can then be added to, and dispersed within, a food product. When micro-encapsulation technology is used for n-3 HUFA enrichment of food products DHA and EPA concentrates, rather than a complete fish or microalgal oil, may be chosen because this increases the efficacy of the enrichment procedure.

Animal feeds containing linseed or linseed oil are being used to manipulate the fatty acid compositions of farm produce, such as eggs and meat. One disadvantage with these food products is that, just like the fillets of fish fed linseed oil (Fig. 3), they contain most of the n-3 fatty acids as ALA rather than n-3 HUFAs. An alternative approach involves the incorporation of DHA-rich, dried marine microalgae into broiler chicken and laying-hen feeds; this results in the production of DHA-enriched eggs and chicken meat with an enhanced n-3 HUFA content. Based upon what we know about fatty acid bioconversions, it should be clear that DHA-enriched eggs and meat are better food alternatives than those in which most of the n-3 fatty acids are present as 18C ALA.

Looking to the future

Marine fish, and their oils, have a long tradition as sources of essential nutrients in the human diet. An expanding human population is placing an increasing demand on fish resources, and alternative sources of the essential nutrients that fish supply should be sought. When thinking about the n-3 HUFAs a sustainable long-term solution to the problem may lie in the fact that fish do not synthesize n-3 fatty acids themselves, but obtain them via their diet. The source of these fatty acids lies at the base of the marine food chain, with a range of bacteria and microalgae being capable of synthesizing these essential fatty acids. The various micro-organisms have fatty acid profiles that differ in amounts and percentages of DHA and EPA; some produce a DHA-rich oil with small amounts of EPA and almost no n-6 fatty acids, whereas others produce oils that are rich in EPA, but with only a small amount of DHA. Some of these alternative oils have been tested in humans, and there is evidence that the daily consumption of capsules containing oils extracted from marine micro-organisms can meet our requirement for bioactive n-3 HUFAs. A few are commercially available as dietary supplements, and others are undergoing clinical trials to treat patients with cardiovascular or neurological diseases.

As an alternative to the use of marine micro-organisms as a sustainable source of n-3 HUFAs, the genetic engineering of oilseeds is also being explored. Terrestrial plants are capable of synthesizing 18C n-3 and n-6 fatty acids, but not those with long carbon chain lengths. Genes encoding for the elongase and desaturase enzymes involved in the biosynthesis of n-3 HUFAs have been identified in a range of micro-organisms. Some of these genes have been isolated and inserted into oilseed plants, such as linseed and soya, that appear to have potential as transgenic producers of n-3 HUFAs. These transgenic (GM) plants can produce EPA, and to some extent DHA, but the fatty acid compositions and levels in the oils are not equivalent to those seen in marine fish oils and microalgal oils. For example, the oils produced by the GM plants contain several n-6 fatty acids that are not present in the oils extracted from marine organisms. As such, there remain a number of obstacles before the goal of producing GM plant oils substantially equivalent to those from marine sources is achieved. Firstly the levels of EPA, and especially DHA, must be increased if GM plant oils are to closely resemble marine oils, and secondly the levels of n-6 fatty acids must be substantially reduced. Nevertheless, the demonstration that GM oilseed plants can produce n-3 HUFAs indicates that some progress is being

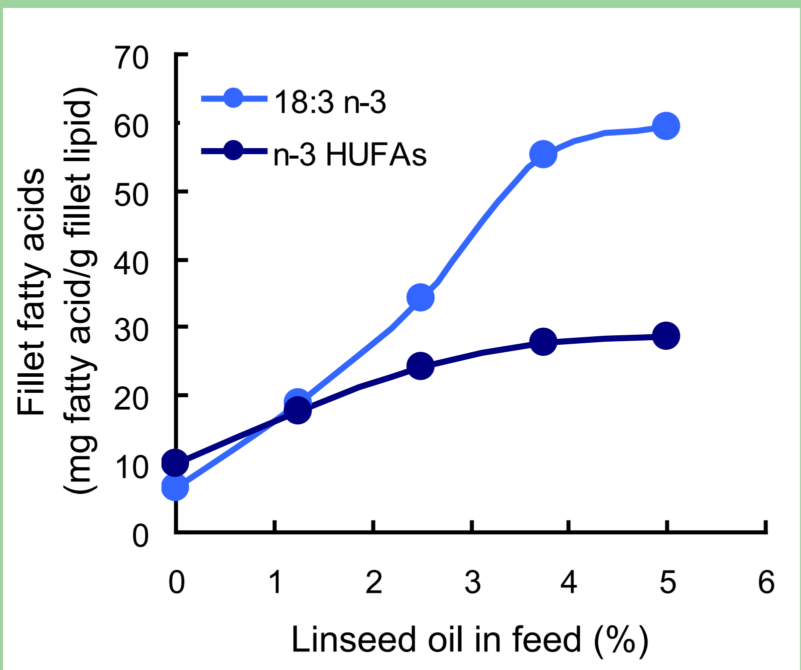

Figure 3. Nile tilapia (Oreochromis niloticus) can convert ALA (18:3 n-3) to EPA (20:5 n-3) and DHA (22:6 n-3). Feeding tilapia with linseed oil (rich in ALA) results in an increase in the deposition of n-3 fatty acids in the fillet fat (lipid); a large increase in the deposition of ALA, but also some increase in concentrations of n-3 HUFAs (e.g. EPA and DHA). (data from Visentainer et al. (2005) Food Chemistry 90, 557-560)

made towards finding sustainable terrestrial alternatives to marine fish oils.

How might these futuristic GM plant oils be used? They could pass directly into the human food chain by being used in the manufacture of margarines, spreads, yoghurts and other dairy products. However, given the resistance of many consumers to GM foods this may not be the best option available. An alternative would be to introduce the n-3 HUFAs these oils contain into the human food chain via an indirect route. The GM plant oils could be used as oil sources in animal feeds, resulting in enhanced levels of n-3 HUFAs in the meat, eggs and milk produced by these animals. Perhaps a more obvious indirect route would be the aquatic one, in which farmed fish are provided with feeds in which marine oils are replaced by n-3 HUFA enhanced GM plant oils. Irrespective of the strategy employed to introduce GM plant oils into the human food chain it will be important that procedures are transparent. There must be an initial rigorous testing, adequate regulatory procedures must be in place and consumers must be given sufficient information to enable them to make a well-founded decision about whether or not to purchase the products.

ADDITIONAL READING

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Internet source: The Lipid Library: http://lipidlibrary.aocs.org

BOX 1: FATS AND FATTY ACIDS

In normal daily conversation when we talk about fats we will usually be referring to the group of chemical compounds that chemists and biochemists call lipids. In the formal language of the chemist and biochemist, fats are a specific group of compounds within the lipids; fats are the lipids that are most frequently encountered in biological tissues where they are used as energy stores.

Lipids: A diverse class of organic compounds that are insoluble in water but soluble in organic solvents such as ether, acetone, ethanol and chloroform; include acylglycerols, phospholipids, glycolipids and steroids. Some lipids are essential components of biological membranes, and others act as energy stores.

Fats: Simple lipids that are esters of fatty acids with the trihydric alcohol glycerol; formally called acylglycerols. Important energy sources in feeds, and the primary form of energy storage in animals.

Fatty acid: A long chain organic acid with the general formula $CH_3(C_XH_Y)COOH$. The hydrocarbon chain is either saturated (Y = 2X) or there are double bonds between some of the adjacent carbon atoms (unsaturated fatty acid).

Saturated fatty acid (SFA): A fatty acid that is completely hydrogenated, and therefore lacks double bonds between adjacent carbon atoms, e.g. palmitic acid (16:0).

Monounsaturated fatty acids (MUFAs): Fatty acids with a single double bond in the hydrocarbon chain, e.g. oleic acid (18:1 n-9).

Polyunsaturated fatty acids (PUFAs): Fatty acids with 2-4 double bonds in the hydrocarbon chain, e.g. alpha-linolenic acid (ALA; 18:3 n-3) and linoleic acid (LA; 18:2 n-6).

Highly-unsaturated fatty acids (HUFAs): Fatty acids with 4 or more double bonds in the carbon chain, e.g. arachidonic acid (ARA; 20:4 n-6), eicosapentaenoic acid (EPA; 20:5 n-3), docosahexaenoic acid (DHA; 22:6 n-3).

Essential fatty acid (EFA): A fatty acid that cannot be synthesized by animals, and which must be present in the diet. Fatty acids of the n-3 and n-6 series are the EFAs.

n-3 fatty acid: A PUFA or HUFA in which the first double bond in the carbon chain occurs in the link between the third and fourth carbon atoms from the methyl end of the molecule.

n-6 fatty acid: A PUFA or HUFA in which the first double bond in the carbon chain occurs in the link between the sixth and seventh carbon atoms from the methyl end of the molecule.