### WHERE TO FIND OMEGA-3 FATTY ACIDS AND HOW FEEDING ANIMALS WITH DIET ENRICHED IN OMEGA-3 FATTY ACIDS TO INCREASE NUTRITIONAL VALUE OF DERIVED PRODUCTS FOR HUMAN : WHAT IS ACTUALLY USEFUL ? (a)

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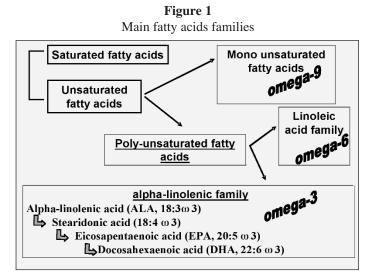
Abstract: Omega-3 polyunsaturated fatty acids have two major field of interest. The first lies in their quantitative abundance and their role in the development and maintenance of the brain. The second is their role in the prevention of different pathologies, mainly the cardiovascular diseases, and more lately some psychiatric disorders, from stress to depression and dementia. Thus, dietary omega-3 fatty acids are very important to ensure brain structure and function, more specifically during development and aging. However, concerning essential alpha-linolenic acid (ALA), most occidental diets contain about 50 % of the recommended dietary allowances. The problem is to know which foods are naturally rich in this fatty acid, and to determine the true impact of the formulations (enriched in omega-3 fatty acids, either ALA or EPA and DHA) in chows used on farms and breeding centres on the nutritional value of the products (meat, butter, milk and dairy products, cheese, and eggs, etc), and thus their effect on the health of consumers, especially to ensure adequate quantities in the diet of the aging people. The consequences (qualitative and quantitative) of modifications in the composition of animal foods on the value of derived products consumed by humans are more marked when single-stomach animals are concerned than multi-stomach animals. Because, for example, hydrogenating intestinal bacteria of the latter group transform a large proportion of polyunsaturated fatty acids in their food into saturated fatty acids, among others, thus depriving them of any biological interest. Under the best conditions, by feeding animals with extracts of linseed and rapeseed grains for example, the level of ALA acid is increased approximately two-fold in beef and six-fold in pork, ten-fold in chicken, and forty-fold in eggs. By feeding animals with fish extracts or algae (oils) the level of DHA is increased about 2-fold in beef, 7-fold in chicken, 6-fold in eggs, and 20-fold in fish (salmon). To obtain such results, it is sufficient to respect only the physiological needs of the animal, which was generally the case with traditional methods. It is important to stress the role of fish, whose nutritional value for humans in terms of lipids (determined by omega-3 fatty acid levels) can vary considerably according to the type of fats the animals have been fed. The aim of preventing some aspects of cardiovascular disease (and other pathologies) can be achieved, or on the contrary frustrated, depending on the nature of fatty acids present in fish flesh, the direct consequence of the nature of fats with which they have been fed. It is the same for eggs, "omega-3 eggs" being in fact similar to natural eggs, were used in the formulation of certain formula milks for infants, whose composition was closest to that of breast milk. In fact, the additional cost on the price paid by the consumer is modest compared to the considerable gain in nutritional value in terms of omega-3 fatty acids content. Interestingly, in aged people, ALA recommendations in France are increased (0.8% daily energy intake in adult, 0.9 % in aged) and DHA is multiplied by 2 (0.05 % daily energy intake in adult, 0.1 % in aged; as well as in pregnant and lactating women).

Key words: Animal feeding, omega-3 fatty acids, polyunsaturated, diet, nutrition, aged, nutritional value, cost.

Abbreviation used: FA: fatty acid. PUFA: polyunsaturated fatty acid. ALA : alpha-linolenic acid. EPA : eicosapentaenoïc acid. DHA : docosahexaenoïc acid.

#### Introduction

Recent reviews in this journal have recently focused attention on the role of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various age and during aging (1) and, more specifically, the importance of dietary omega-3 fatty acids in psychiatry (2). The first element of the omega-3 fatty acids (figure 1) is the essential fatty acid alphalinolenic acid (ALA, 18:3(n-3),  $18:3\omega3$ , 18:3 omega-3); other members of this family are derivatives of ALA with longer, more unsaturated hydrocarbon chains. The main ones are eicosapentaenoïc acid (EPA, 20:5(n-3),  $20:5\omega3$ , 20:5 omega-3) and docosahexanoïc acid, (DHA, 22:6(n-3),  $22:6\omega3$ , 22:6 omega-3).



For example, all the omega-3 FA are required for the prevention and treatment of cardiovascular disease, particularly ischemic obstruction. ALA has been shown to have positive effects on the cardiovascular system. The main indication for DHA and EPA from fish oil is that they reduce hypertriglyceridemia. Other areas, such as inflammation, certain rheumatological and dermatological conditions (psoriasis), cancers, and most recently psychiatric disorders, are under active investigation. Polyunsaturated FAs (PUFAs) are involved in ageing: their dietary lack may alter membrane lipid turnover, including the brain.

The omega-3 FA, especially DHA, are implicated in brain structures and cognitive functions. ALA was one of the first macronutrients for which experiments showed an effect on brain structure and function. Its most important actions are to form part of membranes. It was first shown that the differentiation and function of brain cells in culture required not just ALA, but also DHA and arachidonic acid (3). It was then shown that a lack of ALA altered the composition of the membranes of brain cells, neurons, oligodendrocytes and astrocytes, together with myelin and nerve terminals (4). This lack led to changes in membrane fluidity, resulting in biochemical and physiological disturbances and their associated neurosensory and behavioural changes (5). Consequently, the nature of polyunsaturated FA (especially omega-3 FA) in infant formulas (for premature and full term babies) influences their visual, cerebral and intellectual capacities (6, 7). Omega-3 FAs are important nutrients that actively contribute to the structure of the brain, and hence to its function, as discussed in popular science books (8), one of which focuses on the omega-3 FA (9).

This review evaluates the real contribution, in terms of the daily requirement, to human consumption of animal products whose omega-3 FA content has been increased by using high omega-3 FA feeds, that contain either ALA or EPA and DHA. Feeds enriched by directly adding omega-3 FA (such as milk enriched with microcapsules containing fish oil, bread enriched

in the same way or with linseed) are not covered in this review. Bioavailability of omega-3 fatty acids in capsules is briefly presented in the discussion. The aim is to evaluate the relevance of improving the omega-3 fatty acid (FA) content of animal products by feeding animals with appropriate lipids; while bearing in mind that ALA has quite different properties from EPA and DHA, so that each must be considered in the light of its specific properties. In fact, diet in occidental countries is not adequate, at least for ALA.

#### A lack of dietary omega-3 FA in occidental countries

The French (10) daily recommended intake of ALA is 2 g for men (for 2200 kcal/day) and 1.6 g for women (for 1800 kcal/day) and 1,5 g in ageing people (for 1700 Kcal/day). A recent study carried out in Aquitaine showed that women of child-bearing age obtain only 40% of the recommended daily ALA requirement from their diet (11). Similar results were obtained in Brittany in a study on a small sample of men and women (12), throughout France in the SUVIMAX study, as reported by the AFSSA (13) (Table IA) and in other countries: Canada (14), Sweden (15) and US (16). The actual intake of EPA and DHA is not clearly documented. The estimated consumption of the long chain omega-3 FA in France has yet to be published, but the Brittany study showed that the intake of DHA and EPA was 110 mg per day in this region (12), which is less the recommended daily requirement, 120 mg DHA per day for men and 100mg DHA for women and 100 mg for aged people. Table 1B presents the recommendations for EPA and DHA.

 Table 1A

 Consumption of omega-3 fatty acids in France

	All France SUVIMAX	Aquitaine	Brittany	Recommended daily intake (g/day)
ALA men (g/day) women (g/day) pregnant (g/day) men + women	1 0.8	0.7 0.8	0.75	2 (for 2200 Kcal/day) 1.6 (for 1800 Kcal/day)
Linoleic/ALA Men women men + women	$\begin{array}{c} 11.1\\ 10.8 \end{array}$	15	14.9	5 5

From SUVIMAX, AFSSA report 2003. French recommended daily intake (10). The Aquitaine study was done on women only (11). The Brittany study (12) gave no separate data for men and women; they consumed 110 mg EPA+DHA per day. The consumptions of EPA and DHA for the whole of France have not yet been determined. According to ISSFAL (17), there are various international recommendations of poly-unsaturated fatty acids intakes. In fact, the ALA recommendations are 1% of daily energy (i.e. 2,0 g/day for 2000 kcal) for five different bodies/countries in Australia, Japan, the Netherlands. It is 0.68% of daily energy for the food and nutrition board in USA and Canada (1,35 g/day) and 0.7 % for ISSFAL itself.

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	Date	Omega-6/omega-3 Ratio recommended	Specific recommendations
Norway (National nutrition council) NATO workshop Canada (Scientific Review Committee France CNERNA-CNRS GB (British Nutrition Task force) FAO-WHO Expert committee on fats and oils in human nutrition ISSFAL-USA Expert workshop Crete, Eurodiet-nutrition France CNERNA-CNRS; AFSSA	1989 1989 1990 1992 1992 1994 2000 2000 2001	none none 5:1 - 6:1 4:1 - 10:1 6:1 5:1 - 10:1 none none 5:1	0.5 % DE for omega-3 (1-2 g/day) 0.27 % DE (0.8g/day) EPA-DHA Omega-3 at least 0.5 % DE 0.5 - 1 % DE for ALA EPA 0.2 - 0.5 % DE,DHA 0.5 % DE Pre-formed DHA in pregnancy EPA + DHA 0.3 % DE, or 0.65 g/day 200 mg long chain poly-unsaturates per day 0.8 % DE for ALA, 0.2 %. DE per day long chain polyunsaturates (500 mg for men, 400 mg for women), DHA min 0.05 % DE (120 mg for men and 100 mg for women; 0.1 % DE for aged
Netherlands (Health Council)	2001	none	(100mg/day) 200 mg/day long chains omega-3

 Table 1B

 Specific recommendations for EPA and DHA

From ISSFAL (17) and AFSSA (10). DE: daily energy.

The only foods that are effective dietary components (10), providing the tens of grams of ALA required daily, are the oils prepared from rapeseed, soybean and walnuts, plus a type of egg defined as "omega-3" or "benefic" eggs (in France) or "Columbus eggs" (in US and most European countries) from laying hens fed linseed or eventually algae (quite apart from their other qualities, including 'organic' or 'special') and walnuts themselves. Table Il lists the main sources of ALA. Wild fatty fish generally have a very high DHA content, as do farmed fish provided they are fed correctly.

Table 2Human dietary sources of ALA

	ALA (g/100g)	Linoleic/ALA
Mother's milk	0.6	10
Fats		
lard	1	9
Beef dripping	0.5	4
Mutton dripping	0.2	0.7
Butter	0.5	1.5
Margarine " oméga-3 "	1.2	12
Oils		
Rapeseed	10	1.9
Soybean	7	6.7
Walnut	12	4.4
Olive	0.7	9
Meat		
Horse	0.10 to 0.8	0.6
Rabbit	0.10 to 0.5	2 to 5
Chicken	0.04 to 0.2	6.3
Duck	0.04 to 0.2	9.8
" omega-3 " eggs	0.4	3.4

From (9, 13). Although linseed oil contains 54 g ALA per 100g, it is presently banned for human consumption in France, but not in many other countries. There are no data for ruminant (sheep, goats, cattle) meat as their ALA, EPA and DHA contents are very low.

It is clear that the effects of animal feed on the nutritional value (for mankind) of derived products destined for human consumption is in fact much greater than the influence of methods used to grow vegetables on their nutritional value. The variety and the species of plant influence the compositions of plant products much more than the growing methods. For example, the polyunsaturated FA contents of peanut oils produced from African and American peanuts are very different. The same is true for olive oils. A study of olive cultivars in Tunisia (18) showed that they differed significantly in their contents of oleic and linoleic acids and ALA. This is not so for animals, whose lipid reserve may be very sensitive to the type of fatty acids obtained from the diet.

#### Modulating the lipid composition of mammalian meats

A practical distinction must be made between structural lipids (most of which are more or less genetically determined and are mainly found in membranes) and reserve lipids that can vary with the dietary intake. The structural lipids include the polar lipids (mainly the phospholipids), whose quality and quantity depend on their constituent fatty acids rather than on the diet. Their incorporation into biological membranes is under overall genetic control as each membrane has a functionspecific fatty acid profile that is only partly dependent on the diet. In contrast, the quality of reserve lipids (neutral fats, mainly triglycerides) varies considerably according to their anatomical location and the fat content of the animal. The fatty acid profile is characteristic of the species, or even the breed, but above all, the diet.

The biochemical and physiological parameters of the digestive system produce changes, sometimes radical ones, in foodstuffs, particularly lipids. This is why meat from monogastric mammals like pigs, horses and rabbits differ somewhat from polygastric animals like cows, sheep and goats. Hence pig meat has a much higher polyunsaturated FA content than does beef. The bacteria in the rumens of polygastric

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animals hydrogenate plant PUFA so that their meat has much less (< 50%) of these FA than does the meat of monogastric animals. This means that attempts to improve the PUFA content by feeding linseed (12), or seafood or derivatives are much more successful in pigs and rabbits than in cattle or sheep. Indeed, progress in genetics and a better knowledge of dietary needs has allowed to improve growth performances, to increase muscle weight in poultry and pig; and, in the pig, to strongly decrease carcass adiposity (19).

In pig, the fatty acid composition of lipids deposited with rapeseed oil diet may alter the technological qualities of adipose tissues and that of meat (20), as well as nutritional interest for human. For example, feeding pigs with ALA (linseed) up to 10g/kg feed, the ALA in their backfat could reach a maximum of 6.8g/100g (21). Interestingly, extra intake of ALA may stimulate growth in growing-finishing pigs, this effect being independent of the conversion of ALA into EPA and DHA (22). Curiously, rabbit have not been extensively studied (23), although being an important element of the Cretan diet.

Several possible ways of enriching the human diet in omega-3 FA by modifying pork and beef by changing the diet fed to the animals are shown in Tables III, IV and V.

Formaldehyde treated whole linseed given to lamb raised the PUFA: saturated fatty acids ratios in the liver and adipose tissue, but not in muscle (24); algae can also be used (25). It has been shown that the sheep rumen has a relatively great hydrogenating capacity that is able to saturate both mono- and polyunsaturated FA; but the very long polyunsaturated FAs seem to be less well hydrogenated (26). Biohydrogenation in cow is high, and, for instance, supplemental dietary ALA is hydrogenated to various trans fatty acids (27). In contrast to sheep, the long chain fatty acid pattern in the cow duodenum show a high degree of hydrogenation of 20 and 22 carbon fatty acids (28). Interestingly, the widespread idea that linseed oil decreases the digestibility, arising from studies with sheep, do not seem to apply to lactating cows fed 3% linseed oil (29). Feeding lactating cows with flaxseed increases milk protein percentage and its omega-3-to-omega-6 ratio (30); but fish oil significantly decrease feed intake, increases milk yield, decreases protein, casein and fat concentration (31). In fact, the label "omega-3 enriched butter" for butter, and to a lesser extent cheese, is not nutritionally acceptable because, although there is a marked enrichment, the amounts of omega-3 FA are minute compared to the dietary requirement and the quantity of saturated FA is overwhelming. Moreover, the omega-3 FA content of butter also varies enormously with the season, from 0.35 g/ 100 g in spring to 0.43 g/100 g in summer and 0.26 g/100 g in winter (32). In addition to feeding the livestock linseed (12) and soybean, it clear that incorporating fish oil in the diet of cows (table VI) is a promising way of increasing the EPA content of milk (33).

Table 3				
Pork (filet, neutral lipids)				

	Pigs fed control diet	Pigs fed linseed
ALA (% fatty acids) DHA (% fatty acids)	$1.10 \\ 0.07$	7.90 0.05

As % fatty acids (99). Fed a diet containing 15% linseed for 42 days. Quantitative data (g/100g meat) were not calculated.

 Table 4

 Charolais beef (filet, neutral lipids)

As % fatty acids (100). The DHA content of polar lipids was not assayed, as being fond in phospholipid. Feeding for 120 days. The cattle were fed silage at 2 pm, concentrate at 9 am and at 4.30 pm. The linseed content was 213 g/Kg feed and that of fish oil was 54g/kg feed. The linseed/fish oil mix contained 213 g linseed and 27g oil.

Table 5Lamb (filet, triglycerides)

Lamb	Control	Fish	Fish oil
ALA (g/100g meat)	0.021	0.008	0.0120
DHA (g/100g meat)	0	0.004	0.0017

As % fatty acids (101, 102). The diet was fed for 46 days. The "fish" diet contained 9% fish. The "fish oil" diet contained 1.5% fish oil.

 Table 6

 Composition of milk produced by cows fed fish oil

g fish oil/ cow / day	0	150	300	450
ALA (g/100g fat)	0.30	0.33	0.37	0.32
EPA (g/100g fat)	0.07	0.10	0.15	0.53
DHA (g/100g fat)	0.05	0.06	0.12	0.17

Diet fed for 6 weeks (33).

Micronization of flaxseed do not reveal any advantage over raw flaxseed (34). But, clearly, if the PUFAs could be protected from hydrogenation by rumen bacteria by, for example, using microcapsules or some chemical treatment, they could find their way in some measure into the meat and milk of ruminants. Such a trial has been done on goats (35) and is currently assayed in other species. In contrast, extrusion could increase luminal and whole tract nutrient degradabilities of flaxseed (36).

#### Fish

Although ALA accounts for less than 0.5% of the total fatty acids in fish (37), fish contain a great deal of EPA and DHA. It

has been known for several years that farmed fish generally have a poorer omega-3/omega-6 ratio than do wild fish: it is 2 for farmed trout and 7 for wild trout, 2 for farmed eels and 5 for wild eels, and 6 for farmed salmon and 11 for wild fish (38). The omega-3 FA content of fish can vary considerably, from 1 to 20, depending on the feed (39). The difference is even more spectacular for the fattiest fish. Unfortunately, the lipids in the feed of farmed fish are more likely to be selected as a function of world prices rather than for their nutritional value for the fish. Fish oils are presently widespread and easy to obtain because of the requirements for animal protein and thus the production of fish meal for animals. But vegetable oils have been used in the recent past. This may occur again in the near future because fish oils have been contaminated with dioxins in some regions, so that the EU forbids the mixing of oils from different regions. The main vegetable oils used are palm oil and coconut oil, which should be avoided for human nutrition as it contains too much saturated FA. Fish fed these oils contains minute amount of omega-3 fatty acids, but high amount of saturated fatty acids, thus providing diet not recommended for human.

In fact including components like linseed and rapeseed in fish feeds is effective only if the fish are, like carp, vegetarians. These fish have the enzymes required to transform ALA into EPA and DHA. In contrast, such a seed-based diet is not very effective for carnivorous fish, which have little or none of these enzymes. This is also true of the land- based feline family. And about 75% of the fish used for human consumption are obligate carnivores that are poorly able of transforming ALA into DHA and EPA. Hence, the quantity of DHA in the meat of trout or salmon (carnivores) varies directly as the amount in their diet. Feeding salmon a diet containing rapeseed oil or pork fat results in the same fish tissue concentrations of DHA and EPA, while a diet containing herring oil results in much higher concentrations (40). Salmon are much like trout, as it is unsatisfactory to feed them olive oil or one variety of sunflower oil (called Oleisol, as it is rich in oleic acid). Diets containing these FA result in lower omega-3 FA contents than does a diet containing herring oil (41). Feeding trout with herring oil or lard produces great differences in their omega-3 FA content (Table VII) (42). Feeding farmed trout a diet containing ALA results in the production of only 5% of the DHA in the fish meat that could be produced by a more suitable diet (43).

Table 7				
Trout fed herring oil or pork fat.				

	Flesh of trout fed herring oil	Flesh of trout fed pork fat
EPA (% fatty acids)	3.50	1.40
EPA (g/100g, recalculated)	0.17	0.06
DHA (% fatty acids)	11.50	4.30
DHA (g/100g, recalculated)	0.55	0.18

From (42). The diets contained 8% herring oil or pork fat. The herring oil diet contained 1.7% fatty acids as DHA and 2.2% EPA, the pork fat diet contained 0.5% FA as DHA and 0.5% as EPA. No ALA was detected. Initial fish weight: 30 g. Fish were fed for 124 days. Adding 1.5 g/kg alpha-tocopherol to the diet improved the amounts of DHA and EPA in the trout flesh; those fed herring oil increased from 8.9% fatty acids to 11.3%. The data shown in the table were obtained with a diet containing 50 mg/kg alpha tocopherol. The flesh of the trout fed pork fat had a lower lipid content (4.2%) than that of trout fed herring oil (4.8%). These data were used to recalculate the amounts of EPA and DHA as g/100g trout flesh.

In practice, it would be useful to determine the time prior to harvest that carnivorous fish should be fed omega-3 FA (EPA and DHA) in order to provide the consumer with a product of acceptable composition.

Many hundred papers have been published on the effect of omega-3 fatty acids in fish oil to prevent cardio-vascular diseases, especially ischemic ones, and brain ischemic stroke, in number of animals models and in human. Most studies in human have shown that there is an inverse association between fish consumption and the risk of coronary heart disease. Moreover, both consumption of fatty fish and higher blood concentration of omega-3 fatty acids are associated with a reduced risk of sudden death. Several trials have assessed the positive effects of fish (and fish oil supplements) on coronary heart disease, mainly after cardio-vascular events, mainly myocardial infarction. The possible mechanism of action of omega-3 fatty acids are: antiarrhythmic, antithrombotic, antiatherosclerotic, anti-inflammatory, lowers blood pressure, lowers triglyceride concentration in blood, improve endothelial function, improve membrane fluidity, etc. Fish oils capsules are now recognized as true drug, mainly to prevent hypertriglyceridemia. However, the specific effect of EPA and DHA on coronary heart disease is not yet clearly determined; and even the relative merit of oily fish compared with fish oil capsules require further investigations, although recent guidelines from American Heart Association has supported the use of fish oil supplements for patients with documented coronary heart disease. The specific effect of purified EPA or DHA, as ethyl esters for example, is not significantly documented. Moreover, the optimal intake of long chains omega-3 fatty acids is not firmly established, nor their mechanism of action; although French recommended dietary allowances (ANC) provides data for DHA only.

#### Poultry

The intestinal physiology of birds results in the relatively good preservation of their dietary PUFAs, especially those from fish oil (Table VIII). There is a dose-response effect in the poultry tissues. This is most evident for the ALA content, and less so for DHA as diets with a high linseed content slightly reduce the amounts of this FA, while increasing the ALA content (Table IX). Poultry are thus considered to be an excellent means of appropriately modifying the dietary intake of omega-3 FA by Americans (44). Certain plants with a high omega-3 FA content, particularly sub-tropical ones like chia have even been suggested (45).

## Table 8Chicken leg, with skin

	Leg of chicken Leg of chickenLeg of chicken			
	fed corn oil fed linseed oil fed fish o			
ALA (% fatty acids)	1.4	11.4	1.1	
DHA (% fatty acids)	0.2	0.4	1.4	

Diet fed for 7 - 8 weeks, starting on day one after hatching. Diet contained 2.5% corn, linseed or fish oil (103). The data from this publication could not be used to calculate the amounts as g/100g meat.

 Table 9

 Effect of increasing amounts of dietary linseed oil on the fatty acid composition of checken legs (with skin)

	1.0%	2.5%	5.0%
ALA (% fatty acids)	4.4	11.4	21.9
DHA (% fatty acids)	0.4	0.5	0.3

As % total fatty acids (103). See legend to Figure VIII.

De novo fatty acids synthesis in birds occurs mainly in the liver, and adipose tissue growth and subsequent fattening depend on the bioavailability of plasma triglycerides, which are transported ass components of lipoproteins. Indeed, enhanced fatty acid uptake by adipose tissue represents a major factor in determination of adiposity in the chicken (46), and difference in fattening between genotypes seems to be due to both increased VLDL secretion and VLDL removal form plasma without difference in VLDL characteristics (47). In fact, lipoprotein metabolism is clearly related to fattening in poultry (48). The effect of omega-3 fatty acids remains to be determined. Usefulness in human diet of fats from palmipeds in south-west of France is due to the huge presence of corn in the diet of ducks and gooses, thus their fat contains mainly oleic acid and minute amount of ALA, as both are present in corn oil. Changing corn with other fat will alter the nutritional value for human.

The fatty liver used in human diet is another problem. Susceptibility to liver steatosis was studied in various species (49) and differential channelling of liver lipids in relation to susceptibility to hepatic steatosis in the goose examined (50). Overfed palmiped de novo hepatic lipogenesis prevails over dietary lipid intake to modulate lipid composition of the fatty liver plasma membrane (51). Interestingly, hepatic lipid characteristics of laying hen respond to omega-3 fatty acids (52).

## Eggs as a source of alpha linolenic acid and DHA for the human diet

With fish, eggs are just about the perfect model of dietary transfer, as the type of FA in the egg yolk is closely linked to the type of lipid consumed by the hen. It was held for many years that the yolk composition was constant whatever the diet fed to the laying hen, so that the hen acted as a sort of filter/regulator. However, the omega-6/omega-3 ratio (for all hydrocarbon chain lengths) is 1.3 for wild Cretan Greek eggs, but it is 19.9 for "industrial" eggs. The amounts of ALA are 40-fold higher, while those of DHA are 5-fold greater (53). One explanation is that Cretan Greek laying hens eat snails, slugs and purslane, a type of green plant.

Poultry fed flaxseed contains significantly more omega-3 fatty acids into their eggs (54). The enrichment is proportional to the amount of omega-3 FA in the hen's diet. It is greater with a diet containing linseed than with one containing rapeseed (55). This relationship holds for the ALA content of the diet, provided by the linseed or rapeseed, and the concentration of this FA in the egg yolk (Table X) (56).

The biological effects of dietary omega-3 FA incorporated into eggs on the physiological parameters of animals have been studied and the findings may be extrapolated to humans. Several lipid parameters reflecting the structure and function of membranes, particularly brain membranes (57-60), were measured (61, 62). The consumption of these eggs by humans had beneficial effects, especially on lipid parameters (63, 64). Feeding hens a diet containing 0%, 10% or 20% linseed oil increased the ALA in the eggs (28, 261, and 527 mg per egg) and the DHA to 51, 81, and 87 mg per egg without altering the amount of cholesterol in the eggs (65). In the trial, 28 men each ate 4 eggs a day (for the experiment, not to promote egg consumption). There was no significant difference in their total cholesterol, HDL cholesterol, or total plasma triglycerides. But those who had eaten omega-3 FA eggs had higher blood plasma ALA and DHA concentrations and a lower omega-6/omega-3 ration in their platelet phospholipids. The authors conclude that these eggs are a promising dietary constituent that complies with the (Canadian) government guidelines and could increase omega-3 FA consumption.

	Control	Laying hens fed fish oil	Laying hens fed linseed oil	Laying hens fed soybean oil	Laying hens fed safflower oil
ALA					
% total fatty acids EPA	0.4	0.4	9.4	1.7	0.06
% total FA DHA	-	2.1	0.3	-	-
% total FA	0.6	6.3	2.2	1.8	0.2
Total ω3 % FA	0.6	10.1	12.6	3.1	0.3
Total $\omega 6 + \omega 3$					
% FA	18.8	19.7	32.3	28.3	36.2
ω6 /ω3	17.8	1.0	1.6	8.1	120

 Table 10

 Effect of adding oils to the diet of laying hens on the composition of the eggs laid by them

Laying hens were White Leghorns. Egg yolk. As % total fatty acids (56). The diets contained 10% fish, linseed or safflower oil. The controls were fed a diet containing 2.9% of unidentified lipid. The data in the paper could not be used to calculate the amounts as g/100g egg yolk. The fish oil was Maxepa oil.

Omega-3 FA enriched eggs alter the blood lipid profile of patients suffering from hypercholesterolemia, reducing plasma triglycerides and platelet aggregation. Omega-3 FA could cause a shift in the LDL size towards particles that are less artherogenic (66). Naturally enriched eggs are presently on sale in several countries, particularly those enriched in omega-3 FA, vitamin E and carotenoids (lutein and zeaxanthine), such as "omega-3 eggs", "benefic eggs", "Columbus eggs". They are said to improve several human physiological parameters (67).

Extracts of egg yolk, usually lecithins (the eggs taken from hens fed a special diet, based upon algae and linseed), have been added to several brands of baby formula, so that these formulas provides the same quantities of very long chain FA (especially omega-3 FA) as mother's milk (68-72). The fatty acids in blood plasma and red blood cells of these babies fed the omega-3 FA enriched formula were identical to those of breast-fed babies (73). Similarly, the milk of women eating omega-3 rich eggs also contained more of these fatty acids (74).

## Reconstitution of a human diet using products from animals raised on linseed

In France, in Brittany, a group of volunteers, 32 men and 43 women aged 25 – 45 years, was fed one of two diets for 35 days (12). The control diet was produced by normal farming; while the test diet was based on livestock (beef, milk, butter and cheese, pork, poultry and eggs) fed partially on linseed, which is particularly rich in ALA. The feeding period was interrupted by a 35-day washout period. The enriched diet had a most interesting effect on the human diet (Table XI). The ALA intake was doubled from 0.75 to 1.65 g/day, while the total omega-3 FA intake increased from 0.86 to 1.91 g/day. The linoleic acid/ALA acid ratio was halved, from 14.9 to 6.8, which is close to the recommended ratio of 5. There were measurable effects on the blood lipids, too, with the blood serum ALA concentration increasing from 0.44 to 0.93, the

total omega-6 FA/ omega-3 FA ratio dropping from 14.3 to 10.2 and the linoleic/ALA ratio increasing from 71 to 34. Thus the trial showed that the test diet was effective.

# Table 11 ALA, EPA and DHA contents of foods and their effects on the amounts absorbed by humans

	ALA		EPA + DHA	
	Control	Lin	Control	Lin
Milk % total FA	0.3	0.9	0.1	0.1
Eggs % total FA	0.6	6.6	0.8	1.6
Pork % total FA	1.1	2.9	0.2	0.2
Chicken % total FA	1.4	6.0	0.2	0.4
Absorption by human volunteers (g/day)	0.75	1.65	0.11	0.25

From (12). Beef was not tested. The exact cuts of meat used were not specified.

#### Influence on the taste of the human diet

Actually, the main constituents involved are the lipids as they directly influence the flavour of food. The taste of meat is linearly dependent on its quality, and the phospholipids are the main source of flavour (75). In addition to the Maillard reaction, the nature of the polyunsaturated FA in beef has a verifiable influence on the flavour as a result of the production of volatile compounds during cooking (76). Certain substances, like 2-alkyl-thiapyrans and 2-alkylthiophenes, have been found in cooked beef and lamb (77).

For instance, a study carried out on enriched pork (fed a diet containing 2% fish oil and 1% rapeseed oil) showed that the enrichment in omega-3 FA did not alter either the colour or taste of the meat (78). Some sensory deficits are noted when using high levels of stabilised tuna fishmeal (79). Trained panellists in triangle tests are able to identify bacon from pigs

fed 10 and 15% flaxseed (80). On the contrary, high dietary concentrations of linseed (15% of the feed) can sometimes slightly alter the taste of the meat, increasing the intensity, with more frequent defects (81). Feeding large amount of vegetable oils contribute to decrease flavour of goat dairy products (31). Feeding poultry a diet high in linseed or menhaden fish oil may alter the taste of the meat (82). But the eggs laid by hens fed this fish oil taste quite normal (83).

## Slightly increased cost to the consumer, but considerably increased nutritional value

It is thus quite possible to improve considerably the nutritional value of foodstuffs by modifying the diets fed to slaughter livestock to make it more traditional, if not natural (6, 7). This is especially true for the quality of the lipid fatty acids and the contents of several essential or non-essential micronutrients. Products with high omega-3 FA contents are presently available, so that is now possible to evaluate the cost of this enrichment (Table XII). The cost of the feed for hens laying omega-3 eggs is 5% more than the cost of feed for hens laying "label" or free range eggs. This increases the production cost by only about 1% and results in an approximately 4% increase in the consumer price. But the nutritional value is increased 9-fold, thus reducing the price of a unit of omega-3 FA by this much.

## Table 12 Cost of alpha-linolenic in eggs compared to that of vegetable oils

	Euros per 1g ALA (50% daily requirement)	
Rapeseed	0.015	
Soybean	0.03	
Walnut	0.05	
Eggs - " linseed " standard	0.9	
Eggs oméga-3 M	1.3	
Isio-4 oil	0.12	
Standard eggs	8	
Free range eggs	12	

The cost of 50% of the daily requirement for DHA is about 0.02 euro, provided by less than 10 g of mackerel or sardines. From (8, 9).

#### Conclusion

The major source of ALA for human is rapeseed oil (canola oil), the cheapest source of ALA; and walnut oil. Soyabean oil is also interesting, but contains too high amount of linoleic omega-6 acid; plus a type of egg defined as "omega-3" or "benefic" eggs (in France) or "Columbus eggs" (in US and most European countries) Interestingly, vegetable oils contributed surprisingly little, just 9% of the ALA intake in Aquitaine, while total plants and their derivatives account for

27% (26). Consequently, it is easy to increase ALA by using rapeseed oil, for instance. Some other food could be useful to provide amount of ALA, such as flaxseed oil and flaxseed: up to 50 g high ALA flaxseed per day is palatable, safe and nutritionally beneficial in human by raising the omega-3 fatty acids in plasma and erythrocytes and by decreasing postprandial glucose responses (84), without compromising antioxidant status (85). In human subjects, by adding ground flaxseed and flaxseed oil to one or two daily meals, it is possible to obtain significant effects on serum levels of enterolactone and ALA (86). But these seeds are not commonly used in diets, so it is interesting to enrich animal products by feeding animals with omega-3 rich diets, made with rapeseed, linseed or linseed oil, or fish products, so as to improve a balanced diet.

Omega-3 fatty acids are involved for many years in cardiovascular diseases and brain development. Very recently, most experimental data has focused on the prevention of psychiatric problems (like depression, dementias and bipolar disorder) by consuming fatty fish. The main feature of these fatty fish is their high omega-3 fatty acid content; but other components, such as iodine, which has a considerable effect on the brain, and selenium, may also have positive effects. Rigorous clinical trials have yet to provide incontrovertible proof that capsules containing omega-3 fatty acids as fish oil extracts are effective. The same applies to purified omega-3 fatty acids, generally supplied as their ethyl esters, as not nearly enough experiments or clinical trials have been done to provide conclusive, convincing evidence in psychiatric diseases. Moreover, the absorption of fish oils and concentrate remains not clear (87). Difficulties have arisen because clinical trials have used fish oils of unspecified composition; and some trials are made with either ethyl esters or even free fatty acids. Moreover, there are at least 3 route of absorption in human: pre-duodenal route, lymphatic route via chylomicron and route via the portal vein to the liver. In addition, positions of fatty acid on glycerol molecule (or on the phospholipid) participate to the absorption process. Some, authors have found that bioavailability of ethylesters is 20% of fatty acids bond to triglycerides (88), but is enhanced by co-ingestion with high fat meal (89). For another, omega-3 fatty acids given as ethyl-esters or triglycerides are equally absorbed (90).

Usefulness of capsules as dietary complement is not evident, if not containing natural fish oil triglycerides (or phospholipids), bioavailability of triglycerides from algae is poorly documented. Omega-3 fatty acids are not isolated pure compounds in their natural state, but are parts of large natural molecules, triglycerides and phospholipids, that are used by the human body. To obtain capsules containing enriched in either EPA or DHA, these compounds must be destroyed (hydrolysed) and the omega-3 fatty acids purified and stabilised as ethyl esters. This results in a product that is no longer natural, but a chemical, and there is no definite proof that this is a medication for instance in psychiatric diseases or to prevent

cognitive decline during aging. A clear distinction must be made between dietary requirements and pharmacological doses, which are much more important. For instance, the safest approach is therefore to look for these essential omega-3 fatty acids in foods that naturally contain them.

In a balanced diet, both ALA and longer chains must be found in the diet, as conversion of ALA into EPA and DHA is variable according to sex (91), and is altered in older men (92). For instance, supplementing lactating women with flaxseed oil increases ALA and EPA in their milk (and their plasma and erythrocytes), but not DHA (93). In young men, EPA and docosapentaenoïc acid (and not DHA) are the principal products of ALA metabolism (94). Moreover, DHA itself is speculated to be essential for human (95). In fact, co-ingestion modifies the kinetics of omega-3 fatty acid metabolism in human: fish based diet reduce the synthesis of DHA (96). Desaturases activities during development in human and animal have been recently reviewed (97, 98). Concerning aged people, it is important to note that ALA recommendations are increased in France (0.8% daily energy intake in adult, 0.9% in aged) and multiplied by 2 for DHA (0.05 % in adult, 0.1 % daily energy intake in aged, as well as in pregnant and lactating women).

The consumer must still select the best cuts of meat or fillets of fish and know how to prepare them. And each type of meat has lean and fatty cuts that require specific treatment. Thus stewing cuts can have very different lipid contents, ribs have 16% fat while shoulder has only 4%. A minimum of ribs adds richness and flavour; without them, the dish is too "dry". The cooking method also influences the fat content, but less than is generally believed. Grilling does not remove all the fat, while using fat in a preparation does not always determine the fat content. For example, a grilled entrecote steak still contains about 12% fat, while a normal serving of Burgundy beef has only 7%.

The way a dish is cooked is also fundamental. Steamed plaice has only 2% fat, but fried plaice has 16%. The difference is similar for lemon sole cooked plain or breaded. Herrings have a high fat content, whether raw or fried, but they are not the same. The fat of this oily fish dissolves into the frying fat when they are fried, so that the natural oils are replaced by those from the fryer. Thus the nutritional value of a breaded or fried fish depends almost completely on the nutritional value of the cooking oil. Fish may thus prevent or aggravate cardiovascular disease, depending, in part, on how it is cooked (9).

It is now essential that the regulations governing the scheduling, raw materials and food programs of restaurants do not just define minimum contents of toxic substances, but also defines the real nutritional value of foodstuffs, which must nourish but not poison. This is necessary, and urgently so for such things as fish and eggs. The omega-3 FA content in the feed of food animals may considerable influence fish and eggs, but is less important for poultry, pork, and beef and lamb. It has essentially no influence on butter and dairy products.

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