

The supply of fish oil to aquaculture: a role for transgenic oilseed crops?

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Summary

The importance of an alternative and sustainable supply of omega-3 long chain polyunsaturated fatty acids (LC omega-3) has long been established. As these biologically active fatty acids have an established role in nutrition and health, there is an ever increasing demand for oils containing LC omega-3 e.g. eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). These fatty acids are produced by microrganisms and enter our diet through the consumption of fish. However, in order that the nutritional requirements of fish in aquaculture are met and sufficient levels are deposited to meet the requirements of human consumers, EPA and DHA must be supplied in excess. Given the importance of the aquaculture industry in delivering healthy foodstuff, the question of how to resource the supply of LC omega-3 then arises; traditional sources of EPA and DHA (fish oil) are challenged, whilst vegetable oils do not contain EPA or DHA. Therefore research efforts have focused on the successful reconstitution of LC omega-3 biosynthesis in oilseed crops. The production of EPA and DHA in the seed oil of agricultural crops has the capacity to deliver large volumes of these fatty acids., The expression of optimised combinations of the genes required to produce these fatty acids in the seed of the crop *Camelina sativa* has been achieved and the utility of this approach demonstrated, despite the difficulties confronted. . Furthermore, the stability and viability of the omega-3 LC trait has been proven in the field; and in separate studies, EPA-Camelina oil has been shown to be a suitable substitute for fish oil. Taken together this represents a significant breakthrough - the provision of an effective alternative to the use of omega-3 fish oil by the global aquaculture industry.

Keywords

Aquaculture; fish oil; nutrition; metabolism; sustainability; GM plants; Omega-3; plant biotechnology

Abbreviations

CVD, cardiovascular disease; DHA, docosahexaenoic acid (22:6n-3); EFA, essential fatty acid; EPA, eicosapentaenoic acid (20:5n-3); FM, fishmeal; FO, fish oil; GM, genetically modified; LC-PUFA, long-chain polyunsaturated fatty acids ($\geq C_{20} \geq 3$ double bonds); LC omega-3, LC-PUFA predominantly EPA and DHA; MT, metric million tonnes; TAG, triacylglycerol; VO, vegetable oil.

1. Introduction

In 2010, fish accounted for around 17 % of the global population's intake of animal protein and almost 7% of all protein consumed.¹ As well as being an important dietary sources of protein, minerals and vitamins,² fish and seafood are also the major sources of long chain (LC) omega-3 polyunsaturated fatty acids, particularly eicosapentaenoic (EPA; 20:5 ω 3) and docosahexaenoic (DHA; 22:6 ω 3) acids,³ that have well-known beneficial effects in human health, including cardiovascular and inflammatory diseases, and important roles in neural development.^{4,5} However, global marine fisheries are stagnating and over 60% of fish stocks require rebuilding⁶ so that an increasing proportion of fish are farmed, reaching almost 50% in 2012¹. Paradoxically, feeds for many farmed fish species are dependent on fishmeal and fish oil, themselves derived from marine fisheries, for the supply of LC omega-3.⁷ Reliance on finite marine resources was an unsustainable practice⁸ and the continued growth of aquaculture has been dependent upon the development of more sustainable feeds with alternative ingredients, generally derived from terrestrial agriculture, that lack LC omega-3.⁹ This has important consequences for the supply of these nutrients to human consumers and so there has been a global drive to find alternative supplies of LC omega-3 fatty acids for aquaculture.¹⁰ The most promising and viable option for entirely new sources of LC omega-3 are transgenic oilseed crops.

2. Long chain omega-3 and human health

It has long been appreciated that dietary LC omega-3 can have important, generally beneficial effects on human health.¹¹ The strongest evidence has been found in relation to heart and cardiovascular disease (CVD)¹²; today a large number of national health agencies and government bodies recognize the importance of increasing dietary intake of EPA and DHA to promote cardiac health and decrease the risk of CVD.^{12,13} Recently, the current advice and guidelines worldwide were comprehensively reviewed¹⁴ and recommendations of over 50 organisations were compiled by the Global Organisation for EPA and DHA.¹⁵ Most recommendations suggest between 250 and 500 mg/d of EPA and DHA for reducing CVD risk or 1 g/d for secondary prevention in existing CVD patients, with a dietary strategy for achieving 500 mg/d being to consume two fish meals (200 – 250 g) per week with at least one of oily fish^{14, 16, 17} (Table 1).

In addition to cardiac health and CVD, dietary LC omega-3 can be beneficial for inflammatory diseases obtained in rheumatoid arthritis, in which the dose required to gain benefit is set higher than for CVD (around 3g per day¹⁸). There is also increasing evidence for beneficial effects of dietary LC omega-3 in Inflammatory Bowel Diseases (IBD) such as Crohn's disease and ulcerative colitis.¹⁹ The effects of dietary LC omega-3 on cancers has been more controversial. Epidemiological studies indicated that, in general, dietary LC-omega-3 may decrease risk of colorectal, breast and prostate cancers²⁰ and some studies suggest beneficial effects in chemotherapy²¹. However, one research group suggested that LC-omega-3 may be associated with increased risk of prostate cancer;^{22,23} although latterly a second systematic review and meta-

analyses of all studies concluded that the results did not support an association between LC omega-3 and prostate cancer.²⁴

With regard to development, there is robust evidence that decreased DHA status can lead to cognitive and visual impairment and that DHA supplements have positive beneficial outcomes in pre-term infants.²⁵ There have also been several reports of potential beneficial effects of dietary DHA supplementation in a number of psychological/behavioural/ psychiatric disorders including attention deficit hyperactivity disorder and depression although there are insufficient studies and data to draw firm conclusions.²⁶ However, it is becoming generally recognized that LC omega-3 are potential key nutrients to prevent various pathological conditions associated with the normal aging process,²⁷ which has prompted research into the effects of LC omega-3 on dementia, including Alzheimer's disease and other age-related cognitive impairments.²⁸ In general, DHA supplementation trials in patients with some pre-diagnosed cognitive impairment indicated that this appeared to slow progression of Alzheimer's.²⁹

3. Aquaculture: requirement for LC omega-3

Vertebrates, including fish, cannot synthesise polyunsaturated fatty acids (PUFA) because they lack the enzymes required for their production from monounsaturated fatty acids and so they are essential in the diet. Terrestrial plants can be rich in medium chain PUFA such as linoleic acid (18:2 ω 6) and α -linolenic acid (LNA, 18:3 ω 3), but the biologically active fatty acids in fish are the long-chain PUFA, primarily EPA and DHA. Some freshwater and salmonids species of fish can produce EPA and DHA from LNA, but most marine fish cannot. In consequence, which PUFA can satisfy the essential fatty acid (EFA) requirements in fish vary with species.^{30, 31} The actual EFA requirement in fish can be described at three levels. The amount of EFA a fish requires to prevent nutritional pathology is low, often around 1% of the diet.^{30, 31} However, a higher level of EFA may be required to support optimum growth and health, which is similar to the situation in humans where few people suffer from EFA deficiency but, rather, LC omega-3 is required for optimal development and health. The final EFA requirement level in fish is that to maintain nutritional quality based on LC omega-3 content of the flesh.⁹ As such, this is not a requirement of the fish, but rather that of human consumers.³² To satisfy this level, EPA and DHA need to be supplied to the fish well in excess of the requirements for optimal health and growth so that they are deposited and stored in the fish. For example, to produce farmed salmon with the level of EPA and DHA required to supply the weekly human requirement of 3.5 g in one 130 g portion, it would be necessary for EPA plus DHA to be at 6 - 7% of diet.³³

This level of supplementation would make fish-farming commercially uneconomical.

4. Fish Oil

In 2013 around 75% of total global fish oil supply was used in aquaculture, with 83% of that consumed by salmonids (60%) and marine fish (23%), and a further 21% used for direct human consumption³⁴ (Figs.1A &B). Despite continued growth of aquaculture¹, the use of fish oil for feeds

has been relatively stable over the last decade with, on average, around 0.8 million metric tonnes (MT) being used³⁴ (Fig.1C). The primary constraint with fish oil is that it is a finite resource with production limited through strict regulation of fishing and catch quotas.^{35, 36} Although production of fish oil has declined in recent years, largely due to regulation and quotas in South America,³⁷ this has been partially offset by increased use of seafood and aquaculture by-products including by-catch and trimmings to produce fish meal and, to a lesser extent, fish oil.³⁶ However, fish oil production averages around 1 million MT annually and there is little to no prospect of that increasing.

Production of fish oil is also subject to environmental influences and acute phenomena such as El Niño have well-known consequences, considerably reducing supply.³⁸ Sustainability issues are also key drivers limiting fish oil supplies, and these will have an increasing impact with the many initiatives currently being developed with respect to both national and international standards and certification of marine ingredients, including fish oil.³³ Although increasing adoption and implementation of these standards will further improve sustainability, they will likely have impacts on the availability of fish oil and its use in aquaculture. A further factor constraining the use of fish oil is the presence of contaminants/undesirables such as persistent organic pollutants (POPS) including dioxins and PCBs.^{9, 39} With some fish oils, such as those from the Baltic, levels can be higher than permitted for use in animal feeds and these require decontamination before they can be used. However, this is an issue that has been diminishing to some extent with the increasing replacement of dietary fish oil and as a result levels of these undesirable marine environmental contaminants in feeds are decreasing.⁴⁰

To put demand for fish oil (viz. LC omega-3) for aquaculture into perspective, to supply the salmon industry alone would require more than 1 million MT annually (i.e. greater than the average global supply). Thus the only way aquaculture has continued to grow has been by increasing substitution with alternative oils.¹⁰ Global oil and fat production was more than 185 million MT in 2012, with total production of vegetable oils at over 160 million MT, and animal fats including tallow, lard and butter totalling another 25 million MT. Thus, alternative oils are plentiful although they all lack LC omega-3 (Table 2). Terrestrial plants do not produce EPA and DHA and so they are not components of any vegetable oil, and animal fats are dominated by saturated and monounsaturated fatty acids with only low levels of PUFA (Table 3). The consequence of this has been the increasing substitution of fish oil with alternative oils, effectively spreading the available fish oil thinner in the feeds, which has inevitably led to a reduction in LC omega-3 levels in both feeds and farmed fish¹⁰ (Table 4).

However, the issues surrounding the limited supply of LC omega-3 transcend aquaculture. Based on the most commonly recommended dose for cardiac health (500 mg/day¹⁵), the demand for LC omega-3 is over 1¹/₄ million MT whereas global supply is optimistically estimated at just over 0.8 million MT indicating a shortfall of over 0.4 million MTs⁴¹ (Table 5). The majority of supply (almost 90%) is from capture fisheries, whether as food fish or via fish oil and meal, with small

additional amounts estimated from seafood by-products, unfed aquaculture and algal sources. The calculations in Table 5 contain assumptions and estimates and so the precise extent of the difference can be argued, however, that there is a gap between supply and demand is not in question irrespective of how it is calculated.⁴² There is a fundamental, global lack of LC omega-3 to supply human needs, whether by direct consumption or via aquaculture.

5. Alternative sources of omega-3 LC-PUFA

There is an urgent need for alternative sources of the LC omega-3, EPA and DHA.⁴¹ As the primary producers of almost all LC-omega-3 are marine microalgae and bacteria, the only alternatives to traditional fish oil are other oils sourced from the marine environment including lower trophic levels (zooplankton), mesopelagic fish, by-catch/by-products and microalgae themselves.

Lower trophic levels

Zooplankton such as krill and calanoid copepods in the southern and northern hemispheres, respectively, are possible options but, although biomass at lower trophic levels is large, there are inherent dangers associated with fishing down the marine food web.⁴³ Potentially, zooplankton can be good oil sources, but harvesting of krill and copepods poses significant technological challenges and cost.⁴⁴ For most species, lack of schooling behaviour makes harvest by traditional trawling technology an expensive economic option.⁴⁴ Antarctic krill, which do form schools, are the only species being targeted for commercial harvest, apart from a small scientific quota (~1000 MT) of the calanoid copepod, *Calanus finmarchicus*.⁴⁵ Krill meals, that contain residual oil and therefore some LC omega-3, are currently used in some premium feeds focussed on health benefits and are generally used sparingly. These krill meals are therefore not being used as primary sources of LC omega-3 and, currently, krill oils are used almost exclusively for the human nutraceutical market. Although there may be evidence that harvesting krill, and potentially copepods, could be sustainable, there are still significant environmental and ecological concerns.⁴⁴ For instance, Antarctic krill are near the base of a food chain that includes whales and penguins, suggesting there could be opposition to greatly increased exploitation.⁴⁶

Mesopelagic fish

Mesopelagic fish that inhabit the intermediate pelagic water masses between the euphotic zone at 100m depth and the deep bathypelagic zone at 1000m are available in potentially large quantities (1 - 6 billion MT), with lantern fish, myctophids, constituting about 60% of biomass.⁴⁷ Different species can contain between 16 and 60 % of dry weight⁴⁸ as oil and most are potentially good sources of LC omega-3.⁴⁴ On the positive side, they are resources that, so far, have not been the subject of commercial exploitation, and they do not compete with existing or potential human feed production. Negative points include biological (seasonal variation), ecological (mixed fishery difficult to manage), technical (capture methods and on-board processing), and nutritional issues.

By-catch and seafood processing by-products

In almost all fisheries there are non-target catch and/or discarded target catch that, together, make up the by-catch. However, both the precise definition and resultant estimates of by-catch can be controversial.⁴⁹ In 2005, the discard rate was estimated at around 7 million MT/year or 8% of the global catch.⁵⁰ By its nature, by-catch is a diffuse resource⁵¹ and this imposes a major limitation to its usefulness as a source of FO, although processing of by-products, including oil production, at sea is an increasing trend⁵². Another limitation is that by-catch includes a multitude of species, not necessarily “oily”, which limits the quantity and quality of the oils produced.⁵¹

Seafood industry by-products including viscera, heads, carcasses and trimmings, particularly those produced from pelagic fisheries and aquaculture are another potential source of marine oil although this is largely dictated by species. Thus, by-products from oily species including salmon, herring and mackerel can be a source of substantial oil whereas by-products from other pelagic (white fish) fisheries have generally low oil contents.⁵³ Liver from species like cod and halibut have traditionally been used for fish oil production, but production is now relatively small (~ 40,000 MT) and goes mostly for direct human consumption as vitamin supplements as much as sources of LC omega-3.⁵⁴ Oil is now being actively recovered from aquaculture species waste, particularly salmon farming, with around 20,000 MT reportedly recovered in Norway⁵⁵ and 50,000 MT in Chile in 2006.⁵⁶

Marine microalgae

Potentially, culture of the main primary producers, marine microalgae, could offer a long-term solution to the sustainable supply of LC omega-3.⁵⁷ Various photosynthetic microalgae are already commonly used in fish hatcheries to supply both EPA (e.g. diatoms) and DHA (e.g. flagellates) in the rearing of larval marine crustacean and fish species.⁵⁸ Production usually employs medium- to high-density batch, semi-continuous or continuous culture in relatively small volumes⁵⁹. Up-scaling of production to the volumes required for algal oil and/or algal biomass to supply the amount of LC omega-3 required to replace fish oil in commercial aquafeed production has significant biological and technological challenges.⁶⁰ Economic production of LC omega-3 would require algae to demonstrate simultaneous high growth and high oil content with high proportions of EPA and DHA. These are almost exclusive traits as oil deposition is usually associated with conditions which occur only when growth is limited (e.g. N limitation).⁶¹ Technical challenges include efficient capture of light energy in high-density culture with effective temperature control. These issues remain to be solved but there are several research strategies targeting their solution. These include exploiting culture conditions to direct metabolism towards lipid production, to improve biomass productivity or oil yield by mutagenesis and selective breeding, and to improve strains by genetic modifications to optimize light absorption and increase biosynthesis of EPA and DHA⁶⁰. Therefore, in the future, algal species with favourable biological characteristics may be found

and/or developed, and photo-bioreactor technologies may improve considerably enabling economically sustainable production of microalgae rich in EPA and DHA for use in aquafeed.

In contrast, heterotrophic microalgae species including *Cryptocodinium* and thraustochytrids such as *Schizochytrium* are already being utilised for the commercial production of DHA using large-scale biofermentor technology.⁶² Even so, the high production costs are generally limiting the use of these products to direct human consumption mainly in the form of DHA supplementation of infant formulae.⁶³ However, the DHA-rich products from heterotrophic microalgae may have niche markets in marine hatcheries, particularly for high-value species. Production volumes would have to be increased and costs reduced before these products could be viable for wider application in aquaculture.

6. Plants as a source of omega-3 LC-PUFA

It has long been the hope of researchers in the field of plant lipid biotechnology to produce a terrestrial supply of vegetable oil with a composition (EPA/DHA) matching that of fish oil. However, metabolic engineering on this scale is not trivial. Synthesis of LC omega-3 commonly occurs in marine microalgae via a series of sequential aerobic desaturation and elongation reactions. These reactions typically follow two routes – the ‘ $\Delta 6$ pathway’ (Fig 2a) beginning with the $\Delta 6$ -desaturation of the C18 substrate, C2 elongation and $\Delta 5$ -desaturation or the much rarer ‘ $\Delta 8$ pathway’ where the C18 substrate undergoes a C2 elongation, followed by two rounds of desaturation ($\Delta 8$, $\Delta 5$). To then generate DHA in either pathway requires a further C2 elongation and ($\Delta 4$) desaturation. Through whichever aerobic route, a minimum of three genes are required to synthesise EPA and five for DHA production. A further anaerobic route to LC omega-3 has been identified in some bacteria and unicellular marine eukaryotes and functionally characterised; this pathway uses a processive polyketide synthase-like enzymatic system to convert malonyl-CoA to EPA and DHA with the production of intermediates.^{64,65}

The activities and genes for each of the biochemical routes to LC omega-3 FA production have been characterised and are available for use by the metabolic engineer to reconstitute EPA/DHA synthesis in a suitable heterologous host.⁶⁴ However, success can only be achieved by addressing some significant challenges: (1) the C18 di- or tri-unsaturated substrate must be pre-existing; (2) reconstitution of the pathway in a specific tissue (e.g. seed) requires the co-ordinated and targeted expression of multiple genes and activities; (3) the production of novel LC omega-3 and their correct acyl-exchange between lipid pools depends on the capacity of native endogenous enzymes that are unfamiliar with the fatty acids; and (4) the processes underpinning the biochemical acyl-exchange or flux of fatty acids between lipid species within plant cells is only now starting to be appreciated. It is entirely possible for biosynthetic intermediates to be channelled into ‘metabolic cul-de-sacs’ rendering them unavailable for further desaturation, elongation or storage in seed oil.⁶⁵

Historically, many of the individual genes necessary for LC omega-3 production were individually characterised in plants, indicating that in combination they would be successful. The first demonstration of EPA production in a transgenic plant was published by Qi *et al.*⁶⁶ expressing the algal alternative pathway in the leaves of *Arabidopsis thaliana*. The approach used a series of sequential transformations of individual genes and resulted in the vegetative accumulation of EPA, predominantly in phospholipids. To be of economic value in the context of aquaculture as described above, it is necessary to have an engineered oilseed crop where the target LC omega-3 would be produced and stored in the neutral lipids (triacylglycerol) of seeds at high levels. The possibility of LC omega-3 biosynthesis targeted to seeds was shown by Abbadi⁶⁷ in which the conventional $\Delta 6$ pathway was expressed in transgenic linseed. Abbadi⁶⁷ identified the presence of low levels of EPA in seeds, but concomitant with high levels of C18 $\Delta 6$ -intermediates. The unwanted intermediates were the result of the poor acyl-exchange of intermediates between the acyl-CoA pool (elongation) and glycerolipid pool (desaturation). Following these proof-of-concept studies efforts have continued to drive up the accumulation of LC omega-3 in seed oil and reduce the presence of intermediates e.g. $\Delta 6$ desaturation products like γ -linolenic acid. Such iterative engineering followed two strategies, the first utilised by Petrie and co-workers (CSIRO) made use of *Agrobacterium*-mediated transient expression of multiple gene combinations in *Nicotiana benthamiana*.⁶⁸ Although testing was undertaken in leaves, the co-expression of the Lec2 transcription factor or master regulator essentially 'reprogrammed' the leaf to replicate the lipid composition of a seed; a system that permitted the rapid selection of optimal gene combinations. The second approach, adopted by Heinz *et al.* (University of Hamburg) and Napier *et al.* (Rothamsted Research) was to directly assess gene combinations and their regulatory elements in the seeds of stably transformed plants (namely the oilseed model *A. thaliana*).^{69, 70} In combination with the development lipidomic approaches, it then enabled the evaluation of gene combination efficacy in the seed. Critical to the progress of these efforts was the use of omega-3 desaturases (reducing the accumulation of un-wanted omega-6 fatty acids) and the use of acyl-CoA dependent desaturases (breaking the desaturase/elongase substrate dichotomy).⁶⁵

7. Transition to Crops

As described, the majority of the systematic work undertaken to produce a seed oil composition akin to fish oil (i.e. ~20% EPA and DHA in total fatty acids) was undertaken in model plant species i.e. *Arabidopsis* and tobacco. However, to produce the volumes of LC omega-3 required by aquaculture necessitates the production of EPA and DHA in agricultural oilseed crops. The opportunities of scale associated with such crops provide the only chance to generate a fish oil substitute in significant quantities. From the available oilseed crops, screening has identified two as appropriate hosts for the production of LC omega-3; the first canola, a cultivar of rapeseed (*Brassica napus* L.) and secondly Camelina (*Camelina sativa* L.). At this time no public data are available to assess the expression of the LC omega-3 trait in canola. However, two laboratories

(Rothamsted and CSIRO) have demonstrated the success of Camelina as a host for the accumulation of EPA and DHA in seed oil.^{71, 72} Camelina is an oilseed crop of the Brassicaceae adapted to temperate growing regions, with a low input requirement, short crop cycle and disease resistance. In terms of performance, Camelina has an acceptable yield (levels over 3000 kg/ha have been achieved) with an oil content of ~40%. Of interest to metabolic engineers, Camelina is an excellent platform for the production of tailored oils; it has very low outcrossing levels, and fully developed tools for breeding and molecular trait improvement due to the high sequence identity with *Arabidopsis*. Furthermore, genetic transformation is possible using *Agrobacterium*-mediated floral dip. However, it is the unique fatty acid composition of Camelina (unusual for a *Brassicaceae* the polyunsaturated α -linolenic acid (18:3) and linoleic acid (18:2) – substrates for LC omega-3 synthesis - are the major fatty acids, whilst erucic acid (22:1) is low) that engenders this host to genetic engineering approaches⁷³.

Although different approaches were taken by Rothamsted and CSIRO, both demonstrated the suitability of Camelina as a host for the production of LC omega-3 in seeds. Researchers at Rothamsted used knowledge acquired from earlier engineering in *Arabidopsis*, introducing two optimised constructs into Camelina – one producing EPA alone and the other making both EPA and DHA. Analysis of the seed fatty acid composition from the stable transgenic lines revealed the average level of EPA alone was 24%, and for EPA and DHA, 11 and 8% respectively⁷². Significantly in both constructs only very low levels of C18 intermediates were recorded. Overall, the average total level of LC omega-3 was 19% (EPA + DHA) and much higher (25%) in some single seeds (see Fig 2)⁷² Authors are these % of seed dm or of lipid?. The reported work from CSIRO showed significant accumulations of DHA with all the introduced constructs (12% with construct combination GA7 and mod-F), however levels of EPA were low (~3% for mod-F).⁷¹ The differences in the accumulations of LC omega-3 in Camelina can be attributed to a number of factors e.g. selection of seed-specific promoter, choice of Camelina strain, activity of each enzyme choice, constructs design/orientation, and possibly site of integration. For example, it is likely that the use of a highly active $\Delta 5$ -elongase resulted in the specific difference in EPA accumulation⁶⁵. Moreover, field evaluation of the LC omega-3 trait (EPA + DHA) in Camelina was undertaken at Rothamsted, where the feasibility was demonstrated (by comparison to replicated glasshouse experiments) of producing LC omega-3 in the field without any yield penalty⁷⁴. Thus, the choice of the oilseed crop Camelina as a host for the successful expression of the LC omega-3 trait has been established.

8. Utility of Camelina LC Omega-3 Oil in Aquaculture

The essential question must be; can the novel LC omega-3 oils generated in Camelina be used as a substitute for fish oil? To address this Betancor *et al.*⁷⁵ recently completed a study in which the EPA-containing Camelina oil described by Ruiz-Lopez *et al.*⁷² was tested as a substitute for fish oil

in a salmon feeding trial. In this study, fish oil (control), wild type (WT) and EPA-containing Camelina oil (GM) were fed to juvenile Atlantic salmon within an iso-energetic diet for a period of seven weeks. At the end of the feeding trial all the fish had doubled in weight and displayed no indicators of ill health; moreover fatty acid analysis indicated the expected accumulation of EPA. Further transcriptomic analysis produced a similar pattern of gene up regulation in fish receiving WT and GM Camelina oil; reflecting the fact that the GM Camelina oil is a modified vegetable oil and therefore contains a different chemical e.g. sterol composition to fish oil⁷⁵. As formulations for aquafeed diets are now composed of blended fatty acids from marine and vegetable sources, the Betancor study successfully demonstrated how LC omega-3 Camelina oil is appropriate for use in aquaculture. (Authors can fish desaturate and elongate EPA to 22:6 (n-3) ? probably not!)

9. Conclusion

As discussed above there is a clear need within aquaculture for an alternative, sustainable supply of LC omega-3. The potential of engineered oilseed crops to meet this demand have been developed by a number of laboratories over the last fifteen years, but only now, at least with Camelina, has it been possible to demonstrate the viability of oilseed crops as a source of EPA and DHA. However, it is clear there are a number of hurdles before a Camelina LC omega-3 oil can become a commercial reality. It must be hoped that, subject to the appropriate regulatory and safety approvals, a forward looking aquafeed industry would be willing to adopt LC omega-3 Camelina oil in fish diets, thereby reducing the pressure on oceanic stocks. Camelina engineered to produce EPA and DHA, when grown in sufficient volume could make a significant contribution to reducing the demand from capture fisheries (200 000 ha of LC omega-3 Camelina could produce 150 000MT of oil or 15% of the global oceanic harvest).⁶⁵ The area required seems large, but it is in fact modest when placed in the context of global vegetable oil production. For example, such a hypothetical area would be less than 3% of the Canadian oilseed sowing area of a crop like canola⁶⁵. Of course before such numbers can be achieved on farms a number of issues must be resolved; crucially regulatory approval must be sought and obtained, the agronomy and breeding of Camelina must be addressed, oil formulations tested, and the views of consumers understood. All of these steps require significant effort and resolve, however the demand for LC omega-3 is likely to grow, therefore it is our hope that this experimental terrestrial oilseed source of LC omega-3 can be converted to a commercial reality.

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Table 1 International recommendations for dietary long-chain n-3 LC-PUFA consumption in humans.

Source and reference	Date	Recommendation			
		Total n-3 % of Metabolizable energy	ALA g/d	EPA+DHA mg/day	n-3:n-6
NATO workshop	1989	—	3	800	4:1
UK Committee on Medical Aspects of Food Policy	1994	0.2	—	100-200	—
ISSFAL workshop	1999	1	2.2	650	—
Apports Nutritionnels Conseilles (France)	2001	0.8-1	1.8	450 (DHA, 110-120)	5:1
Eurodiet	2000	—	2	200	—
Health Council of the Netherlands	2001	1	—	200	7.5:1
American Heart Association	2002	—	—	~1 g/d (2ry prev CHD)	—
US National Academics of Science, Institute of Medicine	2002	—	1.4	~140	—
European Society of Cardiology	2003	—	—	~1 g/d (2ry prev CHD) 400-1000	—
WHO FAO	2003	1-2	—	(1-2 fish meals/wk)	—
ISSFAL	2004	—	1.6	≥500	—
UK Scientific Advisory Committee on Nutrition	2004	—	—	~450 (Min 2 portions fish/wk)1 oily	—

ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAO, Food and Agriculture Organization; ISSFAL, International Society for the Study of Fatty Acids and Lipids; NATO, North Atlantic Treaty Organization; WHO, World Health Organization.

Table 2. World oil and fat production in 2012.^a

	Million tonnes (Mt/yr)
Total production	~186
Palm	50.7
Soybean	42.4
Rapeseed	24.3
Sunflower	15.1
Palmkernel	5.9
Cottonseed	5.3
Groundnut	5.1
Coconut	3.6
Olive	3.4
Corn	2.5
Sesame	0.8
Linseed	0.6
<u>Castor</u>	<u>0.6</u>
Total vegetable oil	160.3
Tallow	~ 9
Lard	~ 8
Butter	~ 7
<u>Fish</u>	<u>~ 1</u>
Total animal fat/oil	~ 25

^a <http://lipidlibrary.aocs.org/market/fo6-07.htm> (Updated 03/2013; accessed 10/2014)

Table 3. Fatty acid (FA) composition (Mol%) of major vegetable oils and animal fats.

Oil/fat	16:0	18:0	18:1n-9	18:2n-6	18:3n-3	Significant other FAs
<u>High saturated</u>	44	4	39	11	0	
Palm	9	3	15	2	0	70 (sat.* <16:0)
Palm kernal	9	2	7	2	0	79 (sat. < 16:0)
Coconut						
<u>High 18:1n-9</u>	4	1	62	22	10	
Rapeseed	10	2	78	7	0.6	
Olive						
<u>High 18:2n-6</u>	11	4	23	54	8	
Soybean	6	5	20	69	tr	
Sunflower	22	3	19	54	0.7	
Cottonseed	12	3	37	41	0.3	7 (LC-sat./mono.*)
Groundnut	11	2	25	60	1	
Maize (Corn)	9	6	38	45	0.6	
Sesame	44	4	39	11	0	
<u>High 18:3n-3</u>						
Linseed	6	3	17	14	60	
Perilla	6	2	12	16	64	
Camelina	5	2	12	15	47	19 (C20 mono.)
<u>High D6 products</u>						
Borage	10	4	18	36	tr	26 (18:3n-6), 6 (C20/22)
Evening primrose	8	2	11	68	tr	10 (18:3n-6)
Echium	7	4	16	15	29	13 (18:3n-6), 12 18:4n-3)
Buglossoides	4	2	7	11	39	5 (18:3n-6), 18 (18:4n-3)
<u>Animal fats</u>						
Tallow	25	19	43	2	1	4 (16:1)
Lard	26	14	44	9	tr	3 (16:1)
Poultry	23	6	42	19	tr	6 (16:1)

*Sat., saturated FA; Mono., monounsaturated FA

Table 4. Effect of complete or partial replacement of dietary fish oils by vegetable oils on fatty acid compositions (percentage of weight of total lipid) of flesh of Atlantic salmon. FO, fish oil; LO, Linseed oil; PO, palm oil; RO, rapeseed (Canola) oil; SO, sunflower oil; VO, vegetable oil blend; ^a Initial wt. 55 g / 100 % replacement / 30 weeks, ^b Initial wt. 80 g / 100 % replacement / 17 weeks, ^c Initial wt. 22 g / 100 % replacement / 9 weeks, ^d Initial wt. 127 g / 100 % replacement / 40 weeks), ^e Initial wt. 0.16g / 100 % replacement, RO/PO/LO [3.7:2:1] / 22 months. Authors, this is unclear to me

	FO	PO ^a	FO	RO ^b	FO	SO ^c	FO	LO ^d	FO	VO ^e
14:0	4.2	1.6	3.9	1.4	5.2	2.2	4.7	0.7	4.9	0.8
16:0	17.8	23.6	17.3	10.3	17.8	12.0	12.9	8.3	13.0	12.9
18:0	2.9	3.7	2.7	2.7	4.8	5.6	1.9	3.6	2.6	3.1
Total saturated	25.9	29.2	24.5	14.8	28.9	21.0	19.9	12.7	21.1	17.0
16:1n-7	4.9	1.3	5.0	1.8	6.8	2.6	7.5	0.8	4.4	0.9
18:1n-9	18.3	37.2	17.1	39.1	16.3	19.8	16.0	18.6	14.6	41.3
18:1n-7	2.9	1.6	2.6	3.2	3.5	2.7	3.8	1.3	2.7	2.5
20:1n-9	4.8	3.4	7.6	4.1	1.4	1.0	16.6	1.6	11.4	2.7
22:1n-11	4.8	1.8	8.9	2.6	NR	NR	9.9	1.1	12.3	1.3
Total monoenes	37.6	46.2	42.3	51.8	30.8	26.7	57.1	24.2	48.2	49.0
18:2n-6	3.4	10.6	2.5	12.3	3.5	33.2	3.9	13.1	3.3	14.4
20:2n-6	0.5	1.0	0.4	1.2	0.3	1.4	0.4	0.7	0.4	1.2
20:3n-6	0.1	0.6	0.1	0.5	NR	NR	0.2	0.1	0.1	0.7
20:4n-6	0.5	0.4	0.5	0.4	0.9	0.7	0.3	0.1	0.7	0.9
Total n-6 PUFA	4.8	12.7	3.7	14.6	5.4	37.6	4.9	14.0	4.5	17.0
18:3n-3	1.8	0.5	1.4	5.6	NR	NR	0.8	38.7	1.1	8.2
18:4n-3	2.0	0.4	2.0	0.7	3.0	1.1	1.5	1.2	1.5	1.2
20:4n-3	1.5	0.4	1.5	0.7	1.2	0.5	1.2	1.8	1.4	0.9
20:5n-3	5.7	1.9	5.8	2.8	9.8	2.8	4.3	1.3	4.6	1.6
22:5n-3	1.9	0.6	2.1	1.1	3.4	1.0	1.5	0.4	2.1	0.6
22:6n-3	16.3	7.7	14.8	7.2	14.0	8.0	8.1	3.1	12.7	3.4
Total n-3 PUFA	29.5	11.5	27.7	18.4	31.3	13.5	17.5	49.1	23.4	16.0

Table 5. Potential annual demand for n-3 LC-PUFA based on recommended intake for reduction of risk of cardio-vascular disease compared with global annual supply from major sources.

	Million tonnes	Notes
Demand (3.5g/week x 52 weeks x 7 billion)	1.274	Based on 500 mg/day (ISSFAL, 2004)
Supply (see below)	0.84	Less than $\frac{2}{3}$. See below for breakdown
Gap (Demand - Supply)	0.434	
Capture fisheries (food)		
Landed	75.0	FAO, 2014.
Edible yield (50%)	37.5	
Lipid content (5%)	1.875	
n-3 LC-PUFA content (20%)	0.375	
Capture fisheries (animal feed)		
Landed	20.0	FAO, 2014.
Fishmeal produced (22%)	4.4	Fishmeal yield generally constant at 22%
Lipid content (10%)	0.44	
n-3 LC-PUFA content (25%)	0.11	
Fish oil produced	1.0	Oil yield is variable
n-3 LC-PUFA content (25%)	0.25	
Seafood by-products (incl. aquaculture)		
Fishmeal produced	2.0	About 50 % of by-products are produced from aquaculture and, therefore, the
Lipid content (5%)	0.1	n-3 LC-PUFA is mainly derived from
n-3 LC-PUFA content (10%)	0.01	fishmeal and fish oil and , as such,
Fish oil produced	0.2	already counted above.
n-3 LC-PUFA content (10%)	0.02	
Aquaculture (unfed)		
Produced	20.5	Mainly filter-feeding carps (7.1 mt) and bivalves (13.4 mt) (FAO, 2014)
Edible yield (50%)	10.25	
Lipid content (5%)	0.525	
n-3 LC-PUFA content (10%)	0.05	
Aquaculture (endogenous production)		
Macroalgae	NA	Salmonids and fed freshwater species ¹
Produced	23.8	Including species used for carageenin, algin, agar and iodine (~16 mt)
Food or feed use	7.0	Human consumption & aquaculture
Lipid content (2%)	0.14	
n-3 LC-PUFA content (15%)	0.02	

Production figures are from 2012 (FAO, 2014). All lipid and n-3 LC-PUFA contents/yields are estimated averages over the wide range of species in each category. ¹Salmonids include salmon and trout spp. Freshwater species includes eels, tilapia and other freshwater species that still utilise some fishmeal and fish oil (13% of total fish oil used in aquaculture) in feeds. NA, not available. Although there will be some endogenous production of n-3 LC-PUFA inversely related to fishmeal and oil contents of feeds, this cannot be accurately estimated, but

will likely be minor based on volume of fish oil consumed by these sectors (75 % of all fish oil used in aquaculture).

Figure 1. The Consumption and use of global fish oil supply. In 2013 around 75% of total global fish oil supply was used in aquaculture, 83% of that consumed by salmonids (60%) and marine fish (23%), and 21% went human consumption (a & b). The use of fish oil for feeds has been relatively stable over the last decade on average around 0.8 million metric tonnes³⁴ (c).



