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n-3 Oil sources for use in aquaculture – alternatives to the unsustainable harvest of wild fish

Matthew R. Miller[1,2]*, Peter D. Nichols[1] and Chris G. Carter[3]

[1]*CSIRO Food Futures Flagship and Division of Marine and Atmospheric Research, GPO Box 1538, Hobart, Tasmania 7001, Australia*
[2]*Crop & Food Research, Processed Foods Group, PO Box 5114, Nelson, New Zealand 7000, New Zealand*
[3]*National Centre for Marine Conservation and Resource Sustainability, Locked Bag 1370, Launceston, Tasmania 7250, Australia*

The present review examines renewable sources of oils with *n*-3 long-chain ($\geq C_{20}$) PUFA (*n*-3 LC-PUFA) as alternatives to oil from wild-caught fish in aquafeeds. Due to the increased demand for and price of wild-caught marine sources of *n*-3 LC-PUFA-rich oil, their effective and sustainable replacement in aquafeeds is an industry priority, especially because dietary *n*-3 LC-PUFA from eating fish are known to have health benefits in human beings. The benefits and challenges involved in changing dietary oil in aquaculture are highlighted and four major potential sources of *n*-3 LC-PUFA for aquafeeds, other than fish oil, are compared. These sources of oil, which contain *n*-3 LC-PUFA, specifically EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) or precursors to these key essential fatty acids, are: (1) other marine sources of oil; (2) vegetable oils that contain biosynthetic precursors, such as stearidonic acid, which may be used by fish to produce *n*-3 LC-PUFA; (3) single-cell oil sources of *n*-3 LC-PUFA; (4) vegetable oils derived from oil-seed crops that have undergone genetic modification to contain *n*-3 LC-PUFA. The review focuses on Atlantic salmon (*Salmo salar* L.), because it is the main intensively cultured finfish species and it both uses and stores large amounts of oil, in particular *n*-3 LC-PUFA, in the flesh.

Long-chain PUFA: Aquaculture: Fish oil: Vegetable oil: Atlantic salmon: Phytosterols

Introduction

Sustainability of wild fish stocks

Historically, the intensive culture of Atlantic salmon (*Salmo salar* L.) has relied on natural fisheries to supply fishmeal and oil as ingredients for aquafeeds. Therefore the stability and sustainability of the wild fishery are of vital importance to the security of ingredients for aquafeeds. Worldwide, capture fisheries have plateaued at about 85–95 million tonnes per annum even though fishing effort has intensified[1,2]. However, there is a growing concern about the health of global fisheries stocks and the ecological effects of industrial fishing, with evidence that many fisheries are fully or over-fished[1,3–6]. Fish oil and meal production is strongly dependent on the availability of wild fisheries and the mismatch between demand and expected supply of fish oil is expected to reach 40 million tonnes by 2030[7]. Dramatic decreases, even collapse, can occur in fish populations of the species involved in fish oil production[1,8]. Problems facing most fisheries are complex and stock decreases can be caused by numerous environmental, biological and ecological factors, not necessarily fishing impacts[8]. For instance, climatic events such as El Niño can significantly decrease fish populations and therefore increase pressure on fish oil and meal supply[9–11]. One-quarter of the world's fish oil supply and one-third of the fish meal for aquaculture diets come from one anchoveta (*Engraulis ringens*) fishery off the coast of Peru[8,12]. Collapse of this fishery alone would increase the pressure on world fish meal and oil supply and threaten the security of global aquaculture production[8,12].

Sustainable Atlantic salmon farming

Aquaculture is the fastest growing food-producing sector in the world[7]. Atlantic salmon aquaculture production has grown from 55 000 tonnes in 1985 to more than 1·2 million tonnes in 2006[13,14]. By 2010 it is estimated that 85 % of

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Abbreviations: ALA, α-linolenic acid; LC, long-chain ($\geq C_{20}$); PCB, polychlorinated biphenyls; SDA, stearidonic acid.
* **Corresponding author:** Dr Matthew Miller, fax + 63 3 546 7049, email MillerM@crop.cri.nz

global fish oil will be needed for salmon and trout production[15]. However, fish oils are increasingly being used in the nutraceutical and agricultural industries, and thus both demand and price have substantially increased, stimulating the need for replacement oils. Replacement of fish oil with renewable land-based products such as vegetable oils has been extensively researched and the results incorporated into currently used commercial aquafeeds[16–19].

The present review evaluates how an ecologically sustainable salmon aquaculture industry might be achieved via use of secure and sustainable n-3 long-chain ($\geq C_{20}$) (LC)-PUFA oil options that are not based on wild fish catch. The review is also relevant to the sustainable culture of other fish species.

Discussion

Salmon are carnivorous and require dietary protein and lipid that have traditionally been supplied from wild-caught marine sources. Feed efficiency for Atlantic salmon aquaculture is continually improving through the use of highly specified aquafeeds; currently about 1 kg of fish is produced per kg of feed. Although an estimated 3·2 kg of wild fish stock is required to produce 1 kg of aquafeed for salmon aquaculture[1,2], aquaculture has a significant ecological advantage over wild salmon capture since 1 kg of growth in the wild equates to 10–15 kg of fish eaten by carnivorous fish or captured as by-catch[20,21]. The efficiency of the aquaculture industry is also continually improving as nutritional requirements are better understood. However, further research into sustainability and security of feed ingredients is vital for the growth of the industry.

Lipid content and nutrition of aquafeeds

Lipids provide the main source of metabolic energy in aquafeeds for many carnivorous fish, particularly salmonids. Current extrusion technologies allow aquafeeds to contain up to 40 % oil. The natural marine diet of Atlantic salmon contains high concentrations of n-3 LC-PUFA, in particular EPA and DHA, low concentrations of n-6 PUFA, and moderate amounts of MUFA and SFA (Table 1).

The lipid component of aquafeeds requires the inclusion of n-3 and n-6 essential fatty acids which are necessary for cellular metabolism (synthesis of prostaglandins, eicosanoids, leucotrienes and other essential fatty acid metabolites) and for maintaining cell membrane structure and integrity[22,23]. Digestion, absorption, transport, accumulation, biosynthesis and metabolism of lipids, in particular essential fatty acids and n-3 LC-PUFA, have been studied in Atlantic salmon and reviewed elsewhere[22,24–27].

Atlantic salmon can show nutritional 'diseases' or pathologies due to lipid imbalances[28–30]. They can display reduced growth, poor feed efficiency, evacuolated pyloric caeca tissue and increased incidence of pancreatic disease with essential fatty acid-deficient diets[28]. It is therefore a requirement that aquafeeds supply n-3 LC-PUFA as a part of the oil component if it is not supplied by residue oil in the fish meal[22]. It has been demonstrated that a diet consisting of 100 % vegetable oil and therefore lacking n-3 LC-PUFA causes severe heart lesions, thinning of ventricular walls, muscle necrosis and can influence the devolvement of arteriosclerotic changes in Atlantic salmon[29,30].

Currently, n-3 LC-PUFA for aquafeeds are mainly sourced from marine oil obtained from wild fisheries. Over the past 10 years, the intensive aquaculture industry has been using fish oil blended with vegetable and animal oil in the manufacture of aquafeeds to reduce cost and, to a lesser extent, to decrease the pressure on this finite resource. Many trials with replacement oils have demonstrated that the fatty-acid profile of the salmon closely reflects that of its diet[13,16,31–42]. It is suggested that in Atlantic salmon, 75 % of dietary fish oil can be replaced by vegetable oil without compromising growth and performance or significantly affecting fish health or welfare if n-3 LC-PUFA requirements are met[19,35,43]. Vegetable oils do not contain any n-3 LC-PUFA, but can contain higher concentrations of SFA (in the case of palm oil), MUFA and n-6 PUFA[44]. Salmon fed replacement vegetable oils have a reduced n-3 LC-PUFA content, which is a reflection of these diets.

It is important to monitor any potential aquafeed oil source for xenobiotics such as dioxins and dioxin-like polychlorinated biphenyls (PCB). Recently, there has been increased interest in the quality and toxicological properties of fish oil as an ingredient in aquafeeds for salmon aquaculture[45–50]. There are considerable health concerns associated with the presence of dioxins and dioxin-like PCB residues in fish oil[45,46]. Concentrations of such residues vary greatly in fish oil sources from around the world with seasonal and/or spatial variations common[45]. Dioxins and PCB are fat-soluble xenobiotics that are carcinogenic to humans[47,51] and are also known to cause skin ailments, liver disease, reproductive disorders and neurological problems[52]. Furthermore, dioxins and PCB are lipophilic and resistant to degradation, and therefore can accumulate in significant concentrations in fish oil. Lastly, dioxins and PCB can persist in the environment for many years and they bioaccumulate up the food chain, with potential harmful effects for the human consumer. Alternative oils such as vegetable or single-cell oils have a very minor possibility of containing dioxins and PCB, making them favourable as replacement oils in aquafeeds[53].

n-*3 and* n-*6 PUFA*

Recently, it has been generally acknowledged that when incorporating alternative lipid sources in aquafeeds, the lipid composition should be targeted to achieve proper fatty acid profiles to meet the requirements of fish and maximise human health benefits[54]. Over the past 20 years evidence has increased that n-3 LC-PUFA, in particular EPA and DHA, have unique nutritional and health benefits to the human consumer[55–64]. As salmon have the ability to store considerable amounts of n-3 LC-PUFA in their flesh, they are considered an excellent source of these key essential fatty acids.

However, excess intake of n-6 PUFA in humans, in particular linoleic acid (18 : 2n-6), has been associated with many disorders including CVD, cancer, and inflammatory and autoimmune disease[65]. It is thought that n-6 PUFA, in particular linoleic acid, is excessive in most Western diets,

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Table 1. Fatty acid profiles (g/100 g) of possible plant or vegetable replacement oils for fish oil

	Palm oil*	Rapeseed oil†	Linseed oil‡	Sunflower-seed oil§	Olive oil‖	Soya oil¶	Echium oil**	Thraustochytrid oil††	Fish oil‡‡
14 : 0	1·2	0·0	0·0	0·0	0·0	0·0	0·0	8·9	6·2
16 : 0	46·7	4·7	6·3	6·4	10·8	9·7	7·5	26·1	16·4
18 : 0	5·2	2·1	5·1	4·1	3·3	3·5	3·6	0·8	3·5
Other SFA	0·3	0·6	0·0	1·5	0·6	0·2	0·0	1·0	1·2
Total SFA	53·5	7·4	11·4	12·0	14·6	13·4	11·2	36·7	27·3
16 : 1*n*-7c	0·0	0·0	0·0	0·0	0·7	0·7	0·0	0·6	8·2
18 : 1*n*-9c	33·8	58·3	18·3	25·3	75·4	22·5	17·2	1·3	21·1
18 : 1*n*-7c	1·3	4·3	1·3	2·0	2·5	1·8	1·0	0·4	3·6
20 : 1*n*-9	0·0	1·1	0·0	0·0	0·0	0·0	0·8	0·1	3·8
Other MUFA	0·0	0·0	0·0	0·0	0·0	0·0	0·9	0·2	6·2
Total MUFA	35·1	63·7	19·6	27·2	78·6	25·0	19·9	2·5	42·9
18 : 3*n*-3	0·0	7·3	53·3	0·0	0·0	6·4	28·1	0·1	0·6
18 : 4*n*-3	0·0	0·0	0·0	0·0	0·0	0·0	11·4	0·4	1·5
20 : 4*n*-3	0·0	0·0	0·0	0·0	0·0	0·0	0·0	1·0	1·0
20 : 5*n*-3	0·0	0·0	0·0	0·0	0·0	0·0	0·0	2·2	12·7
22 : 5*n*-3	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·7	1·7
22 : 6*n*-3	0·0	0·0	0·0	0·0	0·0	0·0	0·0	36·7	7·3
Other *n*-3	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·5
Total *n*-3	0·0	7·3	53·3	0·0	0·0	6·4	39·6	41·1	25·3
18 : 2*n*-6	11·4	21·6	15·7	60·7	6·8	55·2	19·5	0·6	3·4
18 : 3*n*-6	0·0	0·0	0·0	0·0	0·0	0·0	9·8	0·3	0·0
20 : 3*n*-6	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·5	0·0
20 : 4*n*-6	0·0	0·0	0·0	0·0	0·0	0·0	0·0	2·4	1·0
22 : 5*n*-6	0·0	0·0	0·0	0·0	0·0	0·0	0·0	15·8	0·0
Other *n*-6	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·1
Total *n*-6	11·4	21·6	15·7	60·7	6·8	55·2	29·3	19·6	4·5
n-3:*n*-6	0·0	0·3	3·4	0·0	0·0	0·1	1·3	2·1	5·6
Oil price January 2007 ($US/tonne)§§	572	712	759	657	3245	641	4000–8000‖‖	20 000–100 000‖‖	817
Oil price January 2008 ($US/tonne)§§	1039	1569	1475	1645	3894	1174	4000–8000‖‖	20 000–100 000‖‖	1615

* Aoroma, Hallam, Vic, Australia.
† Steric Trading Pty Ltd, Villawood, NSW, Australia.
‡ Melrose Laboratories, Mitcham, Vic, Australia.
§ Meadowlea Foods, Mascot, NSW, Australia.
‖ Island Olive Grove, Cambridge, Tasmania, Australia.
¶ Carolina Soy Product, Warsaw, NC, USA.
** Croda Chemicals, East Yorkshire, UK.
†† Martek, Columbia, MD, USA.
‡‡ From jack mackerel (*Trachurus symmetricus* L.), Skretting Australia, Cambridge, Tasmania, Australia.
§§ Prices taken as an average of January oil prices in 2007 and 2008 from Hamburg market prices, OIL WORLD ISTA Mielke GmbH, Hamburg, Germany.
‖‖ Estimated current costs; however, costs may reduce when the volume of production increases.

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which are dominated by vegetable oils and processed foods. It is increasingly recognised that a greater ratio of *n*-3:*n*-6 PUFA plays a positive role in human health(65–67) and is also important in aquafeeds, because this ratio best represents the natural diets of salmon(25). Eating oily, *n*-3 LC-PUFA-rich fish such as salmon is proposed to be a good way to improve the dietary *n*-3:*n*-6 ratio(68).

Biosynthetic pathway of n-3 *and* n-6 *PUFA*

Atlantic salmon can naturally biosynthesise *n*-3 LC-PUFA from dietary precursors (Fig. 1). Understanding and utilising this biosynthetic capacity through the provision of different precursors may ensure that farmed salmon receive their required *n*-3 LC-PUFA. Salmon lack Δ12 and Δ15 fatty acid desaturases and cannot produce linoleic acid and α-linolenic acid (ALA; 18 : 3*n*-3) from the precursor oleic acid (18 : 1*n*-9)(24). However, salmon can biosynthesise dietary ALA into *n*-3 LC-PUFA(24). Atlantic salmon are anadromous: the adult fish live in the sea, but breed and have their early development stages in fresh water. The ability of Atlantic salmon to biosynthesise *n*-3 LC-PUFA from precursors changes throughout their life cycle(69).

The conversion of ALA to *n*-3 LC-PUFA has been demonstrated in freshwater fish, which have high concentrations of ALA and limited DHA in their natural diet(26,70,71). However, the conversion of ALA to EPA and DHA is inefficient in marine fish, which have high concentrations of LC-PUFA in their natural diet(72). Therefore the evolutionary pressure of fatty-acid availability has affected the ability of fish to biosynthesise *n*-3 LC-PUFA. There have been many studies to determine the capacity of Atlantic salmon to biosynthesise *n*-3 LC-PUFA from precursor dietary fatty acids(36,69,73–81). Salmon have shown a very limited capacity to produce *n*-3 LC-PUFA from ALA in both *in vivo* and *in vitro* trials(36,69,73–75,77–80). However, there has been limited research on other biosynthetic precursors such as stearidonic acid (SDA; 18 : 4*n*-3) in salmon(76,81,82). Understanding the complex interactions between gene expression, synthesis of enzymes (protein expression) and fatty acid composition will give a better understanding of biological responses at the cellular level, including, for example, how Atlantic salmon endogenously produce, use and store *n*-3 LC-PUFA.

Advantages and disadvantages of vegetable- or plant-based oils

The major advantages that vegetable oils have over fish oil as ingredients in aquafeeds are that they are produced in large volumes, are renewable, can be reliably sourced and importantly are currently less expensive. However, the main disadvantage is that they do not contain any *n*-3 LC-PUFA or *n*-6 LC-PUFA and therefore they are, as a sole oil source, unable to meet the nutritional requirements for these components by Atlantic salmon. Vegetable oils generally have high concentrations of oleic acid, linoleic acid and in some instances ALA.

Other disadvantages of terrestrial oils include significant environmental, social and economic issues surrounding their production(83,84) including increasing oil and grain prices due to competing global demand for biofuel, and an associated acceleration in unsustainable or inappropriate land-use practices, for example replacing rain forest with palm oil plantations(83,84).

Nonetheless, aquafeed producers are increasingly using blends of vegetable oils. Blending oils gives the feed producers flexibility to meet dietary nutritional requirements and also allows seasonal adjustment of diets in response to factors such as cost and availability. The major sources of vegetable oils that have been extensively researched for Atlantic salmon include sunflowerseed(16,32), linseed(33–36), rapeseed(34,37–40), soyabean(35,41), olive(32,38) and palm oil(13,32,35,39). The fatty acid profiles of the major replacement oils for aquaculture diets differ greatly (Table 1).

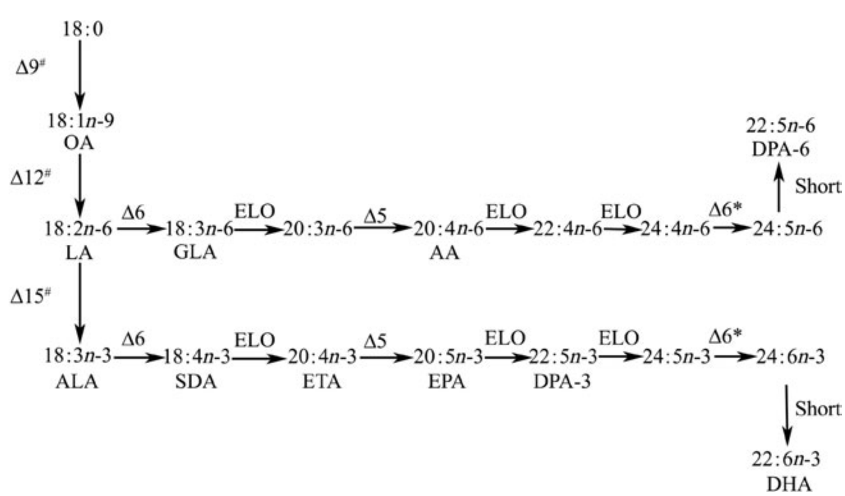

Fig. 1. Representation of the *n*-3 and *n*-6 long-chain (≥ C_{20}) PUFA biosynthetic pathways from their C_{18} fatty acid precursors in Atlantic salmon (*Salmo salar* L.). OA, oleic acid; LA, linoleic acid; Δ5, Δ 6 and Δ6*, fatty acyl desaturases; GLA, γ-linolenic acid; ELO, fatty acyl elongases; AA, arachidonic acid; DPA-6, docosapentaenoic acid (*n*-6); Short, fatty acyl peroxisomal chain shortening; ALA, α-linolenic acid; SDA, stearidonic acid; ETA, eicosatetraenoic acid; DPA-3, docosapentaenoic acid (*n*-3). Δ6* may or may not be the same desaturase enzyme as Δ6. Δ9#, Δ12# and Δ15# are not present in Atlantic salmon. Adapted from Tocher(24).

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The fatty acid profile of vegetable oils can vary with location, season and species. Alongside fatty acid profile, cost and production characteristics of these oils are considerable factors in their selection as ingredients in aquafeeds. Globally, soya and palm are the most abundant oil crops and have the lowest market price. The cheapest oils for aquafeeds in non-European Union countries are poultry and animal fats (lard, $US 930 per tonne, Hamburg market prices, January 2008, OIL WORLD ISTA Mielke GmbH, Hamburg, Germany) in which the fatty acid profiles are dominated by SFA, in particular palmitic acid (16 : 0) and stearic acid (18 : 0). There are significant economic benefits of including poultry and animal fats in aquafeeds, but regulations in Europe following the outbreaks of BSE forbid the use of animal lard in aquafeeds. The rise in vegetable oil prices shown in Table 1, and their predicted increase in the future, is largely attributed to increased use and demand for oil in biofuels[84].

Phytosterols

Minor components of vegetable oil that are not present in the natural diets of fish need to be investigated and monitored. For example, a potential benefit of the increasing replacement of fish oil and meal with plant-based ingredients in salmon aquafeeds is the increased amounts of phytosterols in the fish diet. Phytosterols are naturally occurring molecules found in vegetable oils and meals which are structurally related to cholesterol[85]. Phytosterols are known to affect cholesterol metabolism and have been shown to reduce LDL-cholesterol levels in humans, reducing the risk of CHD[85–87]. Phytosterols are lipophilic and have been introduced to margarines, butters, spreads and breakfast cereals and promoted as ‘functional foods’ to reduce CHD[88–90]. With the increased use of vegetable oils in aquafeeds, increased natural abundances of phytosterols will occur in Atlantic salmon diets. There has only been one investigation of the digestion, accumulation and metabolism of phytosterols in Atlantic salmon when fed replacement diets containing plant-based oils[91]. That study showed that Atlantic salmon were able to digest and accumulate low levels of phytosterols[91]. The low amount of phytosterols available in the diet, coupled with their low digestibility, suggests at this stage that Atlantic salmon are unlikely to be a major delivery source of this cholesterol-lowering agent. However, in combination with the health benefits of n-3 LC-PUFA, phytosterols may act in unison to increase the benefits of eating farmed salmon for the consumer. Minor components of vegetable oils such as phytosterols need to be monitored to determine whether they have positive or negative effects not only for the consumer, but also for fish health.

Regiospecificity of fatty acids

Lipids are important molecules in the body for storing energy and for maintaining cell membrane integrity. Regiospecificity refers to how individual fatty acids are positioned on the glycerol backbone of both storage TAG and membrane polar lipids. Regiospecificity of lipids plays an important role in their function and bioavailability. The bioavailability of n-3 LC-PUFA is of vital importance if Atlantic salmon is to be marketed as a good source of n-3. Regiospecific characteristics of dietary and possibly endogenously biosynthesised n-3 LC-PUFA stored as TAG by Atlantic salmon is yet to be understood fully by fish nutritionists. Therefore, it is important to assess the regiospecificity of novel sources of n-3 LC-PUFA oil. Traditional profiling of lipid class and fatty acid composition provides important information, but does not reveal the regiospecific nature of the molecules, which can play a key role in their function[92,93]. With advances in analytical and computing facilities, new techniques and methods can be used to examine lipids with the emphasis on the regiospecific distribution of fatty acids.

The middle *sn*-2 position of the storage TAG molecule is thought to be the most bioavailable position for the digestion of particular fatty acids. This was first shown in fat absorption by infants fed breast or formulated milk[94]. How Atlantic salmon regiospecifically store n-3 LC-PUFA has the potential to affect the bioavailability of fatty acids for the human consumer. Structural lipids, such as polar lipids, are important components of cell membrane structure. The composition of the molecular species in the cell membrane can be influenced by many factors including temperature and diet[95]. The composition of cell membranes has a major effect on the health of the cell and therefore the health of the fish. To date, limited research has been conducted to identify changes in the regiospecific composition of membrane lipids and TAG in Atlantic salmon as a result of changes in the fatty acid profile of their diet[96]. Regiospecific analysis of the lipid profiles of Atlantic salmon has shown recently that DHA has a high affinity for the *sn*-2 (middle) position in both the TAG and polar lipid fractions[97].

Influence of temperature on lipids

Temperature has a major influence on the membrane and storage lipids of exothermic animals, such as Atlantic salmon, which need to adapt to seasonal and occasionally abrupt changes in environmental temperature[72,98–100]. The Tasmanian Atlantic salmon industry commonly encounters temperatures (over 19°C) that approach the upper threshold for salmon survival[31]. A possible outcome of climate change is increased sea temperature, which may affect aquaculture not only in Tasmania but worldwide. Replacement oil blends may assist by adapting the fatty acid profiles of salmon diets to meet the different nutritional requirements for raised temperatures.

Fish may exploit the structural diversity of lipids within their membranes to adapt to change in ambient water temperature. Membrane lipids may adapt in several ways to a change in temperature: by altering the unsaturation and chain-length of the fatty acids[101]; by changing the distribution of fatty acids within the phospholipid molecules[102,103]; by altering the composition of the polar head group of the phospholipids[104,105]. In general, colder temperatures lead to an increase in unsaturation in gill lipids, thus maintaining membrane fluidity[103,106]. Most studies have investigated temperature influence on Atlantic salmon lipids within the range of 2–12°C[41,107–109], but only one has examined salmon at higher temperatures such

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as 19°C[97]. An increased water temperature of 19°C resulted in adaptation of both structural and storage lipids with significant reduction in PUFA occurring, in particular in the tissue concentrations of EPA[97]. As water temperatures rise, there is reduced need for high levels of *n*-3 LC-PUFA in polar lipids to maintain optimal cell membrane function. The converse is true for decreasing water temperatures. This suggests that changes in Atlantic salmon diets, in particular the amount of *n*-3 LC-PUFA, during periods of high water temperature may maintain the health and performance of fish. With global water temperatures increasing it is pertinent to monitor membrane structure and oil storage in salmon at higher temperatures.

Temperature plays a significant role in the digestibility of lipids in salmon diets[108,110–112]. Reduced water temperature decreases the digestibility of SFA and therefore changing the dietary source of oil may have implications during winter conditions. Highly saturated oils, such as palm, have been demonstrated to have reduced digestibility at very low water temperature; however, the digestibility of MUFA and PUFA was not affected[110]. The fatty acid profile of novel sources of oil needs to be considered, in regard to digestibility, when formulating diets.

Potential sources of n*-3 long-chain PUFA for aquaculture*

Other than traditional fish oil sources, the current and future possible sources of oil that contain beneficial essential fatty acids or their precursors are:

(1) Other marine sources including by-catch and marine invertebrates such as krill and copepods;
(2) Vegetable oils that contain biosynthetic precursors that can be used by Atlantic salmon to biosynthesise *n*-3 LC-PUFA;
(3) Several different microbial taxa at the base of the marine food chain that produce single-cell oils that are rich in *n*-3 LC-PUFA;
(4) GM organisms including single-celled micro-organisms and terrestrial plants that have undergone genetic modification and contain enriched amounts of *n*-3 LC-PUFA.

Each of these sources of oil will be discussed in turn.

Other marine sources

Improving our seafood processing byproducts as well as an increased use of by-catch may create a possible source of *n*-3 LC-PUFA oil for aquafeeds. Seafood processing byproducts exist in large quantities (over 30 % of processed seafood is inedible) and can contain high levels of LC *n*-3 oils, including EPA and DHA. Therefore, byproducts from seafood processing operations could supply, in part, the *n*-3 LC-PUFA required for aquaculture. However, before the use of recycled oil and meal from seafood processing can occur, several factors need to be assessed. These include understanding the risk of prions and other disease transmission vectors associated with feeding byproducts from one species back to the same or similar species[113]. As previously stated, regulations in Europe forbid the use of land animal products in aquafeeds. It is yet to be determined whether the benefits (ecological and economic) outweigh the risks (human health) of using various types of seafood byproducts as feed. Furthermore, contaminants in oil from seafood byproducts, such as PCB and dioxins, have the potential to bioaccumulate in farmed fish[45,46]. Careful monitoring of these contaminants in seafood byproducts and oil derived from them is needed. Finally, the socio-economic and environmental aspects of the use of seafood byproducts need to be assessed before they can be used in aquafeeds.

Other marine sources, such as marine invertebrates, may be a future source of *n*-3 LC-PUFA oil. Southern Ocean krill (*Euphausia superba*) biomass is estimated at up to 700 million metric tonnes, and the current regulatory catch quota is almost 6 million metric tonnes[114,115]. However, due to factors such as the remoteness of the fishery, the current catch is only 0·2 million metric tonnes[116]. Krill contain oil which has high concentrations of EPA and DHA plus high levels of phospholipids and antioxidants such as the carotenoid, astaxanthin[115,117]. The fatty acid profile of krill oil can vary markedly with the region and time of year of harvest, with other factors also influencing its profile[118,119]. Considerable care will be required with management practices in the setting of local catch limits for krill harvest to protect not only this sensitive species, but also key Southern Ocean predators. Krill is at the base of the Southern Ocean's food chain, and is also particularly sensitive to environmental changes including climate change[116,120]. Over-fishing of krill, particularly concentrated fishing efforts in localised regions, could severely undermine the food web and devastate marine life[120]. As such, increasing fishing pressure for krill is worrisome and the catch and ecological consequences should be closely monitored.

Zooplankton, specifically copepods, may also provide a minor alternative source of *n*-3 LC-PUFA oil. The commercial harvesting of wild copepods is not expected to meet the demand, quality and constant supply of *n*-3 LC-PUFA[121]. Cultured copepods may be a future source of feed including oil; however, the scale of production to supply aquaculture's growing demand for quality *n*-3 LC-PUFA-rich oil is beyond its scope[121]. However, copepod oil, more likely the total biomass, may provide niche products for segments of the aquaculture industry in particular in larval rearing[121–123].

Biosynthetic precursors of n*-3 long-chain PUFA*

As mentioned above, fish have an endogenous capacity via fatty acid desaturase and elongase enzymes to biosynthesise *n*-3 LC-PUFA from ALA. The pathways are quantitatively important in freshwater fish, but activity levels are very low in marine species[24,69]. Understanding and utilising biosynthetic precursors further along the LC *n*-3 pathway may provide renewable and sustainable options from the use of specialised vegetable oils for future aquafeeds. Plants such as Patterson's curse (*Echium plantagineum* L.) and blackcurrant (*Ribes nigrum* L.) have a Δ^6 desaturase gene that produces the *n*-3 LC-PUFA biosynthetic precursor SDA from ALA. Echium oil from Patterson's curse has an SDA level $>10\%$ depending on the strain (Table 1). It has been suggested that the Δ^6 desaturation of ALA to SDA is the limiting step in the biosynthetic pathway of *n*-3 LC-PUFA[124]. In this case, dietary SDA bypasses the initial rate-limiting Δ^6 desaturase step in the *n*-3 LC-PUFA biosynthetic pathway (Fig. 1) and potentially enables greater biosynthesis of EPA and DHA via non-limiting steps. For this to occur, subsequent desaturase enzymes must also be present in large enough amounts.

It has been recently shown that freshwater Atlantic salmon parr can maintain concentrations of *n*-3 LC-PUFA, in particular EPA and DHA, in muscle tissue over a 6-week period when fed a diet containing SDA, but with only trace levels of *n*-3 LC-PUFA[81]. This result indicated that SDA-rich aquafeeds may have potential as an alternative to replace *n*-3 LC-PUFA sources such as fish oil in freshwater aquaculture; however, this was only a short trial and occurred over the period before smolting, which has been shown to coincide with a period of peak *n*-3 LC-PUFA production[69]. Smolting involves a series of morphological, physiological and behavioural changes which include both increased lipid deposition and increased accumulation of LC-PUFA, in particular arachidonic acid, before saltwater transfer[125,126]. During this freshwater parr stage, the use of dietary precursor (SDA) oils such as Echium oil may prove most beneficial in aquafeeds.

Further research on Atlantic salmon smolt fed SDA demonstrated an up-regulated expression of genes involved in fatty acid synthesis, which affected the concentration of the direct biosynthetic product (eicosatetraenoic acid; 20 : 4 *n*-3) in all tissues[127]. However, the increased gene expression with use of the SDA-rich diet is not enough to maintain concentrations of *n*-3 LC-PUFA in seawater Atlantic salmon fed SDA at equivalent amounts to those in fish fed with fish oil[127]. Results with Atlantic salmon smolt are analogous with other species of fish, the marine Atlantic cod (*Gadus morhua* L.) and a salmonid species the Arctic charr (*Salvelinus alpinus* L.), that have shown SDA conversion to eicosatetraenoic acid (20 : 4*n*-3), but not to EPA and DHA[128,129]. Selective breeding programmes with Atlantic salmon and other salmonoids have focused on characteristics such as health and growth requirements, but increasingly contain product quality factors such as flesh colour, fat content and fat distribution[130]. In the future, selection traits may be widened to include enhanced biosynthesis of *n*-3 LC-PUFA and/or an ability to store large amounts of the *n*-3 LC-PUFA-rich oil in the fillet. Family lines of salmon with an elevated ability for *n*-3 LC-PUFA biosynthesis and/or storage may lead to a reduced need for the provision of dietary *n*-3 LC-PUFA.

The cost and availability of an SDA-rich oil source, such as Echium oil, is at present not economically viable for aquaculture. The current price of SDA-rich oil from Patterson's curse is 2·5–5 times the price of fish oil (Table 1). In Australia, Patterson's curse is an introduced pest species and considered a noxious weed. Despite a significant proportion of southern Australian agricultural land being covered by Patterson's curse, there are presently no companies in Australia looking to use this resource for its oil content. However, the amount of oil available to be extracted from Patterson's curse is minor compared with commercial oil crops. The only commercially viable source of SDA-rich oil may be through the genetic modification of oilseed crops (see below). Furthermore, the ability of salmon and or other species to digest, accumulate and biosynthesise SDA into longer *n*-3 LC-PUFA needs to be further assessed before it can be considered as a dietary ingredient for aquafeeds.

Single-cell oils

Single-cell oils provide a novel and renewable source of essential fatty acids, in particular EPA and DHA. Single-cell organisms, including thraustochytrids, diatoms, other microalgae and some marine bacteria are the *n*-3 LC-PUFA 'biofactories' of the ocean. Thraustochytrids are heterotrophic protists, commonly found in marine and other saline environments; they can be detritivores, bacterivores and/or parasites[131]. Originally thought to be closely related to primitive fungi, thraustochytrids have more recently been assigned to the subclass Thraustochytridea (Chromista, Heterokonta), aligning them with heterokont algae such as diatoms and brown algae[132]. Thraustochytrids can produce a number of *n*-3 LC-PUFA, especially DHA. Thraustochytrids show potential as a source of oil for aquaculture[31,131,133–136]. Large-scale culture of thraustochytrids may be suitable for commercial aquafeeds as they produce a relatively high biomass and have a high percentage of *n*-3 LC-PUFA-rich lipid[131]. Optimising strain selection and growth conditions can provide single-cell oils with specific qualities such as high DHA (concentrations up to 60 %), low *n*-6 (in particular docosapentaenoic acid, DPA-6, 22 : 5*n*-6), high total *n*-3 LC-PUFA and high *n*-3:*n*-6 ratios[137]. Thraustochytrid biomass (for example, the product Algamac) is already being used commercially as an enriched feed for rotifers (*Brachionus* spp.) and brine shrimp (*Artemia*) before feeding these live feeds to finfish larvae[133–135] and as a fish oil replacement in Atlantic salmon nutrition trials[31,136]. The replacement of fish oil with 100 % thraustochytrid oil in Atlantic salmon parr diets has been demonstrated, without any detriment to growth, to significantly increase the concentrations of DHA in muscle tissue[136]. Thraustochytrid oil (Table 1) from the species *Schizochytrium* L., has a high concentration of DHA (35 %).

Single-cell oils, such as oil from thraustochytrids, provide a major renewable source of *n*-3 LC-PUFA for aquaculture. These organisms could potentially provide a source of *n*-3 LC-PUFA without any foreseeable negative impact on wild fish stocks. Currently, manufacturers of single-cell oils do not have sufficient production capacity and the oils are viewed as too expensive to be considered for use as a replacement oil in diets for Atlantic salmon, although the use of thraustochytrid biomass is now thought to be economically feasible for prawns (Browdy *et al.*[138]; B Bullis, personal communication). There is still considerable scope for the discovery or development of novel strains with other advantageous properties including high *n*-3 LC-PUFA concentrations. With the continual discovery of new strains and improvements in fermentation and molecular engineering techniques, it may be possible to produce single-cell oils or biomass with sufficient amounts of *n*-3 LC-PUFA and at a price suitable to meet the growing demand in aquaculture. It is likely that increased use in biomedical fields will cover the high initial cost of this biotechnology allowing

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aquaculture to access it when production capacity is increased and, importantly, as the price is reduced. Single-cell biomass rather than extracted oil is a logical candidate for aquafeeds as it provides *n*-3 LC-PUFA-rich oil with accompanying marine proteins at a significantly lower price due to a reduction of the processing costs.

Genetic modification of oils

Transgenic oilseed crops and micro-organisms that are engineered to produce the major *n*-3 LC-PUFA by the insertion of various genes encoding desaturases and/or elongases have been suggested as a source of *n*-3 LC-PUFA[139,140]. However, the requirement for coordinate expression and activity of five or more new enzymes encoded by genes from possibly diverse sources has made this goal difficult to achieve and only low yields have generally been obtained[139,141,142].

A gene encoding the Δ^6 desaturase isolated from borage (*Borago officinalis*) was expressed in transgenic tobacco and *Arabidopsis*, resulting in the production of γ-linolenic acid and SDA, the direct precursors of LC-PUFA[143,144]. This initial research provided only the first step to *n*-3 LC-PUFA, but may provide a renewable source of SDA for aquaculture and other uses. Recently more genes encoding the whole pathway have produced EPA[145] and DHA[139], in crops or model plants, including oilseeds. In the model plant *Arabidopsis*, the insertion of five genes resulted in the first oil with DHA[139]. That study observed a total LC-PUFA content (arachidonic acid + EPA + DHA) of 4·2 %. This has subsequently been increased to close to 8 % (SP Singh, SS Robert, XR Zhou, JR Petrie, SI Blackburn, PM Mansour, PD Nichols and Q Liu, unpublished results). Further research using different genes and seed-specific promoters with soyabean has produced an EPA content of 19·5 % and a DHA content of 3·3 %[146]. These two examples demonstrate the complicated nature of engineering multi-genes to produce a sustainable land plant source of *n*-3 LC-PUFA-rich oil.

Nonetheless, GM plants may in the future provide the most economically viable source of *n*-3 LC-PUFA-rich oil for aquaculture. It is estimated that the cost and availability of oils from GM plants would be similar to that of currently available commercial oilseed crops such as rapeseed and soya. Research in this area has the potential for significant commercial, health, social and environmental benefits. However, consumer and industry acceptance of this biotechnology and the requirement for passing health and safety requirements set by regulatory bodies will be needed for oils from GM plants before they can be used by the aquaculture industry. Recent assessments of perceived consumer acceptance of GM land-plant *n*-3 LC-PUFA technologies in Australia and the USA have reported that farmed fish were a preferred delivery mechanism compared with capsules or functional foods[147,148]. Ultimately, as demand for fish oil intensifies, as knowledge about fishing impacts and benefits to human health of *n*-3 LC-PUFA increases, and the potential for salmon prices to decrease occurs due to reduced ingredient cost, consumers may eventually accept oil from a GM crop as an ingredient of aquafeeds.

Future security of n*-3 long-chain PUFA oils and sustainable aquaculture*

Having access to secure sources of *n*-3 LC-PUFA-rich oils is vital for the continued sustainability and growth of the intensive aquaculture industry. The future use of these *n*-3 LC-PUFA-rich oils will depend on the cost and availability of fish oil as a commodity. It is yet unknown whether a premium *n*-3 oil crop (to include *n*-3 LC-PUFA via GM or increased *n*-3:*n*-6 ratio via selective breeding and management methods) would be economically viable. However, as fish oil and grain prices rise, alternative sources of *n*-3 LC-PUFA-rich oils are becoming more financially feasible. Single-cell biomass with high amounts of *n*-3 LC-PUFA will provide an option, but the current high production cost limits their immediate use. New *n*-3 LC-PUFA oils from GM land plants are still in development, with trials including field planting, fish and animal feeding, toxicity and other assessments required before the large-scale consideration of their use by aquafeed companies.

In summary, the short-term forecast for aquafeeds rests in the careful management of the use of fish oil from wild fisheries, the use of vegetable and other oils in blends, and the use of suitable feeding regimens including finishing diets containing higher levels of the *n*-3 LC-PUFA oils than in the longer grow-out phase. These changing and developing practices will be continuously evaluated as new technologies become available to prevent impacts on wild fish stocks, and to increase resource security and market feasibility. Aquaculture will need to increase efforts to find new sources of *n*-3 LC-PUFA, in particular for intensive rearing of marine carnivorous species. Whether replacement sources will be derived from single-cell biomass, from oil from GM land plants, or a combination of the two is yet to be determined, but will ultimately depend on scientific developments, social acceptance, community needs and governmental policy.

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