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(54) **FEEDSTUFFS FOR AQUACULTURE COMPRISING STEARIDONIC ACID FEEDSTUFFS FOR AQUACULTURE**

FUTTERMITTEL FÜR DIE AQUAKULTUR, DIE STEARIDONSÄURE FUTTERMITTEL FÜR DIE AQUAKULTUR ENTHALTEN

ALIMENTS POUR AQUACULTURE COMPRENANT DES ALIMENTS À BASE D'ACIDE STÉARIDONIQUE POUR AQUACULTURE

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Description**FIELD OF THE INVENTION**

5 **[0001]** The present invention relates to feedstuffs for use in aquaculture, as well as methods for producing said feedstuffs. The invention also provides methods for rearing salmon.

BACKGROUND OF THE INVENTION

10 **[0002]** Global production of farmed fish and crustacea has more than doubled in the last 15 years and its expansion places an increasing demand on global supplies of wild fish harvested to provide protein and oil as ingredients for aquafeeds (Naylor et al., 2000). The supply of seafood from global capture fisheries sources is around 100 million tones per annum (FAO, 2001). This amount has not increased since the mid-1980's and will not increase in the future as most fisheries are at or above sustainable levels of production, and are further subjected to sharp, periodic declines, due to climatic factors such as El Niño (FAO, 2001; Barlow 2000). Fish oil stocks are also under increasing demand not only from aquaculture, but from the agriculture and nutraceutical/biomedical industries.

15 **[0003]** Replacement oils for the aquaculture industry have been sourced from a variety of commercial terrestrial plant sources including sunflower (Bransden et al, 2003; Bell et al., 1993), canola/rapeseed (Bell et al, 2003; Polvi and Ackman, 1992), olive, palm (Fonseca-Madriral et al, 2005; Bell et al, 2002) and linseed (Bell et al., 1993; Bell et al., 2004). The inclusion of vegetable oil to replace part or all of the fish oil in fish diets resulted in the same growth rates and feed conversion ratios (Bransden et al., 2003; Polvi and Ackman, 1992; Torstensen et al., 2004; Fonseca-Magriral et al., 2005; Bell et al., 2002; Bell et al., 2004). However, since these plant oils had essentially no ω 3 long-chain (\geq C20) polyunsaturated fatty acids (ω 3 LC-PUFA) and had high levels of monounsaturated fatty acids (MUFA), ω 6 PUFA and low ω 3/ ω 6 ratios, fish fed such diets displayed reduced levels of ω 3 LC-PUFA. This is thought to be associated with reduced health benefits to the consumer compared to fish fed a diet high in fish oil containing greater levels of ω 3 LC-PUFA (Seierstad et al., 2005). Therefore, raising salmon on diets high in vegetable oil has the potential to dilute the important cardiovascular and other benefits which are associated with eating fish.

Pathways of LC-PUFA synthesis

30 **[0004]** Biosynthesis of LC-PUFA from linoleic and α -linolenic fatty acids in organisms such as microalgae, mosses and fungi may occur by a series of alternating oxygen-dependent desaturations and elongation reactions as shown schematically in Figure 1. In one pathway (Figure 1, II), the desaturation reactions are catalysed by Δ 6, Δ 5, and A4 desaturases, each of which adds an additional double bond into the fatty acid carbon chain, while each of a Δ 6 and a Δ 5 elongase reaction adds a two-carbon unit to lengthen the chain. The conversion of ALA to DHA in these organisms therefore requires three desaturations and two elongations. Genes encoding the enzymes required for the production of DHA in this aerobic pathway have been cloned from various microorganisms and lower plants including microalgae, mosses, fungi.

35 **[0005]** Alternative routes have been shown to exist for two sections of the ALA to DHA pathway in some groups of organisms. The conversion of ALA to ETA may be carried out by a combination of a Δ 9 elongase and a Δ 8 desaturase (the so-called Δ 8 desaturation route, see Figure 1, IV) in certain protists and thraustochytrids, as evidenced by the isolated of genes encoding such enzymes (Wallis and Browse, 1999; Qi et al., 2002). In mammals, the so-called "Sprecher" pathway converts DPA to DHA by three reactions, independent of a Δ 4 desaturase (Sprecher et al., 1995).

40 **[0006]** Besides these desaturase/elongase systems, EPA and DHA can also be synthesized through an anaerobic pathway in a number of organisms such as *Shewanella*, *Mortiella* and *Schizochytrium* (Abadi et al., 2001). The operons encoding these polyketide synthase (PKS) enzyme complexes have been cloned from some bacteria (Morita et al., 2000; Metz et al., 2001; Tanaka et al., 1999; Yazawa, 1996; Yu et al., 2000; WO 00/42195). The EPA PKS operon isolated from *Shewanella spp* has been expressed in *Synechococcus* allowing it to synthesize EPA (Takeyama et al., 1997). The genes encoding these enzymes are arranged in relatively large operons, and their expression in transgenic plants has not been reported. Therefore it remains to be seen if the anaerobic PKS-like system is a possible alternative to the more classic aerobic desaturase/elongase for the transgenic synthesis of LC-PUFA.

45 **[0007]** The biosynthetic pathways for PUFA are well known (Sargent et al., 2002). Vertebrates lack ω 12 and ω 15 (ω 3) lipid desaturases and cannot produce linoleic acid (18:2 ω 6, LA) and α -linolenic acid (18:3 ω 3, ALA) from oleic acid (18:1 ω 9, OA) (see Figure 1). The conversion from ALA to eicosapentaenoic acid (20:5 ω 3, EPA) and docosahexaenoic acid (22:6 ω 3, DHA) is inefficient in marine fish, which have high levels of LC-PUFA in their natural diet, but is greater in freshwater fish, which have high levels of LA and ALA and limited DHA in their natural diet. High levels of ω 3 LC-PUFA, which are found in salmon, cannot be biosynthesised from ALA and LA and therefore must be provided to the fish in their diet.

Desaturases

[0008] The desaturase enzymes that have been shown to participate in LC-PUFA biosynthesis all belong to the group of so-called "front-end" desaturases which are characterised by the presence of a cytochrome b_5 domain at the N-terminus of each protein. The $\text{cyt } b_5$ domain presumably acts as a receptor of electrons required for desaturation (Sperling and Heinz, 2001). The enzyme $\Delta 6$ desaturase catalyses the desaturation of linoleic acid (LA) to form gamma-linoleic acid (GLA, 18:3 $\omega 6$) and linolenic acid (ALA) to form stearidonic acid (SDA, 18:4 $\omega 3$) (Figure 1). Genes encoding this enzyme have been isolated from a number of organisms, including plants, mammals, nematodes, fungi and marine microalgae. The C18 fatty acid substrate for $\Delta 6$ desaturases from plants, fungi and microalgae has desaturation in at least the $\Delta 9$ and $\Delta 12$ positions and is generally covalently linked to a phosphatidylcholine headgroup (acyl-PC).

[0009] The enzyme $\Delta 5$ desaturase catalyses the desaturation of C20 LC-PUFA leading to arachidonic acid (ARA, 20:4 $\omega 6$) and EPA (20:5 $\omega 3$). Genes encoding this enzyme have been isolated from a number of organisms, including algae (*Thraustochytrium* sp. Qiu et al., 2001), fungi (*M. alpine*, *Pythium irregulare*, Michaelson et al., 1998; Hong et al., 2002), *Caenorhabditis elegans* and mammals. A gene encoding a bifunctional $\Delta 5$ - $\Delta 6$ -desaturase has also been identified from zebrafish (Hasting et al., 2001). The gene encoding this enzyme might represent an ancestral form of the "front-end desaturase" which later duplicated and evolved distinct functions.

[0010] The last desaturation step to produce DHA is catalysed by a $\Delta 4$ desaturase and a gene encoding this enzyme has been isolated from the freshwater protist species *Euglena gracilis* and the marine species *Thraustochytrium* sp. (Qiu et al., 2001; Meyer et al., 2003).

Elongases

[0011] Several genes encoding PUFA-elongation enzymes have also been isolated (Sayanova and Napier, 2004). The members of this gene family were unrelated to the elongase genes present in higher plants, such as FAE1 of *Arabidopsis*, that are involved in the extension of saturated and monounsaturated fatty acids. An example of the latter is erucic acid (22:1) in *Brassicacae*. In some protist species, LC-PUFA are synthesized by elongation of linoleic or α -linolenic acid with a C2 unit, before desaturation with $\Delta 8$ desaturase (Figure 1 part IV; "A8-desaturation" pathway). $\Delta 6$ desaturase and $\Delta 6$ elongase activities were not detected in these species. Instead, a $\Delta 9$ -elongase activity would be expected in such organisms, and in support of this, a C18 $\Delta 9$ -elongase gene has recently been isolated from *Isochrysis galbana* (Qi et al., 2002).

Transgenic Plants

[0012] Transgenic oilseed crops that are engineered to produce major LC-PUFA by the insertion of various genes encoding desaturases and/or elongases have been suggested as a sustainable source of nutritionally important fatty acids. However, the requirement for coordinate expression and activity of five new enzymes encoded by genes from possibly diverse sources has made this goal difficult to achieve and only low yields have generally been obtained (reviewed by Sayanova and Napier, 2004; Drexler et al., 2003; Abadi et al., 2001).

[0013] A gene encoding a $\Delta 6$ -fatty acid desaturase isolated from borage (*Borago officinalis*) was expressed in transgenic tobacco and *Arabidopsis*, resulting in the production of GLA (18:3 $\omega 6$) and SDA (18:4 $\omega 3$), the direct precursors for LC-PUFA, in the transgenic plants (Sayanova et al., 1997 and 1999). However, this provides only a single, first step.

Feedstuffs for Aquaculture

[0014] Research in feedstuffs for aquaculture have largely focused on enriching salmon diets by increasing the dietary supply of ALA (Bell et al., 1993) and EPA/DHA (Harel et al., 2002; Carter et al., 2003).

[0015] There is a need for further diets for aquaculture which, upon consumption, enhance the production of omega-3 long chain polyunsaturated fatty acids in aquatic animals.

SUMMARY OF THE INVENTION

[0016] The present inventors have determined that salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., can be produced with appropriate levels of LC-PUFA, such as EPA, DPA and/or DHA, without the need to feed these organisms diets which are rich in LC-PUFA. In particular, the LC-PUFA precursor stearidonic acid (SDA) can be provided to the salmon, which is a *Salmo* sp. or *Oncorhynchus* sp. whilst still producing fish or crustaceans with desirable levels of LC-PUFA.

[0017] Thus, in a first aspect, the present invention provides a method of rearing a salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., the method comprising feeding the salmon a feedstuff comprising lipid, the fatty acid of said lipid comprising at least 5.5% (w/w) stearidonic acid (SDA).

[0018] In a preferred embodiment, the lipid comprises a phytosterol.

[0019] In a particularly preferred embodiment, at least 1% of the SDA in the feedstuff was obtained from a plant. The plant may be non-transgenic, such as an *Echium* sp., *Oenothera biennis*, *Borago officinalis* or *Ribes nigrum*, or transgenic. In an embodiment, at least some of the SDA is from oil obtained from seed of the plant.

[0020] In a preferred embodiment, the transgenic plant comprises an exogenous nucleic acid encoding a $\Delta 6$ desaturase. The transgenic plant may further comprise an exogenous nucleic acid encoding a $\omega 3$ desaturase or $\Delta 15$ desaturase, which increases the production of ALA in the plant. The transgenic plant may further comprise an exogenous nucleic acid encoding a $\Delta 12$ desaturase. Examples of suitable transgenic plants include, but are not limited to, canola, soybean, flax, other oilseed plants, cereals or grain legumes.

[0021] The salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., is fed predominantly the feedstuff over a period of at least 6 weeks, preferably at least 7 weeks and even more preferably at least 12 weeks. In an embodiment, after having been fed the feedstuff for at least 6 weeks, the salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., has similar weight, specific growth rate, weight gain, total feed consumption, feed efficiency ratio, hepatosomatic index and/or survival when compared with the same species of salmon fed the same feedstuff but which substantially lacks SDA.

[0022] The salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., after having been fed the feedstuff for at least 6 weeks, has higher SDA and ETA levels in muscle tissue when compared with the same species of salmon fed the same feedstuff but which substantially lacks SDA.

[0023] In a further embodiment, the salmon, after having been fed the feedstuff for at least 6 weeks, has lower SFA levels in muscle tissue when compared with the same species of salmon fed the same feedstuff but which comprises fish oil instead of the plant oil, comprising at least 5.5% SDA. In preferred embodiments, the levels of 14:0 and 16:0 are reduced, for example by at least 10% or at least 20%.

[0024] In another aspect, the present invention provides a feedstuff for a salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., the feedstuff comprising lipid, the fatty acid of said lipid comprising at least 11% (w/w) stearidonic acid (SDA, 18:4 $\Delta 6,9,12,15, \omega 3$). The feedstuff may have any of the characteristics as described herein in the context of the methods.

[0025] In a further aspect, the present invention provides a salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., produced using a method of the invention.

[0026] In yet another aspect, the present invention provides a salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., wherein the fatty acid of the white muscle lipid of said salmon comprises less than 29.6% SFA and at least 18.3% DHA. In certain embodiments, the white muscle lipid of said salmon comprises fatty acid comprising less than 28%, less than 27%, or more preferably less than 26% SFA. In other embodiments, the white muscle lipid of said salmon comprises fatty acid comprising at least 19%, at least 20%, at least 21%, or more preferably at least 22% DHA.

[0027] Disclosed herein is a fish, wherein the fatty acid of the red muscle lipid of said fish comprises fatty acid comprising less than 28.2% SFA and at least 9.6% DHA. As disclosed herein, the red muscle lipid of the fish may comprise fatty acid comprising less than 27%, less than 26%, or more preferably less than 25% SFA. The muscle lipid of the fish may comprise fatty acid comprising at least 10%, at least 11%, or more preferably at least 12% DHA.

[0028] In a further aspect, the present invention provides a salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., wherein the fatty acid of the white muscle lipid of said salmon comprises at least 2.7% SDA. In embodiments of this aspect, the white muscle lipid of said fish or crustacean comprises at least 3%, at least 3.5%, or more preferably at least 4% SDA.

[0029] In a further aspect, the present invention provides a salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., wherein the fatty acid of the white muscle lipid of said salmon comprises at least 2.7% SDA. In embodiments of this aspect, the white muscle lipid of said salmon comprises at least 3%, or more preferably at least 3.5% SDA.

[0030] In yet a further aspect, the present invention provides a method for producing a feedstuff for salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., according to claim 12. Other ingredients may include vitamins, minerals, choline, or pigments such as, for example, carotenoids or carophyll pink.

[0031] Preferably, the plant is transgenic.

[0032] Preferably, the oil is obtained from the seed of the plant.

[0033] In certain embodiments, it is preferred that the fatty acid of the lipid of the feedstuff comprises at least 15%, at least 20%, or at least 30% (w/w) SDA.

[0034] In another aspect, the present invention provides a method for producing a feedstuff for salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., the method comprising admixing a transgenic organism, or extract or portion thereof, with at least one other ingredient, wherein the organism is genetically modified such that it produces SDA and/or produces higher levels of SDA than when compared to a corresponding non-transgenic wild-type organism. The method may comprise the step of extracting the oil from the organism, for example from the seed of a plant. The extraction may comprise physical means such as crushing of seed, chemical means such as extraction with a solvent, heating or other processes, or any combination of these. The oil may be further purified before mixing with other ingredients. The method preferably includes preparation of an extruded product from the mixed ingredients by an extrusion process, suitable for providing to salmon, which is a *Salmo* sp. or *Oncorhynchus* sp.,. The method may comprise the step of analysing the feedstuff, such as for example measuring the level of lipid or the level of SDA in the fatty acid, or other measurements.

[0035] Preferably, the organism is a plant or yeast.

[0036] In another aspect, the present invention provides a feedstuff produced using a method of the invention. The feedstuff may have the characteristics as described above. Other ingredients that may be included in the feedstuff include fish meal, a high protein source other than fishmeal, a starch source, vitamins, minerals, pigments such as, for example, carotenoids or carophyll pink, or any combination of these. Fishmeal is a preferred protein source for the major carnivorous fish such as salmon, trout, tuna, flatfish, barramundi, particularly for Atlantic salmon. Fishmeal, typically about 65% protein, may be added in an amount from 20 to 700g per kg dryweight. A high protein source other than fishmeal may be from a plant or animal source such as, for example, wheat or other cereal gluten, soymeal, meal from other legumes, casein, protein concentrates, protein isolates, meat, meat and bone, blood, feathers. These are typically at least 30% protein and may be milled with or without extraction of oil. Starch may be added, typically at 10-150 g/kg, and may be in the form of cereal grain or meal. For crustaceans, krill meal, mussel meal or other similar components may be added at 1-200g/kg, cholesterol and/or lecithin at 0-100 g/kg. The mixture may comprise a binding agent such as sodium alginate, for example Manuacol from Kelco International.

[0037] Furthermore, the present inventors have found that expressing a $\Delta 6$ desaturase gene in a fibre producing plant results in surprisingly high levels of $\Delta 6$ desaturase PUFA products.

[0038] As will be apparent, preferred features and characteristics of one aspect of the invention are applicable to many other aspects of the invention.

[0039] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0040] The invention is hereinafter described by way of the following non-limiting Examples and with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

[0041]

Figure 1. Possible pathways of $\omega 3$ and $\omega 6$ LC-PUFA synthesis. The sectors labelled I, II, III, and IV correspond to the $\omega 6$ ($\Delta 6$), $\omega 3$ ($\Delta 6$), $\omega 6$ ($\Delta 8$), and $\omega 3$ ($\Delta 8$) pathways, respectively. Compounds in sectors I and III are $\omega 6$ compounds, while those in sectors II and IV are $\omega 3$ compounds. "Des" refers to desaturase steps in the pathway catalysed by desaturases as indicated, while "Elo" refers to elongase steps catalysed by elongases as indicated. The thickened arrow indicates the $\Delta 5$ elongase step. The dashed arrows indicate the steps in the "Sprecher" pathway that operates in mammalian cells for the production of DHA from DPA.

Figure 2. Schematic representation (not part of the invention) of the construct, pVLin-Ed6, used to transform flax. RB, right border of T-DNA; HPT+Cat-1, hygromycin resistance gene interrupted by Cat-1 intron; 35SP, Cauliflower mosaic virus 35S promoter; LinT, Linin terminator; ED6, full length coding sequence of $\Delta 6$ fatty acid desaturase from *Echium*; LinP, linin promoter; LB, left border of T-DNA. P, *Pst*I; A, *Apal*; X, *Xho*I; N, *Not*I.

KEY TO THE SEQUENCE LISTING

[0042]

SEQ ID NO:1 - $\Delta 6$ desaturase from humans (Genbank Accession No: AAD20018).
 SEQ ID NO:2 - $\Delta 6$ desaturase from mouse (Genbank Accession No: NP_062673).
 SEQ ID NO:3 - $\Delta 6$ desaturase from *Pythium irregulare* (Genbank Accession No: AAL13310).
 SEQ ID NO:4 - $\Delta 6$ desaturase from *Borago officinalis* (Genbank Accession No: AAD01410).
 SEQ ID NO:5 - $\Delta 6$ desaturase from *Anemone leveillei* (Genbank Accession No: AAQ10731).
 SEQ ID NO:6 - $\Delta 6$ desaturase from *Ceratodon purpureus* (Genbank Accession No: CAB94993).
 SEQ ID NO:7 - $\Delta 6$ desaturase from *Physcomitrella patens* (Genbank Accession No: CAA11033).
 SEQ ID NO:8 - $\Delta 6$ desaturase from *Mortierella alpina* (Genbank Accession No: BAC82361).
 SEQ ID NO:9 - $\Delta 6$ desaturase from *Caenorhabditis elegans* (Genbank Accession No: AAC15586).
 SEQ ID NO:10 - $\Delta 6$ desaturase from *Echium plantagineum*.
 SEQ ID NO:11 - $\Delta 6$ desaturase from *Echium gentianoides* (Genbank Accession No: AY055117).
 SEQ ID NO:12 - $\Delta 6$ desaturase from *Echium pitardii* (Genbank Accession No: AY055118).
 SEQ ID NO:13 - $\Delta 5/\Delta 6$ bifunctional desaturase from *Danio rerio* (zebrafish).
 SEQ ID NO's 14 to 16 - Conserved motifs of *Echium sp.* $\Delta 6$ desaturases.
 SEQ ID NO's 17 to 22, 30 and 31 - Oligonucleotide primers.

SEQ ID NO:23 - Linin promoter from *Linum usitatissimum*.

SEQ ID NO:24 - Linin terminator from *Linum usitatissimum*.

SEQ ID NO:25 - cDNA sequence encoding $\Delta 6$ desaturase from *Echium plantagineum*.

SEQ ID NO:26 - $\Delta 15$ desaturase from *Perilla frutescens* (Genbank Accession No: AF213482).

SEQ ID NO:27 - $\Delta 15$ desaturase from *Brassica napus* (Genbank Accession No: L01418).

SEQ ID NO:28 - $\Delta 15$ desaturase from *Betula pendula* (Genbank Accession No: AAN17504).

SEQ ID NO:29 - $\Delta 15$ desaturase from *Arabidopsis thaliana* (Genbank Accession No: AAC31854).

DETAILED DESCRIPTION OF THE INVENTION

General Techniques and Definitions

[0043] Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, plant biology, molecular genetics, immunology, immunohistochemistry, fatty acid synthesis, protein chemistry, and biochemistry).

[0044] Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan et al. (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

[0045] As used herein, the term "lipid" generally refers to an organic molecule, typically containing a hydrocarbon chain(s), that is insoluble in water but dissolves readily in nonpolar organic solvents. Feedstuffs of the invention are defined herein relative to the composition of their lipid component. This lipid component includes fatty acids (either free or esterified, for example in the form of triacylglycerols), sterols and polar lipids.

[0046] As used herein, the term "fatty acids" refers to a large group of organic acids made up of molecules containing a carboxyl group at the end of a hydrocarbon chain; the carbon content may vary from C2 to C34. The fatty acids may be saturated (contain no double bonds in the carbon chain) (SFA), monounsaturated (contain a single double bond in the carbon chain) (MUFA), or polyunsaturated (contain a two, three, four or more double bonds in the carbon chain) (PUFA). Unless stated to the contrary, the fatty acids may be in a free state (non-esterified) or in an esterified form such as part of a triacylglycerol, diacylglyceride, monoacylglyceride, acyl-CoA bound or other bound form, or mixture thereof. The fatty acid may be esterified as a phospholipid such as a phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol forms.

[0047] As used herein, the terms "long-chain polyunsaturated fatty acid", "LC-PUFA" or "C20+ polyunsaturated fatty acid" refer to a fatty acid which comprises at least 20 carbon atoms in its carbon chain and at least three carbon-carbon double bonds. Ordinarily, the number of carbon atoms in the carbon chain of the fatty acids refers to an unbranched carbon chain. If the carbon chain is branched, the number of carbon atoms excludes those in side groups. Generally, the long-chain polyunsaturated fatty acid is an $\omega 3$ fatty acid, that is, having a desaturation (carbon-carbon double bond) in the third carbon-carbon bond from the methyl end of the fatty acid. Preferably, the long-chain polyunsaturated fatty acid is selected from the group consisting of; eicosatetraenoic acid (ETA, 20:4 Δ 8,11,14,17, ω 3) eicosapentaenoic acid (EPA, 20:5 Δ 5,8,11,14,17; ω 3), docosapentaenoic acid (DPA, 22:5 Δ 7,10,13,16,19, ω 3), or docosahexaenoic acid (DHA, 22:6 Δ 4,7,10,13,16,19, ω 3). It would readily be apparent that the LC-PUFA that is in (or limited in amount or even excluded from) a feedstuff of the invention, or produced by a salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., fed a feedstuff of the invention, may be a mixture of any or all of the above and may include other LC-PUFA or derivatives of any of these LC-PUFA.

[0048] Use of the term "fish" includes all vertebrate fish, which may be bony or cartilaginous fish.

[0049] As used herein, the term salmon refers to *Salmo* sp. or *Oncorhynchus* sp. More preferably, the salmon is a *Salmo* sp. Even more preferably, the salmon is Atlantic Salmon (*Salmo salar*).

[0050] In an embodiment, the salmon, is at a "larval" or "juvenile" stage. Fish development recognises 5 periods that occur in the following order: embryonic period; larval period; juvenile period; adult period; senescent period. The larval period occurs once the embryo has hatched and has the ability to feed independently of the egg yolk (or mother in rare cases), organ systems develop morphologically and gain physiological function. The juvenile period is when all organ systems are fully formed and functional (bar the gonads) and fish attain the appearance of miniature adults, the period lasts until the gonads become mature. Once the gonads mature the fish attain the adult period, and then senescence

when growth ceases and gonads do not produce gametes (Adapted from Moyle, P.B. & Cech, J.J. 2004. Fishes An Introduction to Ichthyology, 5th Edition, Prentice Hall).

[0051] The "crustacean" may be any organism of the subphylum "Crustacea", and hence the crustacean may be obtained from marine sources and/or freshwater sources. Such crustacea include, but are not limited to, organisms such as krill, clams, shrimp (including prawns), crab, and lobster.

Feedstuffs

[0052] For purposes of the present invention, "feedstuffs" include any food or preparation, for consumption by a salmon, which is *Salmo* sp. or *Oncorhynchus* sp.

[0053] The present invention provides a feedstuff comprising lipid, the fatty acid of said lipid comprising at least 11% (w/w) stearidonic acid (SDA). The invention also provides methods of using said feedstuff for rearing a salmon, which is a *Salmo* sp. or *Oncorhynchus* sp.

[0054] In embodiments of the invention, the fatty acid of said lipid comprises at least 11.0%, at least 15%, at least 20%, or at least 30% (w/w) SDA.

[0055] In further embodiments, the fatty acid of said lipid comprises less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or more preferably less than 8% (w/w) total saturated fatty acids (SFA). In particular, the feedstuff comprises reduced levels of 14:0 and/or 16:0 compared to the corresponding feedstuff made with fishoil rather than plant oil comprising at least 11% SDA.

[0056] Although the level of SDA that may be produced in seedoil of transgenic plants may be in excess of 40% of the fatty acid, the invention may be practised with plant oil that has less SDA, such as for example at least 5.5% SDA. That is, not all of the ALA is converted to SDA in the plant, and the oil may contain both SDA and ALA. Therefore, in yet other embodiments, the fatty acid of said lipid comprises at least 10%, at least 15%, at least 16%, at least 17%, at least 18%, or at least 19% (w/w) α -linolenic acid (ALA 18:3 Δ 9,12,15, ω 3). In an embodiment, the ALA level is in the range 10-45% (w/w).

[0057] Preferably, the lipid of the feedstuff comprises phytosterol, which may provide additional benefit. In embodiments of the invention, the lipid comprises at least 0.025%, at least 0.05%, or at least 0.1% (w/w) phytosterol. It may comprise at least 0.2% phytosterol, typically in the range 0.2-0.8% (w/w) phytosterol. The phytosterol may be any plant derived sterol from plants such as, but not limited to, *Echium* sp., canola, soybean, flax, cereal or grain legume. Examples of phytosterols include, but are not limited to, brassicasterol, campesterol, stigmasterol, β -sitosterol or any combination of these.

[0058] In a further embodiment, the lipid is substantially free of cholesterol, which may be advantageous in limiting the cholesterol level in the fish or crustacean that is produced, in particular for fish. As used herein, the term "substantially free of cholesterol" refers to the lipid comprising less than 0.1% (w/w) cholesterol, preferably at an undetectable level. Typically, lipid obtained from plants is substantially free of cholesterol.

[0059] In other embodiments, at least 25%, at least 50%, at least 75% or at least 90% of the SDA is esterified in the form of triacylglycerol.

[0060] In yet further embodiments, the lipid content of the feedstuff is at least 10, at least 15, at least 20, at least 30, at least 50, at least 100, at least 200, or at least 250 g/kg dry matter. In another embodiment, the lipid content of the feedstuff is no more than 350g/kg dry matter or any range between these figures.

[0061] In other embodiments, the feedstuff comprises at least 0.55, at least 1, at least 2.5, at least 5, at least 7.2, at least 10, at least 12.5, or more preferably at least 14.3 g/kg dry matter of SDA.

[0062] In yet another preferred embodiment, the fatty acid of the lipid content of the feedstuff comprises less than 2% EPA and/or DHA, more preferably less than 1% EPA and/or DHA.

[0063] The SDA can be from any source. In a preferred embodiment, the SDA is provided in the form of a transgenic organism, or extract or portion thereof, wherein the organism is genetically modified such that it produces SDA and/or produces higher levels of SDA than when compared to a wild-type organism. Preferably, the transgenic organism is a plant or yeast. In a particularly preferred embodiment, the SDA is provided in the form of oil extracted from a plant, especially a transgenic plant. Typically, such oil is extracted from the seed of the plant. However, in some embodiments, the SDA may be obtained from a non-transgenic organism which naturally produces SDA, for example, *Echium plantagineum*.

[0064] Salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., can be fed feedstuffs of the present invention in any manner and amount, and according to any feeding schedule employed in salmon, which is a *Salmo* sp. or *Oncorhynchus* sp., cultivation. Feeding rates typically vary according to abiotic factors, mainly seasonal such as temperature, and biotic, in particular the size of the animal. Juvenile fish are typically fed 5-10% of their body weight per day over about 4-6 feeds per day. Larger fish are typically fed at 2-5% of their body weight per day over about 1-2 feeds per day. The salmon may be allowed to feed to appetite.

[0065] Preferably, the salmon are fed at least once per day, more preferably two or more times per day such as, for

example, 2-6 or 4-6 times per day. It is preferred that any excess food be removed after the feeding period, e.g., by flushing out of a raceway system, or through removal out of the bottom of the sea-cage.

[0066] The benefits increase when salmon are fed over longer periods of time, for example over at least 6, 7 or 12 weeks. Feedstuffs other than those described herein may also be used in the time period, however it is preferred that the feedstuff of the invention is used predominantly over the time period if not exclusively.

[0067] As used herein, "predominantly" means at least 50% of the time, occasions or in amount, as the context determines.

[0068] It is preferable that salmon be fed SDA containing feedstuffs as a mixture with other well-known ingredients included in commercial salmon food formulations so as to provide a nutritionally balanced complete food, including, but not limited to, plant matter, e.g., flour, meal, starch or cracked or processed grain produced from a crop plant such as wheat or other cereals, alfalfa, corn, oats, potato, rice, soybeans or other legumes; cellulose in a form that may be obtained from wood pulp, grasses, plant leaves, and waste plant matter such as rice or soy bean hulls, or corn cobs; animal matter, e.g., fish or crustacean meal, oil, protein or solubles and extracts, krill, meat meal, bone meal, feather meal, blood meal, or cracklings; algal matter; yeast; bacteria; vitamins, minerals, and amino acids; organic binders or adhesives; and chelating agents and preservatives. A wide variety of formulations are reported in both the patent and scientific literature. Alternatively, SDA is used to supplement other foods, e.g., commercial salmon foods.

[0069] In one embodiment, the feedstuff comprises fishmeal (which may or may not be defatted) but does not comprise, as a separate ingredient, fish oil. Alternatively, the feedstuff may comprise some fishoil as an added separate ingredient. However, the minimum level of SDA in the fatty acid of the total lipid of the feedstuff should remain at least 5.5%.

[0070] On a commercial scale feedstuffs may conveniently be provided in the form of pressed or extruded feed pellets.

[0071] The components utilized in the feedstuff compositions of the present invention can be of semi-purified or purified origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by *de novo* synthesis.

[0072] With respect to vitamins and minerals, the following may be added to the feedstuff compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Examples of these include Stay C which is a commercial stabilised vitamin C product, trisodium phosphate or Banox E which is an antioxidant. Other such vitamins and minerals may also be added.

Desaturases

[0073] Organisms useful for producing feedstuffs of the invention typically comprise a gene encoding a $\Delta 6$ desaturase, which may be a transgene or an endogenous gene. As used herein, a " $\Delta 6$ desaturase" is at least capable of converting ALA to SDA, and/or linoleic acid (LA, 18:2 Δ 9,12, ω 6) to γ -linoleic acid (GLA, 18:2 Δ 6,9,12, ω 6). Examples of suitable $\Delta 6$ desaturases include, but are not limited to, those which comprises (i) an amino acid sequence as provided as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12, (ii) an amino acid sequence which is at least 50% identical to any one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12, or (iii) a biologically active fragment of i) or ii). In a further embodiment, the $\Delta 6$ desaturase comprises an amino acid sequence which is at least 90% identical to any one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12. In a further embodiment, the $\Delta 6$ desaturase is encoded by the protein coding region of one of the $\Delta 6$ desaturase genes listed in Table 1 or gene at least 75% identical thereto.

[0074] The $\Delta 6$ desaturase may also have other activities such as $\Delta 5$ desaturase activity. Such enzymes are known in the art as a " $\Delta 5/\Delta 6$ bifunctional desaturase" or a " $\Delta 5/\Delta 6$ desaturase". These enzymes are at least capable of i) converting ALA to SDA, and ii) converting eicosatetraenoic acid to eicosapentaenoic acid. A gene encoding a bifunctional $\Delta 5$ -/ $\Delta 6$ - desaturase has been identified from zebrafish (Hasting et al., 2001). The gene encoding this enzyme might represent an ancestral form of the "front-end desaturase" which later duplicated and the copies evolved distinct $\Delta 5$ - and $\Delta 6$ -desaturase functions. In one embodiment, the $\Delta 5/\Delta 6$ bifunctional desaturase is naturally produced by a freshwater species of fish. In a particular embodiment, the $\Delta 5/\Delta 6$ bifunctional desaturase comprises

- i) an amino acid sequence as provided in SEQ ID NO:13,
- ii) an amino acid sequence which is at least 50% identical to SEQ ID NO:13, or
- iii) a biologically active fragment of i) or ii).

Table 1. Examples of $\Delta 6$ desaturases from different sources.

Type of organism	Species	Accession Nos.	Protein size (aa's)	References
Mammals	<i>Homo sapiens</i>	NM_013402	444	Cho et al., 1999; Leonard et al., 2000
	<i>Mus musculus</i>	NM 019699	444	Cho et al., 1999
Nematode	<i>Caenorhabditis elegans</i>	Z70271	443	Napier et al., 1998
Plants	<i>Borago officinalis</i>	U79010	448	Sayanova et al., 1997
	<i>Echium</i>	AY055117 AY055118		Garcia-Maroto et al., 2002
	<i>Primula vialii</i>	A Y234127	453	Sayanova et al., 2003
	<i>Anemone leveillei</i>	AF536525	446	Whitney et al., 2003
Mosses	<i>Ceratodon purpureus</i>	AJ250735	520	Sperling et al., 2000
	<i>Marchantia polymorpha</i>	AY583463	481	Kajikawa et al., 2004
	<i>Physcomitrella patens</i>			Girke et al., 1998
Fungi	<i>Mortierella alpina</i>	AF110510 AB020032	457	Huang et al., 1999; Sakuradani et al., 1999
	<i>Pythium irregulare</i>	AF419296	459	Hong et al., 2002
	<i>Mucor circinelloides</i>	AB052086	467	
	<i>Rhizopus sp.</i>	AY320288	458	Zhang et al., 2004
	<i>Saprolegnia diclina</i>		453	WO02081668
Diatom	<i>Phaeodactylum tricornutum</i>	AY082393	477	Domergue et al., 2002
Bacteria	<i>Synechocystis</i>	L11421	359	Reddy et al., 1993
Algae	<i>Thraustochytrium aureum</i>		456	WO02081668
Fish	<i>Danio rerio</i>	AF309556	444	Hastings et al., 2001

[0075] Organisms useful in producing feedstuffs of the invention generally comprise a gene encoding an " $\omega 3$ desaturase", which may be a transgene or an endogenous gene. As used herein, an " $\omega 3$ desaturase" is at least capable of converting LA to ALA and/or GLA to SDA and are therefore able to introduce a desaturation at the third carbon-carbon bond from the ω end of the acyl substrate. Such desaturases may also be known in the art as $\Delta 15$ desaturases when active on a C18 substrate, for example 18:2 (LA), introducing a desaturation at the fifteenth carbon-carbon bond from the carboxy (Δ) end of the acyl chain. Examples of $\omega 3$ desaturase include those described by Pereira et al. (2004), Horiguchi et al. (1998), Berberich et al. (1998) and Szychalla et al. (1997) or as listed in Table 2. Examples of suitable $\Delta 15$ desaturases include, but are not limited to, those which comprise (i) an amino acid sequence as provided in SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29, (ii) an amino acid sequence which is at least 50% identical to any one of SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29, or (iii) a biologically active fragment of i) or ii). In a further embodiment, the $\Delta 15$ desaturase comprises an amino acid sequence which is at least 90% identical to any one of SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29. In a further embodiment, the $\Delta 15$ desaturase has an amino acid sequence according to an Accession No listed in Table 2, or is encoded by the protein coding region of one of the $\Delta 15$ desaturase genes listed in Table 2, or a protein or gene at least 75% identical thereto.

Table 2. Examples of $\omega 3/\Delta 15$ desaturases.

Type of organism	Species	Accession Nos.	Protein size	References
Plant	<i>Arabidopsis thaliana</i>	NP 850139.1	288	NCBI
		A Y096462.	386	NCBI

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(continued)

Type of organism	Species	Accession Nos.	Protein size	References
5		AAL77744	435	NCBI
	<i>Brassica napus</i>	P48642	383	Arondelet al., 1992
		AY599884	383	NCBI
		JQ2337	377	NCBI
10		AAT65204	378	NCBI
	<i>Brassica rapa subsp. oleifera</i>	AAL08867	302	Tanhuanpaa et al., 2002
	<i>Glycine max</i>	BAB18135	380	NCBI
15		AAO24263	376	Bilveu et al., 2003
		P48621	453	Yadav et al., 1993
	<i>Linum usitatissimum</i>	ABA02173	391	Vrinten et al., 2005
		ABA02172	392	Vrinten et al., 2005
20	<i>Betula pendula</i>	AAN17504	386	NCBI
	<i>Perilla frutescens</i>	AAD15744	391	Chung et al., 1999
		AAL36934	390	NCBI
25		AAB39387	438	NCBI
	<i>Pelargonium x hortorum</i>	AAC16443	407	NCBI
	<i>Malus x domestica</i>	AAS59833	439	NCBI
	<i>Vernicia fordii</i>	CAB45155	387	NCBI
30		AAD13527	437	Tang et al., 1999
	<i>Vigna radiata</i>	P32291	380	Yamamoto et al., 1992
	<i>Prunus persica</i>	AAM77643	449	NCBI
35	<i>Brassica juncea</i>	CAB85467	429	NCBI
	<i>Nicotiana tabacum</i>	P48626	379	Hamada et al., 1994
		BAA11475	441	Hamada et al., 1996
	<i>Betula pendula</i>	AAN17503	444	NCBI
40	<i>Zea mays</i>	BAA22442	398	Berberich et al., 1998
		BAA22441	443	Berberich et al., 1998
	<i>Petroselinum crispum</i>	AAB72241	438	Kirsch et al., 1997
45	<i>Sesamum indicum</i>	P48620	447	NCBI
	<i>Helianthus annuus</i>	AAP78965	443	NCBI
	<i>Capsicum annuum</i>	AAF27933	438	NCBI
	<i>Ricinus communis</i>	P48619	460	VandeLoo et al., 1994
50	<i>Sorghum bicolor</i>	AAT72937	389	Yang et al., 2004
	<i>Oryza sativa</i>	XP 479619	387	NCBI
	<i>Solanum tuberosum</i>	CAA07638	431	NCBI
55	<i>Solanum lycopersicum</i>	AAP82169	435	Li et al., 2003
	<i>Triticum aestivum</i>	BAA28358	383	Horiguchi et al., 1998
	Algae	<i>Chlorella vulgaris</i>	BAB78717	Suga et al., 2002

(continued)

Type of organism	Species	Accession Nos.	Protein size	References
	<i>Synechococcus</i> sp	AAB61352	350	Sakamoto et al., 1997
	<i>Dunaliella salina</i>	AAD48897	196	NCBI
Fungi	<i>Saprolegnia diclina</i>	AAR20444	358	Pereira et al., 2004
NCBI indicates sequences are available from http://www.ncbi.nlm.nih.gov/				

[0076] The % identity of a polypeptide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. Unless stated otherwise, the query sequence is at least 15 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 15 amino acids. More preferably, the query sequence is at least 50 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 50 amino acids. More preferably, the query sequence is at least 100 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 100 amino acids. Even more preferably, the query sequence and a sequence defined herein are aligned over their entire length.

[0077] The term "polypeptide" is used interchangeably herein with the terms "protein" and "enzyme".

[0078] With regard to the defined polypeptides/enzymes, it will be appreciated that % identity figures higher than those provided above will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polypeptide comprises an amino acid sequence which is at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 76%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO.

[0079] As used herein, the term "biologically active fragment" refers to a portion of the defined polypeptide/enzyme which still maintains desaturase activity. Such biologically active fragments can readily be determined by serial deletions of the full length protein, and testing the activity of the resulting fragment.

Cells

[0080] Suitable cells for use in feedstuffs of the invention, or which can be used to produce SDA for feedstuffs of the invention, include any cell containing SDA or that can be transformed with a polynucleotide encoding a polypeptide/enzyme described herein, and which is thereby capable of being used for producing SDA. Host cells into which the polynucleotide(s) are introduced can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Such nucleic acid molecule may be related to SDA synthesis, or unrelated. Host cells either can be endogenously (i.e., naturally) capable of producing proteins described herein or can be capable of producing such proteins only after being transformed with at least one nucleic acid molecule.

[0081] The cells may be prokaryotic or eukaryotic. Host cells can be any cell capable of producing SDA, and include fungal (including yeast), parasite, arthropod, animal and plant cells. Preferred host cells are yeast and plant cells. In a preferred embodiment, the plant cells are seed cells.

[0082] In one embodiment, the cell is an animal cell or an algal cell. The animal cell may be of any type of animal such as, for example, a non-human animal cell, a non-human vertebrate cell, a non-human mammalian cell, or cells of aquatic animals such as fish or crustacea, invertebrates, insects, etc.

[0083] The cells may be of an organism suitable for fermentation. Suitable fermenting cells, typically microorganisms are able to ferment, i.e., convert, sugars, such as glucose or maltose, directly or indirectly into the desired fermentation product. Examples of fermenting microorganisms include fungal organisms, such as yeast. As used herein, "yeast" includes *Saccharomyces* spp., *Saccharomyces cerevisiae*, *Saccharomyces carlbergensis*, *Candida* spp., *Kluveromyces* spp., *Pichia* spp., *Hansenula* spp., *Trichoderma* spp., *Lipomyces starkey*, and *Yarrowia lipolytica*.

Gene Constructs and Vectors

[0084] Transgenic organisms, and/or host cells, producing SDA are typically transformed with a recombinant vector. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid.

[0085] One type of recombinant vector comprises a nucleic acid molecule which encodes an enzyme useful for the

purposes of the invention (such as a polynucleotide encoding a $\Delta 6$ desaturase or $\omega 3$ desaturase) operatively linked to an expression vector. As indicated above, the phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and effecting expression of a desired nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells, including in bacterial, fungal, endoparasite, arthropod, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene expression in yeast, animal or plant cells.

[0086] In particular, expression vectors contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of desired nucleic acid molecules. In particular, recombinant molecules include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells. A variety of such transcription control sequences are known to those skilled in the art.

[0087] Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained.

Transgenic Plants and Parts Thereof

[0088] The term "plant" as used herein as a noun refers to whole plants, but as used as an adjective refers to any substance which is present in, obtained from, derived from, or related to a plant, such as for example, plant organs (e.g. leaves, stems, roots, flowers), single cells (e.g. pollen), seeds, plant cells and the like. Plants provided by or contemplated for use in the practice of the present invention include both monocotyledons and dicotyledons. In preferred embodiments, plant useful for the production of feedstuffs of the present invention are crop plants (for example, cereals and pulses, maize, wheat, potatoes, tapioca, rice, sorghum, millet, cassava, barley, or pea), or other legumes. The plants may be grown for production of edible roots, tubers, leaves, stems, flowers or fruit. The plants of the invention may be: corn (*Zea mays*), canola (*Brassica napus*, *Brassica rapa* ssp.), flax (*Linum usitatissimum*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), sunflower (*Helianthus annuus*), wheat (*Triticum aestivum*), soybean (*Glycine max*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), cassava (*Manihot esculenta*), coconut (*Cocos nucifera*), , olive (*Olea europaea*), oats, or barley.

[0089] In one embodiment, the plant is an oilseed plant, preferably an oilseed crop plant. As used herein, an "oilseed plant" is a plant species used for the commercial production of oils from the seeds of the plant. The oilseed plant may be oil-seed rape (such as canola), maize, sunflower, soybean, sorghum, oil palm or flax (linseed). Furthermore, the oilseed plant may be other *Brassic*as, cotton, peanut, poppy, mustard, castor bean, sesame, safflower, or nut producing plants. The plant may produce high levels of oil in its fruit, such as olive or coconut.

[0090] Examples of cotton of the, and/or useful for, the present invention include any species of *Gossypium*, including, but not limited to, *Gossypium arboreum*, *Gossypium herbaceum*, *Gossypium barbadense* and *Gossypium hirsutum*.

[0091] When the production of SDA is desired it is preferable that the plant species which is to be transformed has an endogenous ratio of ALA to LA which is at least 1:1, more preferably at least 2:1. Examples include most, if not all, oilseeds such as linseed. This maximizes the amount of ALA substrate available for the production of SDA. This may be achieved by transgenic means, for example by introduction of a $\Delta 15$ deaturase gene into the plant to increase the levels of the ALA substrate for conversion into SDA.

[0092] The plants produced for use in feedstuffs of the invention may already be transgenic, and/or transformed with additional genes to those described in detail herein.

[0093] Grain plants that provide seeds of interest include oil-seed plants and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, etc. Leguminous plants include beans, peas, soybeans, lupins and the like. Beans include guar, locust bean, fenugreek, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, etc.

[0094] The term "extract or portion thereof" refers to any part of the plant. "Portion" generally refers to a specific tissue or organ such as a seed or root, whereas an "extract" typically involves the disruption of cell walls and possibly the partial purification of the resulting material. Naturally, the "extract or portion thereof" will comprise SDA. Extracts can be prepared

using standard techniques of the art.

[0095] Transgenic plants, as defined in the context of the present invention include plants and their progeny which have been genetically modified using recombinant techniques. This would generally be to cause or enhance production of at least one protein/enzyme defined herein in the desired plant or plant organ. Transgenic plant parts include all parts and cells of said plants such as, for example, cultured tissues, callus, protoplasts. Transformed plants contain genetic material that they did not contain prior to the transformation. The genetic material is preferably stably integrated into the genome of the plant. Such plants are included herein in "transgenic plants". A "non-transgenic plant" is one which has not been genetically modified with the introduction of genetic material by recombinant DNA techniques. In a preferred embodiment, the transgenic plants are homozygous for each and every gene that has been introduced (transgene) so that their progeny do not segregate for the desired phenotype.

[0096] Several techniques exist for introducing foreign genetic material into a plant cell. Such techniques include acceleration of genetic material coated onto microparticles directly into cells (see, for example, US 4,945,050 and US 5,141,131). Plants may be transformed using *Agrobacterium* technology (see, for example, US 5,177,010, US 5,104,310, US 5,004,863, US 5,159,135). Electroporation technology has also been used to transform plants (see, for example, WO 87/06614, US 5,472,869, 5,384,253, WO 92/09696 and WO 93/21335). In addition to numerous technologies for transforming plants, the type of tissue which is contacted with the foreign genes may vary as well. Such tissue would include but would not be limited to embryogenic tissue, callus tissue type I and II, hypocotyl, meristem, and the like. Almost all plant tissues may be transformed during development and/or differentiation using appropriate techniques known to those skilled in the art.

[0097] A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., Cloning Vectors: A Laboratory Manual, 1985, supp. 1987; Weissbach and Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989; and Gelvin et al., Plant Molecular Biology Manual, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

[0098] Examples of plant promoters include, but are not limited to ribulose-1,6-bisphosphate carboxylase small subunit, beta-conglycinin promoter, phaseolin promoter, high molecular weight glutenin (HMW-GS) promoters, starch biosynthetic gene promoters, ADH promoter, heat-shock promoters and tissue specific promoters. Promoters may also contain certain enhancer sequence elements that may improve the transcription efficiency. Typical enhancers include but are not limited to Adh-intron 1 and Adh-intron 6.

[0099] Constitutive promoters direct continuous gene expression in all cells types and at all times (e.g., actin, ubiquitin, CaMV 35S). Tissue specific promoters are responsible for gene expression in specific cell or tissue types, such as the leaves or seeds (e.g., zein, oleosin, napin, ACP, globulin and the like) and these promoters may also be used.

[0100] In a particularly preferred embodiment, the promoter directs expression in tissues and organs in which lipid and oil biosynthesis take place, particularly in seed cells such as endosperm cells and cells of the developing embryo. Promoters which are suitable are the oilseed rape napin gene promoter (US 5,608,152), the *Vicia faba* USP promoter (Baumlein et al., 1991), the *Arabidopsis* oleosin promoter (WO 98/45461), the *Phaseolus vulgaris* phaseolin promoter (US 5,504,200), the *Brassica* Bce4 promoter (WO 91/13980), the linin gene promoter from flax, or the legumin B4 promoter (Baumlein et al., 1992), and promoters which lead to the seed-specific expression in monocots such as maize, barley, wheat, rye, rice and the like. Notable promoters which are suitable are the barley 1pt2 or 1pt1 gene promoter (WO 95/15389 and WO 95/23230) or the promoters described in WO 99/16890 (promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, the wheat glutelin gene, the maize zein gene, the oat glutelin gene, the sorghum kasirin gene, the rye secalin gene). Other promoters include those described by Broun et al. (1998) and US 20030159173.

[0101] Under certain circumstances it may be desirable to use an inducible promoter. An inducible promoter is responsible for expression of genes in response to a specific signal, such as: physical stimulus (heat shock genes); light (RUBP carboxylase); hormone (Em); metabolites; and stress. Other desirable transcription and translation elements that function in plants may be used.

[0102] In addition to plant promoters, promoters from a variety of sources can be used efficiently in plant cells to express foreign genes. For example, promoters of bacterial origin, such as the octopine synthase promoter, the nopaline synthase promoter, the mannopine synthase promoter; promoters of viral origin, such as the cauliflower mosaic virus (35S and 19S) and the like may be used.

EXAMPLES**Example 1. Materials and Methods**5 *Lipid extraction and isolation*

[0103] Samples were freeze dried and extracted using a modified Bligh and Dyer protocol (Bligh and Dyer, 1959). A single phase extraction, $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, (1:1:0.9, by vol), was used to yield a total lipid extract (TLE).

10 **[0104]** Lipid classes were analysed by an Iatroscan MK V thin-layer chromatography-flame ionization detector (TLC-FID) analyser (Iatron Laboratories, Japan). Samples were spotted onto silica gel SIII Chromarods (5 μm particles size) and developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was hexane: diethyl ether: acetic acid (60:17:0.1, v/v/v) (Volkman and Nichols, 1991). After development for 25 minutes, the chromarods were oven-dried and analysed immediately to minimise adsorption of atmospheric contaminants. Lipid classes were quantified by DAPA software (Kalamunda, WA, Australia). The FID was calibrated for each compound class: phosphatidylcholine; cholesterol; cholesteryl ester; oleic acid; hydrocarbon (squalene); wax ester (derived from fish oil); triacylglycerol (derived from fish oil); and DAGE (purified from shark liver oil).

15 **[0105]** An aliquot of the TLE was trans-methylated in methanol:chloroform:hydrochloric acid (10:1:1, v/v/v) for 1 hour at 100°C. After addition of water the mixture was extracted three times with hexane: chloroform (4:1, v/v) to produce fatty acid methyl esters (FAME). FAME were concentrated under nitrogen and treated with N,O-bis(trimethylsilyl)-trifluoroacetamide (BSFTA, 50 μl , 60°C, 1h) to convert hydroxyl groups to their corresponding trimethylsilyl ethers. Samples were made up to a known volume with an internal injection standard (23:0 or 19:0 FAME) and analysed by gas chromatography (GC) using an Agilent Technologies 6890N GC (Palo Alto, California, USA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50m \times 0.32mm i.d.), and an FID. Helium was used as the carrier gas. Samples were injected, by a split/splitless injector and an Agilent Technologies 7683 Series auto sampler in splitless mode, at an oven temperature of 50°C. After 1 min the oven temperature was raised to 150°C at 30°C min⁻¹, then to 250°C at 2°C per min and finally to 300°C at 5°C min⁻¹. Peaks were quantified by Agilent Technologies GC ChemStation software (Palo Alto, California, USA). Individual components were identified by mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are typically subject to an error of $\pm 5\%$ of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer fitted with an on-column injector with Thermoquest Xcalibur software (Austin, Texas, USA). The GC was fitted with a capillary column similar to that described above.

20 **[0106]** A polar column was used to separate 18:1 ω 9 and 18:3 ω 3 which coeluted on the HP5 column. FAME were analysed with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a flame ionisation detector (FID) at 250°C. FAME samples were injected using a split/splitless injector into a polar BPX-70 fused-silica column (50 m x 0.32 mm i.d.). The carrier gas was helium. The GC oven temperature was initially held at 45°C for 2 min after injection and then increased at 30°C/min to 120°C and at 3°C/min to 240°C, then held isothermal for 10 min.

Statistical analysis

40 **[0107]** Mean values were reported plus or minus standard error of the mean. Percentage data were arcsin transformed prior to analysis. Normality and homogeneity of variance were confirmed and a comparison between means was achieved by 1-way analysis of variance (ANOVA). Multiple comparisons were achieved by Turkey-Kramer HSD. Significance was accepted as probabilities of 0.05 or less. Statistical analysis was performed using SPSS for windows version 11.

45 *Brassica transformation*

[0108] *Brassica napus* (Line BLN 1239) seeds were surface sterilized by soaking them in 70% (v/v) ethanol for 2 min and then rinsed for 10 min in tap water at 55°C. The seeds were sterilized for 20 min in 25% commercial bleach (10 gl⁻¹ sodium hypochlorite) containing 0.1% Tween-20. The seeds were washed thoroughly with sterile distilled H₂O, placed on GEM medium in tissue culture jars and kept in the cold room for two days for germination. The jars were transferred to low light (20 $\mu\text{Mm}^2\text{s}^{-1}$) for about four to six days at 24°C for growth of the cotyledons. Roots and apices were removed under aseptic conditions. Excised hypocotyl segments (10 mm) were washed with 50 ml CIM medium for about 30 min without agitation in the laminar flow cabinet. The CIM was removed and the segments transferred to a 250 ml flask with 50 ml of CIM, sealed with sterile aluminium foil and shaken for 48 hours at 24°C under low light (10 $\mu\text{Mm}^2\text{s}^{-1}$).

55 **[0109]** *Agrobacterium* strains containing plasmid transformation vectors were grown in 5 ml of LB media with appropriate antibiotics at 28°C for about two days, transferred to a 250 ml Erlenmeyer flask with 45 ml of LB without antibiotics and cultured for four hours at 28°C with shaking. The *Agrobacterium* cells were pelleted by centrifugation, washed, and gently re-suspended in about 20 ml BM. The optical density at 600 nm of the resultant *Agrobacterium* suspension was

adjusted to 0.2 with BM. The cell suspension was added to the explants which had been drained of the CIM medium, mixed briefly and allowed to stand for 20 min. The *Agrobacterium* suspension was removed, the hypocotyl explants washed once with 50 ml CIM and co-cultivation continued for 48 hours on an orbital shaker. After this, the medium was slightly milky due to *Agrobacterium* growth. CIM was removed and the explants washed three times with 50 ml CIM for one minute and then twice for one hour on an orbital shaker at 140x g. Following the washes, 50 ml CIM containing 200mg/l Timentin® was added and placed on an orbital shaker for 24 hours. Under sterile conditions, the CIM medium was clear at this stage.

[0110] Regeneration of transformed shoots on SIM was carried out on a two-stage selection process. Initially, the hygromycin concentration in the SIM medium used was 5 mg/l. After about two weeks, explants with developing calli were transferred to SIM containing 20 mg/l hygromycin. When the regenerating shoots had developed leaves longer than one cm, they were excised carefully and were transferred to SEM with 20 mg/l hygromycin. After two weeks, stems usually had elongated and apices were transferred to RIM containing 10 mg/l hygromycin. Non-elongating shoots were subcultured in SEM every two to three weeks until they were long enough to be transferred to RIM. When the roots were about two cm in length, the regenerated plantlets were removed from tissue culture pots and transferred to soil for further growth.

Media recipes

[0111] Composition of the tissue culture media used in this procedure is given below. They contained MS salts (Murashige and Skoog, 1962), MS or B5 vitamins (Gamborg et al., 1968), sucrose and MES. The pH was adjusted to 5.8 with KOH prior to sterilization. For solid media, agar was added and then autoclaved. Media containing agar was allowed to cool to below 50°C and filter-sterilized compounds were added to the melted media before pouring it into either plastic Petri dishes or 250 ml polycarbonate tissue culture jars (Sarstedt, No 75.9922519). The composition of various media with all additives are given below: germination medium (GEM); basal medium (BM); callus-inducing medium (CIM, modified from Radke et al., 1988); washing medium (WM); shoot-inducing medium (SIM, modified from Radke et al., 1988); shoot-elongation medium (SEM) and root-inducing medium (RIM, modified from De Block et al., 1989).

GEM: 1 x MS salts, 1 x MS vitamins, Sucrose (20 g^l⁻¹), MES (500 mg^l⁻¹), Agar (8 g^l⁻¹), pH to 5.8.

BM: 1 x MS salts, 1 x B5 vitamins, Sucrose (30 g^l⁻¹), MES (500 mg^l⁻¹), pH to 5.8.

CIM: 2,4-D (1.0 mg^l⁻¹) and Kinetin (1.0 mg^l⁻¹) added to BM.

WM: 2,4-D (1.0 mg^l⁻¹), Kinetin (1.0 mg^l⁻¹) and Timentin® (200 mg^l⁻¹) added to BM.

SIM: AgNO₃ (500 mg^l⁻¹), Zeatin riboside (0.5 mg^l⁻¹), BAP (2.0 mg^l⁻¹), GA₃ (0.01 mg^l⁻¹), Timentin® (200 mg^l⁻¹), Hygromycin (5 to 30 mg^l⁻¹), and Agar (8 g^l⁻¹) added to BM.

SEM: 0.5 x MS salts, 0.5 x B5 vitamins, Sucrose (10 g^l⁻¹), MES (500 mg^l⁻¹), Timentin® (200 mg^l⁻¹), Hygromycin (20 to 30 mg^l⁻¹), Agar (8 g^l⁻¹), pH to 5.8.

RIM: 0.5 x MS salts, 0.5 x B5 vitamins, Sucrose (10 g^l⁻¹), MES (500 mg^l⁻¹), IBA (0.1 mg^l⁻¹), Timentin® (200 mg^l⁻¹), Hygromycin (20 to 30 mg^l⁻¹), Agar (8 g^l⁻¹), pH to 5.8.

Example 2. Fish fed with food compositions including plant-derived SDA

[0112] Stearidonic acid (SDA, 18:4 ω₃) is an LC-PUFA precursor, derived by desaturation of ALA by Δ₆ desaturase (Figure 1). The Δ₆ desaturase is also involved other steps in the biosynthesis of LC-PUFA in the formation of DHA from EPA in vertebrates (Yamazaki et al., 1992) and 18:2 ω₆ to 20:4 ω₆. Therefore it is possible that the Δ₆ desaturation of ALA is out-competed by the ω₆ pathway in fish and crustacea when diets contain high levels of 18:2 ω₆, present in vegetable oils such as canola and sunflower.

[0113] Oil from a few plant sources such as *Echium plantagineum* have SDA in the fatty acid profile, up to about 15-20% as a percentage of the fatty acid in the oil. To determine whether SDA-rich oil might serve as an efficient substrate for ω₃ LC-PUFA accumulation in fish, a feeding trial was conducted *in vivo* using salmon (*Salmo salar* L.). Diets including an equivalent level of canola oil were used as a control source of ALA, as described in Tables 3 and 4.

Table 3. Ingredient and lipid composition (g/kg dry matter) of experimental diets.

	Diet			
	CO oil (g)	SO oil (g)	Mix oil (g)	FO oil (g)
<i>Ingredient composition (g kg⁻¹)</i>				
Fishmeal (defatted)	150	150	150	150
Casein	150	150	150	150

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(continued)

		Diet			
		CO oil (g)	SO oil (g)	Mix oil (g)	FO oil (g)
5	Wheat Gluten	100	100	100	100
	Hipro soy	226	226	226	226
	Fish oil	0	0	0	130
	Canola oil	130	0	65	0
10	SDA oil	0	130	65	0
	Pre Gel Starch	150	150	150	150
	Vitamin Mix ^a	3	3	3	3
	Mineral Mix ^b	5	5	5	5
	Stay C ^c	3	3	3	3
15	Choline chloride	2	2	2	2
	Bentontie	50	50	50	50
	CMC	10	10	10	10
	Sodium Mono P	20	20	20	20
20	Yttrium Oxide	10	10	10	10
FAME					
	Total SFA	6.7	10.8	12.2	44.9
	Total MUFA	81.2	41.3	56.2	32.9
25	18:3 ω 3 ALA	13.1	25.4	20.9	3.1
	18:4 ω 3 SDA	0.0	14.3	7.2	4.2
	20:5 ω 3 EPA	0.1	0.1	0.0	18.0
	22:6 ω 3 DHA	0.6	0.4	0.0	10.7
	Total ω 3	13.9	40.2	28.6	39.6
30	18:2 ω 6	28.2	25.8	27.0	8.0
	Total ω 6	28.2	26.1	27.0	9.3
	Other PUFA	0.0	11.6	5.9	3.3
	Total PUFA	42.1	77.9	61.5	52.2
35	SO, stearidonic rich oil crossential SA14 from Croda chemicals; CO, canola oil diet; Mix, 1:1 mix diet of canola oil and stearidonic acid oil; FO, fish oil diet, SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CMC, Carboxymethyl cellulose; DHA, Docosahexaenoic Acid; EPA, SDA, Stearidonic acid; Eicosapentaenoic Acid.				
40	^a Vitamin mix (ASV4) to supply per kilogram feed: 2.81 mg thiamin HCL, 1.0 mg riboflavin, 9.15 mg pyridoxine HCL, 25 mg nicotinic acid, 54.35 mg calcium D-pantothenate, 750 mg myo-inositol, 0.38 mg D-biotin, 2.5 mg folic acid, 0.03 mg cyanocobalamin, 6350 IU retinol acetate, 2800 IU cholecalciferol, 100 IU DL α -tocopherol acetate, 5 mg menadone sodium bisulphate, 100 mg Roche rovimix E50.				
45	^b Mineral mix (TMV4) to supply per kilogram feed: 117mg CuSO ₄ ·5H ₂ O, 7.19 mg KI, 1815 mg FeSO ₄ ·7H ₂ O, 307 mg MnSO ₄ ·H ₂ O, 659 mg ZnSO ₄ ·7H ₂ O, 3.29 mg Na ₂ SeO ₃ , 47.7 mg CoSO ₄ ·7H ₂ O				
	^c L-Ascorbyl-2-polyphosphate (Stay-C, Roche Vitamins Australia, French Forest, NSW, Australia).				

[0114] Four diets were formulated to compare canola oil (CO), two different levels of stearidonic acid oil (100% (SO), 1:1 SO:CO (Mix)), and fish oil (FO) (Tables 3 and 4). Fish meal was defatted three times using a 2:1 mixture of hexane and ethanol (400ml 100g⁻¹ fish meal). Soybean (Hamlet Protein A/S, Horsens, Denmark), casein (MP Biomedicals Australasia Pty Ltd, Seven Hills NSW, Australia), wheat gluten (Starch Australasia, Land Cove, NSW, Australia) and BOIIC pre-gelatinised maize starch (Penford Australia Limited, Lane Cove, NSW, Australia) were used. Stearidonic acid rich oil was provided as Crossential SA14 (Croda Chemicals, East Yorkshire, UK). Fish oil was from jack mackerel (Skretting Australia, Cambridge, Tasmania Australia). Stay-C and Rovimix E50 were supplied from Roche Vitamins Australia (French Forest, NSW, Australia), and the remaining ingredients were supplied by Sigma-Aldrich (Castle Hill, NSW, Australia). Yttrium Oxide was used as a digestibility marker. The diets were manufactured into 3 mm diameter pellets using a California Pellet Mill (CL-2), dried and stored at -5°C.

[0115] The feeding experiment was conducted at the School of Aquaculture, University of Tasmania, Launceston,

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Australia. Atlantic salmon (*Salmo salar*) parr were obtained from Wayatinah Salmon hatchery (SALTAS, Tasmania, Australia) and randomly stocked into 300 1 tanks at 25 fish per tank. They were acclimated for 10 days. The tanks were held at a constant temperature of 15.0°C and a photoperiod of 16:8 (light:dark). The fish were held in a partial freshwater recirculation system. Water was treated through physical, UV and biofilters, with a continuous replacement of approximately 15% per day. Dissolved oxygen, pH, ammonia, nitrate, nitrite, and chlorine were monitored daily to ensure water quality remained within parameters recommended for Atlantic salmon (Wedemeyer, 1996).

Table 4. Fatty acid composition of the lipid in the diets (% of total fatty acid).

FA	CO	SE	SO	SE	Mix	SE	FO	SE
14:0	0.23	0.00	0.13	0.02	0.21	0.01	6.38	0.08
16:0	1.58	0.79	4.30	1.24	5.57	0.93	19.23	0.20
18:0	2.58	0.01	3.83	0.02	3.19	0.01	3.90	0.04
Other Sat	0.75	0.01	0.06	0.00	0.44	0.00	5.02	0.01
Total Sat	5.13		8.33		9.40		34.53	
16:1 ω 7	0.28	0.00	0.17	0.03	0.25	0.00	7.06	0.05
18:1 ω 9	52.03	0.17	24.45	0.06	37.54	0.06	10.88	0.19
18:1 ω 7	3.28	0.02	1.04	0.02	2.18	0.02	2.69	0.01
20:1 ω 9	0.96	0.00	0.74	0.01	0.87	0.00	1.66	0.01
Other Mono	5.92	0.06	5.32	0.17	2.42	0.12	3.02	0.03
Total Mono	62.47		31.73		43.26		25.31	
18:3 ω 3	10.07	0.03	19.57	0.04	16.04	0.03	2.39	0.04
18:4 ω 3	0.00	0.00	11.01	0.09	5.57	0.03	3.20	0.06
20:4 ω 3	0.00	0.00	0.00	0.00	0.00	0.00	0.73	0.01
20:5 ω 3	0.05	0.02	0.05	0.02	0.00	0.00	13.85	0.12
22:5 ω 3	0.14	0.04	0.00	0.00	0.00	0.00	1.46	0.02
22:6 ω 3	0.43	0.01	0.33	0.06	0.41	0.01	8.26	0.08
Other ω 3	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.00
Total ω3	10.68		30.96		22.01		30.46	
18:2 ω 6	21.71	0.04	19.82	0.03	20.81	0.01	6.18	0.10
18:3 ω 6	0.00	0.00	8.20	0.06	4.33	0.02	0.64	0.06
20:3 ω 6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:4 ω 6	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.01
22:5 ω 6	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.03
Other ω 6	0.00	0.00	0.23	0.04	0.00	0.00	0.00	0.00
Total ω6	21.71		28.25		25.13		7.82	
Other PUFA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total PUFA	32.40		59.21		47.15		38.28	

[0116] Fish were initially anaesthetized (50mg l⁻¹, benzocaine) and weights and lengths were recorded. Four fish were killed and assessed for initial lipid content and composition. Twenty five fish were randomly allotted into twelve 300 l tanks. Fish weights were not significantly different between tanks (43.6g±0.7). The four diets were fed in triplicate on a ration of 1.1% body weight per day (% BW d⁻¹), in two equal feeds at 0900 and 1700 hrs by automatic belt feeders. Every three weeks all fish were anaesthetized (50mg l⁻¹, benzocaine) and weighed. Fish were starved the day prior to measuring. Every 7 days the total feed consumption (kg DM) was estimated from the amount of feed that was not eaten by collection in sediment collectors. The amount of uneaten feed was estimated from the number of uneaten pellets using the average weight of a pellet from each feed (Helland et al., 1996).

[0117] Specific growth rates (SGR) were calculated as

$$\text{SGR (\% day}^{-1}\text{)} = 100 \times (\ln (W_2/W_1)) \times d^{-1}$$

where W_1 and W_2 were the weights (g) at the two times and d was the number of days.

[0118] At the end of the experiment fish were starved for one day prior to being anaesthetized (50mg l⁻¹, benzocaine) and their weight and fork length measured. Three fish per tank were killed by a blow to the head after immersion in anaesthetic. Samples of tissue were dissected with red muscle and white muscle sampled below the dorsal fin. Samples were frozen at -80°C until analysis.

Results

[0119] No significant difference was found between fish fed the four diets with respect to initial and final weight, weight gain, specific growth rate (SPR), total feed consumption (FC), feed efficiency ratio (FER), hepatosomatic index (HSI) or survival as determined using ANOVA (Table 5).

[0120] After 42 days there was no statistical difference in the composition of flesh lipid with respect to the lipid classes for the different dietary groups, in either red or white muscle (Tables 6 and 7). The predominant lipid class in red muscle was TAG (94.0-96.7%). There was significantly ($p > 0.02$) less TAG in the fed fish (42.0-67.0%) compared to the initial measurement (82.0%) for the white muscle.

[0121] For fatty acid composition, there were significantly ($p > 0.01$) higher levels of 18:3 ω 3 and 18:4 ω 3, in both white and red muscle tissues, in the fish fed SO than in fish fed the Mix diet. Both 18:3 ω 3 and 18:4 ω 3 levels were significantly higher than in the FO and CO fed fish (Tables 6 and 7). There were significantly ($p > 0.01$) higher levels in both muscle tissues of 22:6 ω 3 and total ω 3 in the FO and SO diets compared to the Mix and CO diets. There were significantly ($p > 0.01$) higher levels of 20:5 ω 3 in the FO and SO fed fish compared to the CO fed fish in both the red and white muscle. The ratio of ω 3/ ω 6 was significantly ($p > 0.01$) lower in the CO and Mix diet fed fish compared to the SO and FO diets.

Table 5. Growth and efficiencies of Atlantic salmon fed experimental feeds with Canola oil (CO), Stearidonic acid rich oil (SO), 1:1 CO:SO (Mix) and Fish oil (FO) (mean \pm SE).

	Feed											
	CO			Mix			SO			FO		
Initial weight (g)	46.2	\pm 2.5		44.6	\pm 1.1		44.8	\pm 1.1		42.3	\pm 1.2	
Final Weight (g)	81.4	\pm 8.4		80.1	\pm 1.9		76.9	\pm 2.2		76.5	\pm 3.3	
Weight gain (g)	35.1	\pm 5.9		35.5	\pm 0.8		32.1	\pm 2.0		34.1	\pm 3.1	
SGR (% day ⁻¹)	1.2	\pm 0.2		1.3	\pm 0.0		1.2	\pm 0.1		1.2	\pm 0.1	
Total FC (g DM)	41.4	\pm 2.0		41.9	\pm 0.8		40.5	\pm 0.7		38.0	\pm 1.8	
FER (g/g DM)	0.8	\pm 0.1		0.8	\pm 0.0		0.8	\pm 0.1		0.9	\pm 0.0	
HSI (%)	1.0	\pm 0.1		1.0	\pm 0.1		0.9	\pm 0.2		0.9	\pm 0.1	
Survival	98.7	\pm 1.4		98.7	\pm 1.4		100.0	\pm 0.0		100.0	\pm 0.0	

SO, stearidonic rich oil diet; CO, canola oil diet; Mix, 1:1 mix diet of canola oil and stearidonic acid rich oils; FO, fish oil diet; DM, Dry matter

¹SGR, Specific growth rate = $100 \times (\ln (W_{\text{final(g)}}/W_{\text{initial(g)}})) \times \text{number of days } (d)^{-1}$

²FC, Total feed consumption = Total amount (g DM) consumed by an individual over 42 days.

³FER, feed efficiency ratio = total weight gain (g) / total feed consumption (g DM).

⁴HSI, hepatosomatic index = $100 (\text{liver weight (g WW)} / \text{Total body weight (g WW)})$. Survival during growth experiment.

[0122] In both muscle tissues, the FO diet surprisingly provided significantly ($p > 0.01$) higher levels of 14:0, 16:0 and total saturates compared with CO and Mix fed. The FO diet also provided significantly ($p > 0.01$) higher levels of 14:0 in both muscle tissues and 16:0 and total saturates in the red muscle compared with the SO fed fish. In both muscle tissues, FO and SO fed salmon had significantly ($p > 0.01$) lower levels of 18:1 ω 9 and total MUFA compared to the fish fed CO and Mix diets. There was significantly ($p > 0.01$) higher levels of 18:2 ω 6 and total ω 6 in the fish fed CO and Mix diets compared with FO fed fish.

Table 6. FAME Content and lipid class composition of total lipid of Red muscle samples of Atlantic salmon fed Canola Oil (CO) 1:1 mix of Canola Oil:Stearidonic oil (Mix), Stearidonic oil (SO) diets and Fish oil (FO)

FAME	Initial	SE	CO	SE	Mix	SE	SO	SE	FO	SE	Sig	f
14:0	4.0	± 0.3b	3.3	± 0.2a	3.0	± 0.2a	3.9	± 0.1a	5.2	± 0.2b	0.01	21.9
16:0	16.7	± 0.4b,c	12.9	± 0.2a	12.7	± 0.3a	14.4	± 0.4a,b	16.7	± 0.3c	0.01	14.8
18:0	4.7	± 0.3	4.2	± 0.0	4.6	± 0.1	4.5	± 0.0	4.3	± 0.1		
Other SFA ^e	2.0	± 0.0c	1.7	± 0.0b	1.4	± 0.0a	1.8	± 0.0b	1.9	± 0.0b,c	0.01	11.1
Total SFA	27.3	± 0.7b,c	22.2	± 0.9a	21.7	± 0.4a	24.6	± 1.0a,b	28.2	± 0.4c	0.01	13.9
16:1 ω 7c	5.9	± 0.4c,d	5.0	± 0.2b,c	4.3	± 0.4a	5.8	± 0.2b,c	7.4	± 0.4d	0.01	16.3
18:1 ω 9c	13.4	± 0.6a	30.5	± 1.3b	27.9	± 1.1b	16.1	± 0.5a	14.9	± 0.2a	0.01	26.5
18:1 ω 7c	3.3	± 0.1c,d	3.3	± 0.0b,c	2.9	± 0.1a	3.0	± 0.1a,b	3.5	± 0.1d	0.01	9.3
20:1 ω 9c	1.4	± 0.1b	2.0	± 0.0b	1.3	± 0.4a,b	1.7	± 0.0b	0.4	± 0.4a	0.01	5.0
Other MUFA ^f	2.5	± 0.0a	2.3	± 0.0a	2.5	± 0.0a	2.5	± 0.0a	4.2	± 0.0b	0.01	10.2
Total MUFA	26.4	± 0.5a	43.2	± 2.2b	38.9	± 1.1b	29.1	± 0.6a	30.5	± 0.5a	0.01	28.2
18:3 ω 3	0.7	± 0.0a	2.0	± 0.1b	3.9	± 0.2c	5.7	± 0.2d	2.0	± 0.0b	0.01	65.8
ALA												
18:4 ω 3	2.3	± 0.2a	2.2	± 0.1a	3.7	± 0.0b	4.3	± 0.3c	2.6	± 0.1a	0.01	92.2
SDA												
20:4 ω 3	1.1	± 0.0a	1.0	± 0.0a,b	1.2	± 0.0b	1.4	± 0.0c	1.2	± 0.0a,b	0.01	10.4
20:5 ω 3	8.6	± 0.2b	4.8	± 0.3a	4.4	± 0.3a	6.2	± 0.2b	7.6	± 0.3b	0.01	25.2
EPA												
22:5 ω 3	3.2	± 0.1c	2.3	± 0.1a,b	2.2	± 0.2a	3.1	± 0.1b,c	3.7	± 0.1c	0.01	11.0
DPA												
22:6 ω 3	19.2	± 1.0c	9.6	± 0.5a	9.0	± 0.7a	12.5	± 0.6b	14.4	± 0.7b	0.01	13.6
DHA												
Other ω 3 ^g	1.0	± 0.0	0.8	± 0.0	0.7	± 0.0	1.1	± 0.0	1.3	± 0.0		
Total ω 3	36.2	± 0.6b	22.6	± 1.9a	25.0	± 1.1a	34.3	± 1.1b	32.8	± 0.6b	0.01	16.3
18:2 ω 6	2.8	± 0.1a	7.6	± 0.4b	9.1	± 0.7b	6.2	± 0.6a,b	3.9	± 0.7a	0.01	12.7
LA												
18:3 ω 6	0.2	± 0.0a	0.5	± 0.0b	0.5	± 0.0b	1.5	± 0.2c	0.8	± 0.2a,b	0.01	8.1

(continued)

FAME	Initial	SE	CO	SE	Mix	SE	SO	SE	FO	SE	Sig	f
20:3 ω 6	0.2	± 0.0a	0.6	± 0.0b,c	1.0	± 0.1c	0.7	± 0.1c	0.2	± 0.1a,b	0.01	12.5
20:4 ω 6	1.3	± 0.2a,b	0.5	± 0.0a	0.5	± 0.0a	0.6	± 0.0a,b	0.6	± 0.0b	0.01	5.3
22:5 ω 6	0.3	± 0.0b	0.2	± 0.0a,b	0.2	± 0.0a	0.3	± 0.0a,b	0.3	± 0.0b	0.01	5.5
Other ω 6 ^h	0.8	± 0.0	1.0	± 0.0	0.8	± 0.0	0.8	± 0.0	0.9	± 0.0		
Total ω 6	5.3	± 0.2a	9.9	± 0.9c	11.6	± 0.8c	8.5	± 1.2b,c	5.8	± 0.1a,b	0.01	12.8
Other PUFA ⁱ	4.8	± 0.2	2.0	± 0.0	2.8	± 0.1	3.4	± 0.1	2.7	± 0.1		
Total PUFA	46.3	± 1.3b	34.6	± 2.1a	39.4	± 1.3a,b	46.3	± 1.2b	41.3	± 0.9b	0.01	15.1
Ratios												
ω 3/ ω 6	6.8	± 0.3b	2.3	± 0.4a	2.2	± 0.2a	4.0	± 0.1b	5.6	± 0.2b	0.01	54.5
Lipid Class												
TAG	96.7	± 0.4	96.7	± 0.3	95.4	± 0.2	96.6	± 0.4	94.0	± 0.9		
FFA	0.7	± 0.1a	0.7	± 0.1a	1.8	± 0.1a,b	0.5	± 0.1a	2.5	± 0.6b	0.01	8.0
ST	0.8	± 0.2	1.1	± 0.0	1.0	± 0.1	1.0	± 0.0	0.9	± 0.4		
PL	1.8	± 0.2	1.5	± 0.2	1.7	± 0.2	1.8	± 0.3	2.6	± 0.3		
mg/g Wet ^j	17.8	± 1.0	22.9	± 0.7	22.2	± 1.1	24.5	± 1.8	28.1	± 5.5		

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Lipid Class	mg/g Dry ^j	±	2.8	±	53.6	±	0.9	±	57.0	±	7.7	±	54.1	±	2.9	±	56.6	±	7.1
SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; DHA, Docosahexaenoic Acid; DPA, Docosapentaenoic Acid; EPA, Eicosapentaenoic Acid; SDA, Stearidonic acid; LA, Linoleic acid; ALA, α - Linolenic acid; TAG; Triacylglycerol; FFA, free fatty acid; ST, sterol; PL, polar lipid; WW, wet weight; Sig, Significance; <i>f</i> , Mean sum of squares.																			
^{a,b,c,d} Mean values across the row not sharing a common letter were significantly different as determined by Turkey-Kramer HSD; <i>df</i> = 4, 15.																			
^e Other SFA includes 15:0, 17:0, 20:0, 22:0 and 24:0																			
^f Other MUFA includes 16:1 ω 9, 16:1 ω 5, 18:1 ω 5, 20:1 ω 7, 22:1 ω 9, 22:1 ω 11 and 24:1 ω 9																			
^g Other ω 3 PUFA include 21:5 ω 3 and 24:6 ω 3																			
^h Other ω 6 PUFA include 20:2 ω 6, 20:3 ω 6, 22:4 ω 6 and 24:5 ω 6																			
ⁱ Other PUFA include 16:2 ω 4, 16:3 ω 4 and 18:2 ω 9																			
^j Determined by TLC-FID																			

Table 7. FAME Content and lipid class composition of the total lipid of white muscle samples of Atlantic salmon

FAME	Initial	SE	CO	SE	Mix	SE	SO	SE	FO	SE	Sig	f
14:0	3.9	± 0.4b	2.3	± 0.1a	1.9	± 0.2a	2.3	± 0.1a	3.6	± 0.2b	0.01	13.0
16:0	18.3	± 0.4a,b	14.8	± 0.3a	15.2	± 0.3a	16.8	± 0.6a,b	19.7	± 0.3b	0.01	5.0
18:0	5.7	± 0.3	4.8	± 0.0	5.6	± 0.1	5.6	± 0.1	5.0	± 0.1		
Other SFA ^e	1.8	± 0.0	1.0	± 0.0	0.8	± 0.0	1.0	± 0.0	1.4	± 0.0		
Total SFA	29.7	± 1.1a,b	22.9	± 0.9a	23.5	± 1.7a	25.7	± 1.4a,b	29.6	± 0.5b	0.01	5.1
16:1 ω 7c	5.6	± 0.5b	3.1	± 0.2a	2.7	± 0.4a	3.1	± 0.1a	5.1	± 0.4b	0.01	14.1
18:1 ω 9c	14.2	± 0.7b	27.2	± 1.3c	22.2	± 1.3b,c	11.3	± 0.8a	11.0	± 6.7a	0.01	5.9
18:1 ω 7c	3.2	± 0.1c	2.9	± 0.0b,c	2.5	± 0.1a,b	2.2	± 0.1a	3.1	± 0.1c	0.01	18.0
20:1 ω 9c	1.1	± 0.2	1.5	± 0.0	0.9	± 0.4	1.0	± 0.0	0.5	± 0.4		
Other MUFA ^f	3.3	± 0.1	2.1	± 0.0	2.0	± 0.0	1.7	± 0.0	2.4	± 0.0		
Total MUFA	27.3	± 0.7b	36.8	± 2.3c	30.2	± 2.1b	19.3	± 2.4a	22.0	± 1.1a	0.01	4.7
18:3 ω 3 ALA	1.0	± 0.0a	2.1	± 0.1b	3.5	± 0.7c	6.3	± 0.4d	1.7	± 0.1b	0.01	30.1
18:4 ω 3 SDA	2.0	± 0.2a	1.6	± 0.0a	2.8	± 0.3a	3.9	± 0.1b	2.0	± 0.1a	0.01	10.8
20:4 ω 3	1.1	± 0.0a,b	0.8	± 0.0a	1.2	± 0.0a,b	1.3	± 0.1b	1.0	± 0.0a,b	0.01	4.7
20:5 ω 3 EPA	7.4	± 0.2b,c	4.8	± 0.3a	5.4	± 0.3a,b	7.3	± 0.5b,c	8.6	± 0.3c	0.01	7.0
22:5 ω 3 DPA	3.0	± 0.1b,c	2.1	± 0.1a	2.2	± 0.2a	2.6	± 0.1a,b	3.6	± 0.2c	0.01	10.3
22:6 ω 3 DHA	20.0	± 1.2a,b	16.2	± 0.9a	18.3	± 0.7a	22.2	± 0.6b	24.2	± 0.7b	0.01	8.0
Other ω 3 ^g	0.8	± 0.1	0.5	± 0.0	0.3	± 0.0	0.6	± 0.0	0.9	± 0.0		
Total ω 3	35.4	± 0.2b	28.2	± 2.1a	33.7	± 1.1a,b	44.2	± 2.6c	42.0	± 2.4b,c	0.01	14.4
18:2 ω 6LA	2.9	± 0.2a	7.6	± 0.4b	7.5	± 0.7b	5.6	± 0.6b	3.2	± 0.7a	0.02	4.0
18:3 ω 6	0.6	± 0.2a	0.5	± 0.0a	0.9	± 0.4a,b	1.5	± 0.2b	0.4	± 0.3a	0.01	12.0
20:3 ω 6	0.1	± 0.0a	1.0	± 0.1b	1.1	± 0.1b	0.9	± 0.2b	0.1	± 0.1a	0.02	4.5
20:4 ω 6	1.3	± 0.2	1.0	± 0.0	1.3	± 0.0	1.0	± 0.1	0.9	± 0.0		
22:5 ω 6	0.2	± 0.0	0.4	± 0.0	0.2	± 0.0	0.3	± 0.0	0.2	± 0.0		
Other ω 6 ^h	1.3	± 0.2	0.9	± 0.0	0.6	± 0.0	0.5	± 0.0	0.4	± 0.0		
Total ω 6	5.9	± 0.1a	10.8	± 1.3b	10.6	± 1.8b	8.3	± 1.4a,b	4.7	± 0.2a	0.02	6.2
Other PUFA ⁱ	1.7	± 0.0	1.4	± 0.0	1.9	± 0.1	2.5	± 0.1	1.7	± 0.1		
Total PUFA	43.0	± 1.2a	40.4	± 1.4a	46.3	± 2.3 a,b	55.0	± 1.1c	48.4	± 0.9b		

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FAME	Initial	SE	CO	SE	Mix	SE	SO	SE	FO	SE	Sig	f
Ratios												
ω3/ω6	6.0	± 0.1b,c	2.6	± 0.5a	3.2	± 0.1a	5.3	± 0.1b	8.8	± 0.2c	0.02	16.2
Lipid Class												
TAG	82.0	± 2.6c	46.2	± 4.9a	67.0	± 4.6a	59.9	± 2.0a	50.5	± 12.7a	0.02	3.1
FFA	1.7	± 0.3b,c	1.9	± 0.2c	0.4	± 0.1a	1.8	± 0.1b,c	0.5	± 0.2a,b	0.02	5.9
ST	2.1	± 0.4	4.0	± 0.2	2.1	± 0.2	3.8	± 0.3	2.2	± 0.3		
PL	14.2	± 2.1	47.6	± 4.9	30.5	± 4.7	34.6	± 1.9	46.8	± 12.4		
mg/g Wet	8.4	± 0.3	9.1	± 0.1	9.0	± 0.2	9.2	± 0.1	8.2	± 0.3		
mg/g Dry	10.1	± 1.0a	15.2	± 0.4b	14.2	± 0.6b	14.9	± 0.2b	15.1	± 1.0b	0.02	8.9

Abbreviations and other footnote definitions, see Table 8.

Discussion

[0123] The inclusion of SO at 130 or 65 g/kg of diet for Atlantic salmon parr did not significantly influence growth or feed conversion rates compared to other experimental diets during the 42 day growth trial in freshwater (Table 5). There was little effect between diets in the lipid class profiles (Tables 6 and 7). There was significantly less TAG in the white muscle of the fed fish compared to the diet due to the inclusion of oil in the diet at a level of 130 g/kg compared to the commercial diet (approx. 300 g/kg) they were fed pre-experiment.

[0124] Fish muscle FA profiles were closely related to the FA profile of their diet. It has been shown previously for salmon fed using canola, sunflower and linseed oils, i.e. diets rich in ALA and without EPA and DHA, that there was a significant reduction in total ω 3 and ω 3 LC-PUFA, in particular DHA and EPA (Brandsen et al., 2003; Bell et al., 2003; Polvi and Ackman, 1992; Bell et al., 2004). Therefore, minimal conversion to, or negligible accumulation of, LC-PUFA occurred when fish were fed vegetable oil. In those studies growth rates and the health of fish fed vegetable oils were not affected.

[0125] In the study described here, Atlantic salmon parr sizes were initially $43.6\text{g} \pm 0.7\text{g}$ to a final weight of $72.4\text{g} \pm 1.9\text{g}$. The fish were at an important stage of the growth. Pre-smoltification Atlantic salmon store FA, in particular ω 3 LC-PUFA, prior to the energy requiring transfer to salt water, during which salmon undergo major changes in their lipid metabolism.

[0126] The inclusion of SDA at 14.3 or 7.2 g/kg significantly influenced the FA profiles of the salmon (Tables 6 and 7). Fish fed on the diet containing the higher level of SDA had significantly higher levels of EPA, DPA, DHA and total ω 3 in the muscle samples than fish fed on the CO diet. In some respects, the fatty acid composition of the fish tissues was improved over that of fish fed the FO diet. For example, the level of saturated fat was reduced. The SO diet was also advantageous for this feature in combination with the high levels of LC-PUFA.

[0127] Neither the CO diet nor the SO diet contained EPA or DHA at substantial levels, being $<0.7\%$ of the fatty acid present in the lipid, the trace level probably originating with the fishmeal component. Therefore the increased accumulation of EPA, DPA and DHA in the fish tissues must have represented increased biosynthesis of the fatty acids from SDA in the fish.

[0128] This experiment showed that high levels of total ω 3, DHA and EPA could be maintained in fish such as salmon without their inclusion as dietary FA. This experiment also demonstrated that the levels of fatty acids achieved, as reported in Tables 6 and 7, for example the levels of SDA, EPA, DPA, DHA, total LC-PUFA ω 3, or total ω 3 PUFA (includes C18 fatty acids), were minimum levels that could be achieved through feeding the fish a diet including plant derived SDA, and that even higher levels could be expected by using diets with even higher levels of SDA and/or longer feeding times.

[0129] The conversion of ALA to SDA involves the desaturation at the Δ 6 position of the carbon chain with further chain elongation steps, followed by Δ 5 desaturation to form EPA. The synthesis of EPA to DHA requires additional chain elongations and also involves the Δ 6 desaturation in the conversion of 24:5 ω 3 to 24:6 ω 3 before chain shortening to DHA (Figure 1); this is termed the Sprecher pathway. With the conversion of 18:2 ω 6 to 20:4 ω 6 also using the Δ 6 desaturase, it was possible that the high levels of 18:2 ω 6 in vegetable oils might compete for this enzyme and therefore minimal conversion of ALA to SDA would occur in the ω 3 pathway. We have found here that this problem can be alleviated by adding SDA in the fish diet. The results indicated that a SDA rich plant oil could be used as a source of dietary oil for aquafeeds and, importantly, that the use of SDA oil did not affect the amount of ω 3 LC-PUFA in the FA profile of salmon muscle.

Example 3. Prawn and Lobster feedstuffs

[0130] For feeding of lobsters, prawns or other crustacean with diets high in SDA oil, the following feed compositions can be used (Table 8). Values provided as g/kg dry matter.

Example 4. Isolation of a Gene Encoding a Δ 6-Desaturase from *Echium plantagineum*

[0131] Some plant species such as evening primrose (*Oenothera biennis*), common borage (*Borago officinalis*), black-currant (*Ribes nigrum*), and some *Echium* species belonging to the *Boraginaceae* family contain the ω 6- and ω 3-desaturated C18 fatty acids, γ -linolenic acid (18:3 ω 6, GLA) and stearidonic acid (18:4 ω 3, SDA) in their leaf lipids and seed TAG (Guil-Guerrero et al., 2000). GLA and SDA are recognized as beneficial fatty acids in human nutrition. The first step in the synthesis of LC-PUFA is a Δ 6-desaturation. GLA is synthesized by a Δ 6-desaturase that introduces a double bond into the Δ 6-position of LA. The same enzyme is also able to introduce a double bond into Δ 6-position of ALA, producing SDA. Δ 6-desaturase genes have been cloned from members of the *Boraginaceae*, like borage (Sayanova et al., 1997) and two *Echium* species (Garcia-Maroto et al., 2002).

Table 8. Prawn and Lobster feedstuffs.

	Spiny Lobster	Prawn	
5	Fish meal (defatted)	250	0
	Fish meal (standard)	0	200
	Krill meal	0	185
	Soybean Meal	150	150
10	Wheat gluten	100	100
	<i>Echium plantagineum</i> Oil	110	100
	Cholesterol	2	2
	Lecithin	12	12
	Pre-gel starch	175	100
15	Manuacol	60	60
	Vit Pre-Mix	2.00	2.00
	Banox E	0.20	0.20
	Choline Chloride	0.20	0.20
20	Vitamin C ^a	1.00	1.00
	Carophyll pink	1.50	1.50
	Min Pre-Mix ^b	0.01	0.01
	TSP Phosphate	20.00	20.00
25	Mussel meal	50.00	0.00
	Filler	66.00	66.00
	Total	1000	1000
	SDA	1.54	1.40
30	SO, stearidonic rich oil crossential SA14 from Croda chemicals; ^a L-Ascorbyl-2-polyphosphate (Stay-C, Roche Vitamins Australia, French Forest, NSW, Australia). ^b Mineral mix (TMV4) to supply per kilogram feed: 117mg CuSO ₄ .5H ₂ O, 7.19 mg KI, 1815 mg FeSO ₄ .7H ₂ O, 307 mg MnSO ₄ .H ₂ O, 659 mg ZnSO ₄ .7H ₂ O, 3.29 mg Na ₂ SeO ₃ , 47.7 mg CoSO ₄ .7H ₂ O		
35	Soybean (Hamlet Protein A/S, Horsens, Denmark), wheat gluten (Starch Australasia, Land Cove, NSW, Australia) and BOIIC pre-gelatinised maize starch (Penford Australia Limited, Lane Cove, NSW, Australia) were used. Stay-C and Carophyll pink were supplied from Roche Vitamins Australia (French Forest, NSW, Australia), Mussel meal obtained from New Zealand Greenshell™ mussel, (Sealord P/L Nelson, New Zealand) and the remaining ingredients were supplied by Sigma-Aldrich (Castle Hill, NSW, Australia).		

40 [0132] *Echium plantagineum* is a winter annual native to Mediterranean Europe and North Africa. Its seed oil is unusual in that it has a unique ratio of $\omega 3$ and $\omega 6$ fatty acids and contains high amounts of GLA (9.2%) and SDA (12.9%) (Guil-Guerrero et al., 2000), suggesting the presence of $\Delta 6$ -desaturase activity involved in desaturation of both $\omega 3$ and $\omega 6$ fatty acids in seeds of this plant.

45 Cloning of *E. plantagineum* *EpID6Des* gene

50 [0133] Degenerate primers with built-in *Xba*I or *Sac*I restriction sites corresponding to N- and C-termini amino acid sequences MANAIKKY (SEQ ID NO:14) and EALNTHG (SEQ ID NO:15) of known *Echium pitardii* and *Echium gentianoides* (Garcia-Maroto et al., 2002) $\Delta 6$ -desaturases were used for RT-PCR amplification of $\Delta 6$ -desaturase sequences from *E. plantagineum* using a proofreading DNA polymerase Pfu Turbo® (Stratagene). The 1.35kb PCR amplification product was inserted into pBluescript SK(+) at the *Xba*I and *Sac*I sites to generate plasmid pXZP106. The nucleotide sequence of the insert was determined. It comprised an open reading frame encoding a polypeptide of 438 amino acid residues (SEQ ID NO:10) which had a high degree of homology with other reported $\Delta 6$ -desaturases from *E. gentianoides* (SEQ ID NO:11), *E. pitardii* (SEQ ID NO:12) and *Borago officinalis* (SEQ ID NO:4). It has a cytochrome b₅ domain at the N-terminus, including the HPGG (SEQ ID NO:16) motif in the heme-binding region, as reported for other $\Delta 6$ - and $\Delta 8$ -desaturases (Sayanova et al. 1997; Napier et al. 1999). In addition, the *E. plantagineum* $\Delta 6$ desaturase contains three conserved histidine boxes present in majority of the 'front-end' desaturases (Napier et al., 1999). Cluster analysis

including representative members of $\Delta 6$ and A8 desaturases showed a clear grouping of the cloned gene with other $\Delta 6$ desaturases especially those from *Echium* species.

Heterologous expression of E. plantagineum $\Delta 6$ -desaturase gene in yeast

[0134] Expression experiments in yeast were carried out to confirm that the cloned *E. plantagineum* gene (cDNA sequence provided as SEQ ID NO:25) encoded a $\Delta 6$ -desaturase enzyme. The gene fragment was inserted as an *Xba*I-*Sac*I fragment into the *Sma*I-*Sac*I sites of the yeast expression vector pSOS (Stratagene) containing the constitutive *ADH1* promoter, resulting in plasmid pXZP271. This was transformed into yeast strain S288C α by a heat shock method and transformant colonies selected by plating on minimal media plates. For the analysis of enzyme activity, 2mL yeast clonal cultures were grown to an O.D.₆₀₀ of 1.0 in yeast minimal medium in the presence of 0.1% NP-40 at 30°C with shaking. Precursor free-fatty acids, either linoleic or linolenic acid as 25mM stocks in ethanol, were added so that the final concentration of fatty acid was 0.5mM. The cultures were transferred to 20°C and grown for 2-3 days with shaking. Yeast cells were harvested by repeated centrifugation and washing first with 0.1% NP-40, then 0.05%NP-40 and finally with water. Fatty acids were extracted and analyzed. The peak identities of fatty acids were confirmed by GC-MS.

[0135] The transgenic yeast cells expressing the *Echium EplD6Des* were able to convert LA and ALA to GLA and SDA, respectively. Around 2.9% of LA was converted to GLA and 2.3% of ALA was converted to SDA, confirming the $\Delta 6$ -desaturase activity encoded by the cloned gene.

Functional expression of E. plantagineum $\Delta 6$ -desaturase gene in transgenic tobacco

[0136] In order to demonstrate that the *EplD6Des* gene could confer the synthesis of $\Delta 6$ desaturated fatty acids in transgenic plants, the gene was expressed in tobacco plants. To do this, the gene fragment was excised from pXZP106 as an *Xba*I-*Sac*I fragment and cloned into the plant expression vector pBI121 (Clontech) at the *Xba*I and *Sac*I sites under the control of a constitutive 35S CaMV promoter, to generate plant expression plasmid pXZP341. This was introduced into *Agrobacterium tumefaciens* AGL1, and used for transformation of tobacco W3 8 plant tissue, by selection with kanamycin.

[0137] Northern blot hybridization analysis of transformed plants was carried out to detect expression of the introduced gene, and total fatty acids present in leaf lipids of wild-type tobacco W38 and transformed tobacco plants were analysed as described above. Untransformed plants contained appreciable amounts of LA (21 % of total fatty acids) and ALA (37% of total fatty acids) in leaf lipids. As expected, neither GLA nor SDA, products of $\Delta 6$ -desaturation, were detected in the untransformed leaf. Furthermore, transgenic tobacco plants transformed with the pBI121 vector had similar leaf fatty acid composition to the untransformed W38 plants. In contrast, leaves of transgenic tobacco plants expressing the *EplD6Des* gene showed the presence of additional peaks with retention times corresponding to GLA and SDA. The identity of the GLA and SDA peaks were confirmed by GC-MS. Notably, leaf fatty acids of plants expressing the *EplD6Des* gene consistently contained approximately a two-fold higher concentration of GLA than SDA even when the total $\Delta 6$ -desaturated fatty acids amounted up to 30% of total fatty acids in their leaf lipids (Table 9).

Table 9. Fatty acid composition in lipid from transgenic tobacco leaves (%).

Plant	16:0	18:0	18:1	18:2	GLA	18:3	SDA	Total $\Delta 6$ -desaturated products
W38	21.78	5.50	2.44	21.21	-	37.62	-	-
ET27-1	20.33	1.98	1.25	10.23	10.22	41.10	6.35	16.57
ET27-2	18.03	1.79	1.58	14.42	1.47	53.85	0.48	1.95
ET27-4	19.87	1.90	1.35	7.60	20.68	29.38	9.38	30.07
ET27-5	15.43	2.38	3.24	11.00	0.84	49.60	0.51	1.35
ET27-6	19.85	2.05	1.35	11.12	4.54	50.45	2.19	6.73
ET27-8	19.87	2.86	2.55	11.71	17.02	27.76	7.76	24.78
ET27-11	17.78	3.40	2.24	12.62	1.11	51.56	0.21	1.32
ET27-12	16.84	2.16	1.75	13.49	2.71	50.80	1.15	3.86

[0138] Northern analysis of multiple independent transgenic tobacco lines showed variable levels of the *EplD6Des* transcript which generally correlated with the levels of $\Delta 6$ -desaturated products synthesized in the plants. For example, transgenic plant ET27-2 which contained low levels of the *EplD6Des* transcript synthesised only 1.95% of its total leaf lipids as $\Delta 6$ -desaturated fatty acids. On the other hand, transgenic plant ET27-4 contained significantly higher levels of *EplD6Des* transcript and also had a much higher proportion (30%) of $\Delta 6$ -desaturated fatty acids in its leaf lipids.

[0139] Analysis of the individual tobacco plants showed that, without exception, GLA was present at a higher concentration than SDA even though a higher concentration of ALA than LA was present in untransformed plants. In contrast, expression of *EplD6Des* in yeast had resulted in approximately equivalent levels of conversion of LA into GLA and ALA into SDA. *Echium plantagineum* seeds, on the other hand, contain higher levels of SDA than GLA. *EplD6Des* probably carries out its desaturation *in vivo* in *Echium plantagineum* seeds on LA and ALA esterified to phosphatidyl choline (PC) (Jones and Harwood 1980). In the tobacco leaf assay, the enzyme is most likely desaturating LA and ALA esterified to the chloroplast lipid monogalactosyldiacylglycerol (MGDG) (Browse and Slack, 1981). In the yeast assay, free fatty acid precursors LA and ALA added to the medium most likely enter the acyl-CoA pool and are available to be acted upon by *EplD6Des* in this form.

[0140] In conclusion, the transgenic tobacco plant described herein can be used to produce feedstuffs of the invention.

Functional expression of E. platangineum Δ6-desaturase gene in transgenic seed

[0141] To show seed-specific expression of the *Echium* Δ6-desaturase gene, the coding region was inserted into the seed-specific expression cassette as follows. An *NcoI*-*SacI* fragment including the Δ6-desaturase coding region was inserted into pXZP6, a pBluescriptSK derivative containing a *Nos* terminator, resulting in plasmid pXZP157. The *SmaI*-*Apal* fragment containing the coding region and terminator *EplD6Des*-*NosT* was cloned into pWVec8-Fp1 downstream of the *Fp1* promoter, resulting in plasmid pXZP345. The plasmid pXZP345 was used for transforming wild type *Arabidopsis* plants, ecotype Columbia, and transgenic plants selected by hygromycin B selection. The transgenic plants transformed with this gene were designated "DP" plants.

[0142] Fatty acid composition analysis of the seed oil from T2 seed from eleven T1 plants transformed with the construct showed the presence of GLA and SDA in all of the lines, with levels of Δ6-desaturation products reaching to at least 11% (Table 10). This demonstrated the efficient Δ6-desaturation of LA and ALA in the seed.

Example 5. Transformation of flax with a seed-specific *Echium* Δ6 fatty acid desaturase gene construct

[0143] The full protein coding region of the *Echium* Δ6 fatty acid desaturase gene was PCR amplified with the following primers incorporating an *XhoI* site at the both ends: Ed6F: 5'-ACTCGAGCCACCATGGCTAATGCAATCAA-3' (SEQ ID NO:17) and Ed6R: 5'-CCTCGAGCTCAACCATGAGTATTAAGAG-3' (SEQ ID NO:18). PCR was conducted by heating to 94°C for 2 min, followed by 30 cycles of 94°C for 40 sec, 62°C for 40 sec and 72°C for 1 min 20 sec. After the last cycle, reactions were incubated for 10 min at 72°C. The PCR fragment was cloned into a pGEMTeasy® vector (Promega) and sequenced to ensure that no PCR-induced errors had been introduced. The insert was then digested with *XhoI* and inserted into the *XhoI* site of the binary vector, pWVec8, in a sense orientation between the promoter derived from a seed-specifically expressed flax 2S storage protein gene, linin, and its polyadenylation site/transcription terminator.

Table 10. Fatty acid composition in transgenic *Arabidopsis* seeds expressing Δ6-desaturase from *Echium*.

Plant	Fatty acid (%)									Total Δ 6-desaturation products (%)
	16:0	18:0	18:1 ^{Δ9}	18:2 ^{Δ9,1} (LA)	18:3 ^{Δ6,9,12} (GLA)	18:3 ^{Δ9,12,15} (ALA)	18:4 ^{Δ6,9,12,1} (SDA)	20:0	20:1	
Columbia										
DP-2	8.0	2.8	22.9	27.3	2.5	11.3	0.7	1.6	15.8	3.2
DP-3	7.8	2.7	20.6	25.9	3.0	12.1	0.8	1.7	17.8	3.8
DP-4	7.8	2.8	20.4	28.5	1.2	13.7	0.4	1.7	16.1	1.5
DP-5	8.2	3.2	17.4	29.3	1.2	14.2	0.3	2.1	15.6	1.6
DP-7	8.2	2.9	18.4	26.7	5.0	12.7	1.4	1.7	15.2	6.4
DP-11	9.0	3.5	17.8	28.4	3.0	13.4	0.9	2.1	13.9	3.8
DP-12	8.6	3.0	18.9	27.8	3.3	12.6	1.0	1.8	15.4	4.3
DP-13	8.7	2.9	14.4	27.3	8.5	13.7	2.6	1.7	12.4	11.1
DP-14	9.3	2.9	14.2	32.3	2.1	15.4	0.7	1.8	12.8	2.8
DP-15	8.2	2.9	17.8	30.1	0.3	15.3	0.2	1.9	15.5	0.5
DP-16	8.0	2.8	19.5	29.2	2.7	13.1	0.8	1.7	14.2	3.5

[0144] The binary vector, pWVec8 contained a hygromycin resistance gene as a selectable marker for plant transformation (Wang et al., 1998). The construct, designated pVLin-Ed6 and containing the *Echium* Δ6 desaturase gene for

seed-specific expression was shown schematically in Figure 2. The linin promoter (SEQ ID NO:23) and terminator (SEQ ID NO:24) have previously been shown to confer expression in a highly specific manner in developing flax embryos, being expressed maximally in flax seed at the same time as oil accumulation in flax seeds. Both the linin promoter and terminator elements were able to drive seed specific expression of transgenes in flax at levels comparable to the highly

active bean *phaseolin* promoter.

[0145] Approximately 150 hypocotyls were excised from 6-7 day old seedlings of flax cultivar Ward grown in sterile condition on MS media. This cultivar was found to produce the highest transformation efficiency among many flax cultivars, however many other cultivars were also amendable for gene transformation. The hypocotyls were inoculated and co-cultivated with *Agrobacterium tumefaciens* strain AGL1 harbouring the binary construct pVLin-Ed6 in a similar fashion to that described for *Brassica* transformation in Example 1. Following a co-cultivation period of 3-4 days at 24°C, the hypocotyls were transferred onto selection medium which was MS medium containing 200 mg/l Cefotaxime, 10 mg/l hygromycin, 1 mg/l BAP (6-benzyl-aminopurine) and 0.1 mg/l NAA (naphthaleneacetic acid). Shoot development was initiated after about 2 weeks. Shoots were transferred onto fresh MS medium with the same additives except NAA was reduced to 0.02 mg/l. After 2-3 weeks, healthy green shoots were transferred onto fresh MS media without growth regulators for induction of roots. Rooted shoots were planted in potting mix in glasshouse.

[0146] The transgenic nature of regenerated flax plants was confirmed by PCR amplification of part of the *Echium* $\Delta 6$ fatty acid desaturase sequence with the primers Ed6sl, 5'-ACTCTGTTTCTGAGGTGTCCA-3' (SEQ ID NO:19); and Ed6a1, 5'-CATATTAACCCTAGCCATACACAT-3' (SEQ ID NO:20). DNA extracted from individual, regenerated flax plants was used as template in PCR reactions using the following amplification conditions: denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 40 sec, 58°C for 40 sec and 72°C for 1 min. Seeds set on forty primary transgenic flax plants will be analysed for the presence of SDA and GLA using lipid extraction followed by gas chromatography. It is expected that high levels of SDA will be produced in many of the plants and that SDA levels will be greater than GLA levels.

[0147] Seed from the transformed flax plants or extracts such as the oil or the seed meal can be used in feed compositions for use in feeding fish or crustacea.

Example 6. (not part of the invention) Transformation of cotton with a seed-specific construct expressing an *Echium* $\Delta 6$ fatty acid desaturase gene

[0148] Cottonseed normally contains only negligible amounts (<0.5% of total fatty acids) of α -linolenic acid (ALA). In order to produce ALA at increased levels in cottonseed oil, cotton (*Gossypium hirsutum*) was transformed with a seed-specific gene construct expressing a *FAD3* gene from *Brassica napus* (Arondel et al., 1992) (encoded protein amino acid sequence provided as SEQ ID NO:27). The accession number of the cDNA clone of this gene was L01418. The full protein coding region of the *B. napus* *FAD3* gene was amplified by PCR using the primers BnFAD3-S1, 5'-CTC-CAGCGATGGTTGTTGCTAT-3' (SEQ ID NO:21) and BnFAD3-A1, 5'-AATGTCTCTGGTGACGTAGC-3' (SEQ ID NO:22). The PCR product was cloned into a pGEMTeasy® vector (Promega) and the excised by restriction digest with *NotI*. The *B. napus* *FAD3* coding sequence was inserted in the sense orientation into the *NotI* site between the soybean lectin gene promoter and terminator sequences (Cho et al., 1995), to provide a seed-specific expression construct. This vector contained an NPTII gene conferring kanamycin resistance as a selectable marker for plant transformation. This vector was introduced into *Agrobacterium* and used to transform cotton as described in Liu et al (2002). Independent transgenic plants expressing the *FAD3* gene were obtained and lines accumulating ALA retained.

[0149] Separate cotton transformation experiments were performed using a similar seed-specific lectin cassette expressing a $\Delta 6$ fatty acid desaturase, to convert LA to GLA and ALA to SDA. The full protein-coding region of the $\Delta 6$ desaturase from *Echium plantagineum* (Zhou et al., 2006; SEQ ID NO:25) was amplified by PCR using the following primers incorporating a *SmaI* site at the 5' end, and *SacI* at the 3' end. Ed6F: 5'-ATCCCCGGGTACCGGTCGCCAC-CATGGCTAATGCAATCAAGAAGTA-3' (SEQ ID NO:30) and Ed6R: 5'-TTGGAGCTCAACCATGAGTATTAAGAGCT-TC-3' (SEQ ID NO:31). The PCR fragment was cloned into pGEM-Teasy® vector (Promega) and sequenced to ensure no PCR-induced errors were introduced. The PCR amplified $\Delta 6$ desaturase gene was subsequently cloned into the corresponding *SmaI/SacI* sites in a sense orientation behind the napin (Fp1) promoter and upstream of the nos3' terminator-polyadenylation signal. *Agrobacterium tumefaciens* strain AGL1 harbouring the resulted construct, pGNapin-E6D, was used to transform cotton variety Coker315 by the method described by Liu et al. (2002).

[0150] Nine fertile independently transformed plants were obtained. The transformed cotton plants were positive for the presence of the transgene, and expression in developing seeds, by PCR and Northern blot analysis of the expressed RNA. 15 individual mature seeds from each of these primary transgenic plants were subjected to the analysis of fatty acid composition using gas chromatography (GC) as described above. Surprisingly high levels of γ -linolenic acid (GLA) were found to accumulate in four transgenic lines, while there was no detectable GLA in the non-transformed control plants. Levels of GLA of greater than 15% were observed in many seeds, and the level reached greater than 25% in some seeds that were likely to be homozygous for the introduced $\Delta 6$ desaturase gene. The accumulation of GLA is

mainly at the expense of linoleic acid. Indeed, the conversion of LA to GLA (measured as %GLA x 100/ (%LA + %GLA) in the seedoil) was highly efficient in these cottonseeds relative to seeds of other plants, being greater than 25% in many seed and reaching in excess of 45% in some seed.

[0151] Cotton lines containing both genes will be produced by crossing the transformants expressing the FAD3 gene and transformants expressing the $\Delta 6$ desaturase gene, to produce lines containing SDA. By the methods described above, oilseed plants such as cotton or flax may be produced which produce at least 5.5% SDA on a weight basis in the fatty acid of the seed oil. Preferably, the level of SDA in the fatty acid is at least 1%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45% or at least 50% on a weight basis. The efficiency of conversion of ALA to SDA (measured as %SDA x 100/ (%ALA + %SDA) in the seedoil) is at least 25% and preferably at least 45%. That is, at least 25%, preferably at least 45% of the polyunsaturated fatty acid in the cotton or flax seed that has a carbon chain of C18 or longer is desaturated at the $\Delta 6$ position.

[0152] This application claims priority from US 60/737,946.

[0153] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

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Trp Leu Gln His Asp Tyr Gly His Leu Ser Val Tyr Arg Lys Pro Lys
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Asn Ile Phe His Lys Asp Pro Asp Val Asn Met Leu His Val Phe Val
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 15 Val Ala Phe Tyr Leu Leu Pro Gly Trp Lys Pro Val Val Trp Met Val
 385 390 395 400
 Val Ser Glu Leu Met Ser Gly Phe Leu Leu Gly Tyr Val Phe Val Leu
 405 410 415
 20 Ser His Asn Gly Met Glu Val Tyr Asn Thr Ser Lys Asp Phe Val Asn
 420 425 430
 Ala Gln Ile Ala Ser Thr Arg Asp Ile Lys Ala Gly Val Phe Asn Asp
 435 440 445
 25 Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro
 450 455 460
 Thr Met Pro Arg His Asn Leu Asn Lys Ile Ser Pro His Val Glu Thr
 465 470 475 480
 30 Leu Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Met Ala Ser
 485 490 495
 Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ala Asp Ala Ala
 500 505 510
 35 Ser His Gln Gln Leu Ala Ala Ser
 515 520

<210> 7

<211> 525

40 <212> PRT

<213> Physcomitrella patens

<400> 7

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	Met	Val	Phe	Ala	Gly	Gly	Gly	Leu	Gln	Gln	Gly	Ser	Leu	Glu	Glu	Asn
	1				5					10					15	
5	Ile	Asp	Val	Glu	His	Ile	Ala	Ser	Met	Ser	Leu	Phe	Ser	Asp	Phe	Phe
				20					25					30		
	Ser	Tyr	Val	Ser	Ser	Thr	Val	Gly	Ser	Trp	Ser	Val	His	Ser	Ile	Gln
			35					40					45			
10	Pro	Leu	Lys	Arg	Leu	Thr	Ser	Lys	Lys	Arg	Val	Ser	Glu	Ser	Ala	Ala
		50					55					60				
	Val	Gln	Cys	Ile	Ser	Ala	Glu	Val	Gln	Arg	Asn	Ser	Ser	Thr	Gln	Gly
	65					70					75					80
15	Thr	Ala	Glu	Ala	Leu	Ala	Glu	Ser	Val	Val	Lys	Pro	Thr	Arg	Arg	Arg
					85					90					95	
20																
25																
30																
35																
40																
45																
50																
55																

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Ser Ser Gln Trp Lys Lys Ser Thr His Pro Leu Ser Glu Val Ala Val
 100 105 110
 His Asn Lys Pro Ser Asp Cys Trp Ile Val Val Lys Asn Lys Val Tyr
 5 115 120 125
 Asp Val Ser Asn Phe Ala Asp Glu His Pro Gly Gly Ser Val Ile Ser
 130 135 140
 Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val Phe Ser Ser Phe His Ala
 10 145 150 155 160
 Ala Ser Thr Trp Lys Ile Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu
 165 170 175
 Arg Val Glu Pro Thr Pro Glu Leu Leu Lys Asp Phe Arg Glu Met Arg
 15 180 185 190
 Ala Leu Phe Leu Arg Glu Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr
 195 200 205
 Val Met Lys Leu Leu Thr Asn Val Ala Ile Phe Ala Ala Ser Ile Ala
 20 210 215 220
 Ile Ile Cys Trp Ser Lys Thr Ile Ser Ala Val Leu Ala Ser Ala Cys
 225 230 235 240
 Met Met Ala Leu Cys Phe Gln Gln Cys Gly Trp Leu Ser His Asp Phe
 25 245 250 255
 Leu His Asn Gln Val Phe Glu Thr Arg Trp Leu Asn Glu Val Val Gly
 260 265 270
 Tyr Val Ile Gly Asn Ala Val Leu Gly Phe Ser Thr Gly Trp Trp Lys
 30 275 280 285
 Glu Lys His Asn Leu His His Ala Ala Pro Asn Glu Cys Asp Gln Thr
 290 295 300
 Tyr Gln Pro Ile Asp Glu Asp Ile Asp Thr Leu Pro Leu Ile Ala Trp
 35 305 310 315 320
 Ser Lys Asp Ile Leu Ala Thr Val Glu Asn Lys Thr Phe Leu Arg Ile
 325 330 335
 Leu Gln Tyr Gln His Leu Phe Phe Met Gly Leu Leu Phe Phe Ala Arg
 40 340 345 350
 Gly Ser Trp Leu Phe Trp Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu
 355 360 365
 Ser Pro Val Asp Arg Leu Leu Glu Lys Gly Thr Val Leu Phe His Tyr
 45 370 375 380
 Phe Trp Phe Val Gly Thr Ala Cys Tyr Leu Leu Pro Gly Trp Lys Pro
 385 390 395 400
 Leu Val Trp Met Ala Val Thr Glu Leu Met Ser Gly Met Leu Leu Gly
 50 405 410 415
 Phe Val Phe Val Leu Ser His Asn Gly Met Glu Val Tyr Asn Ser Ser
 420 425 430
 Lys Glu Phe Val Ser Ala Gln Ile Val Ser Thr Arg Asp Ile Lys Gly
 55 435 440 445
 Asn Ile Phe Asn Asp Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu

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	450					455						460							
	His	His	Leu	Phe	Pro	Thr	Met	Pro	Arg	His	Asn	Leu	Asn	Lys	Ile	Ala			
5	465					470					475				480				
	Pro	Arg	Val	Glu	Val	Phe	Cys	Lys	Lys	His	Gly	Leu	Val	Tyr	Glu	Asp			
				485						490					495				
	Val	Ser	Ile	Ala	Thr	Gly	Thr	Cys	Lys	Val	Leu	Lys	Ala	Leu	Lys	Glu			
10				500					505					510					
	Val	Ala	Glu	Ala	Ala	Ala	Glu	Gln	His	Ala	Thr	Thr	Ser						
			515					520					525						

15 <210> 8
<211> 457
<212> PRT
<213> Mortierella alpina

20 <400> 8

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Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1 5 10 15

5 Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
20 25 30

Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
35 40 45

10 Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
50 55 60

Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65 70 75 80

15 Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
85 90 95

Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
100 105 110

20 Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
115 120 125

Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
130 135 140

25 Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145 150 155 160

Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
165 170 175

30 His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
180 185 190

Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
195 200 205

35 His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
210 215 220

Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225 230 235 240

40 Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe

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					245						250					255
	Met	Val	Leu	Asn	Gln	Thr	Trp	Phe	Tyr	Phe	Pro	Ile	Leu	Ser	Phe	Ala
				260					265					270		
5	Arg	Leu	Ser	Trp	Cys	Leu	Gln	Ser	Ile	Met	Phe	Val	Leu	Pro	Asn	Gly
			275					280					285			
	Gln	Ala	His	Lys	Pro	Ser	Gly	Ala	Arg	Val	Pro	Ile	Ser	Leu	Val	Glu
10		290					295					300				
	Gln	Leu	Ser	Leu	Ala	Met	His	Trp	Thr	Trp	Tyr	Leu	Ala	Thr	Met	Phe
	305					310					315					320
	Leu	Phe	Ile	Lys	Asp	Pro	Val	Asn	Met	Ile	Val	Tyr	Phe	Leu	Val	Ser
15				325						330					335	
	Gln	Ala	Val	Cys	Gly	Asn	Leu	Leu	Ala	Ile	Val	Phe	Ser	Leu	Asn	His
				340					345					350		
	Asn	Gly	Met	Pro	Val	Ile	Ser	Lys	Glu	Glu	Ala	Val	Asp	Met	Asp	Phe
20			355					360					365			
	Phe	Thr	Lys	Gln	Ile	Ile	Thr	Gly	Arg	Asp	Val	His	Pro	Gly	Leu	Phe
			370				375					380				
	Ala	Asn	Trp	Phe	Thr	Gly	Gly	Leu	Asn	Tyr	Gln	Ile	Glu	His	His	Leu
25					390						395					400
	Phe	Pro	Ser	Met	Pro	Arg	His	Asn	Phe	Ser	Lys	Ile	Gln	Pro	Ala	Val
				405						410					415	
	Glu	Thr	Leu	Cys	Lys	Lys	Tyr	Gly	Val	Arg	Tyr	His	Thr	Thr	Gly	Met
30				420				425						430		
	Ile	Glu	Gly	Thr	Ala	Glu	Val	Phe	Ser	Arg	Leu	Asn	Glu	Val	Ser	Lys
			435					440					445			
	Ala	Ala	Ser	Lys	Met	Gly	Lys	Ala	Gln							
35		450					455									

<210> 9

<211> 443

<212> PRT

40 <213> Caenorhabditis elegans

<400> 9

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	Met	Val	Val	Asp	Lys	Asn	Ala	Ser	Gly	Leu	Arg	Met	Lys	Val	Asp	Gly
	1				5					10					15	
5	Lys	Trp	Leu	Tyr	Leu	Ser	Glu	Glu	Leu	Val	Lys	Lys	His	Pro	Gly	Gly
				20					25					30		
	Ala	Val	Ile	Glu	Gln	Tyr	Arg	Asn	Ser	Asp	Ala	Thr	His	Ile	Phe	His
			35					40					45			
10	Ala	Phe	His	Glu	Gly	Ser	Ser	Gln	Ala	Tyr	Lys	Gln	Leu	Asp	Leu	Leu
		50					55					60				
	Lys	Lys	His	Gly	Glu	His	Asp	Glu	Phe	Leu	Glu	Lys	Gln	Leu	Glu	Lys
	65					70					75					80
15	Arg	Leu	Asp	Lys	Val	Asp	Ile	Asn	Val	Ser	Ala	Tyr	Asp	Val	Ser	Val
					85					90					95	
	Ala	Gln	Glu	Lys	Lys	Met	Val	Glu	Ser	Phe	Glu	Lys	Leu	Arg	Gln	Lys

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	100					105					110					
5	Leu	His	Asp	Asp	Gly	Leu	Met	Lys	Ala	Asn	Glu	Thr	Tyr	Phe	Leu	Phe
			115					120					125			
	Lys	Ala	Ile	Ser	Thr	Leu	Ser	Ile	Met	Ala	Phe	Ala	Phe	Tyr	Leu	Gln
		130					135					140				
10	Tyr	Leu	Gly	Trp	Tyr	Ile	Thr	Ser	Ala	Cys	Leu	Leu	Ala	Leu	Ala	Trp
	145				150					155						160
	Gln	Gln	Phe	Gly	Trp	Leu	Thr	His	Glu	Phe	Cys	His	Gln	Gln	Pro	Thr
				165						170					175	
15	Lys	Asn	Arg	Pro	Leu	Asn	Asp	Thr	Ile	Ser	Leu	Phe	Phe	Gly	Asn	Phe
			180						185					190		
	Leu	Gln	Gly	Phe	Ser	Arg	Asp	Trp	Trp	Lys	Asp	Lys	His	Asn	Thr	His
			195					200					205			
20	His	Ala	Ala	Thr	Asn	Val	Ile	Asp	His	Asp	Gly	Asp	Ile	Asp	Leu	Ala
		210					215					220				
	Pro	Leu	Phe	Ala	Phe	Ile	Pro	Gly	Asp	Leu	Cys	Lys	Tyr	Lys	Ala	Ser
	225					230					235					240
25	Phe	Glu	Lys	Ala	Ile	Leu	Lys	Ile	Val	Pro	Tyr	Gln	His	Leu	Tyr	Phe
				245						250					255	
	Thr	Ala	Met	Leu	Pro	Met	Leu	Arg	Phe	Ser	Trp	Thr	Gly	Gln	Ser	Val
			260						265					270		
30	Gln	Trp	Val	Phe	Lys	Glu	Asn	Gln	Met	Glu	Tyr	Lys	Val	Tyr	Gln	Arg
			275					280						285		
	Asn	Ala	Phe	Trp	Glu	Gln	Ala	Thr	Ile	Val	Gly	His	Trp	Ala	Trp	Val
		290					295					300				
35	Phe	Tyr	Gln	Leu	Phe	Leu	Leu	Pro	Thr	Trp	Pro	Leu	Arg	Val	Ala	Tyr
	305						310				315					320
	Phe	Ile	Ile	Ser	Gln	Met	Gly	Gly	Gly	Leu	Leu	Ile	Ala	His	Val	Val
				325						330					335	
40	Thr	Phe	Asn	His	Asn	Ser	Val	Asp	Lys	Tyr	Pro	Ala	Asn	Ser	Arg	Ile
			340						345					350		
	Leu	Asn	Asn	Phe	Ala	Ala	Leu	Gln	Ile	Leu	Thr	Thr	Arg	Asn	Met	Thr
			355					360					365			
45	Pro	Ser	Pro	Phe	Ile	Asp	Trp	Leu	Trp	Gly	Gly	Leu	Asn	Tyr	Gln	Ile
		370					375					380				
	Glu	His	His	Leu	Phe	Pro	Thr	Met	Pro	Arg	Cys	Asn	Leu	Asn	Ala	Cys
	385						390				395					400
50	Val	Lys	Tyr	Val	Lys	Glu	Trp	Cys	Lys	Glu	Asn	Asn	Leu	Pro	Tyr	Leu
				405						410					415	
	Val	Asp	Asp	Tyr	Phe	Asp	Gly	Tyr	Ala	Met	Asn	Leu	Gln	Gln	Leu	Lys
			420						425					430		
55	Asn	Met	Ala	Glu	His	Ile	Gln	Ala	Lys	Ala	Ala					
			435					440								

<210> 10

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<211> 448

<212> PRT

<213> Echium plantagineum

5 <400> 10

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Met Ala Asn Ala Ile Lys Lys Tyr Ile Thr Ala Glu Glu Leu Lys Lys
 1 5 10 15
 His Asp Lys Ala Gly Asp Leu Trp Ile Ser Ile Gln Gly Lys Ile Tyr
 5 20 25 30
 Asp Val Ser Asp Trp Leu Lys Asp His Pro Gly Gly Asn Phe Pro Leu
 35 40 45
 Leu Ser Leu Ala Gly Gln Glu Val Thr Asp Ala Phe Val Ala Phe His
 10 50 55 60
 Ser Gly Thr Thr Trp Lys Leu Leu Glu Lys Phe Phe Thr Gly Tyr Tyr
 65 70 75 80
 Leu Lys Asp Tyr Ser Val Ser Glu Val Ser Lys Asp Tyr Arg Lys Leu
 15 85 90 95
 Val Phe Glu Phe Asn Lys Met Gly Leu Phe Asp Lys Lys Gly His Ile
 100 105 110
 Val Leu Val Thr Val Leu Phe Ile Ala Met Leu Phe Gly Met Ser Val
 115 120 125
 Tyr Gly Val Leu Phe Cys Glu Gly Val Leu Val His Leu Leu Ala Gly
 130 135 140
 Gly Leu Met Gly Phe Val Trp Ile Gln Ser Gly Trp Ile Gly His Asp
 145 150 155 160
 Ala Gly His Tyr Ile Val Met Pro Asp Ala Arg Leu Asn Lys Leu Met
 165 170 175
 Gly Ile Val Ala Ala Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp
 180 185 190
 Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Asp Tyr
 195 200 205
 Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Leu
 210 215 220
 Phe Ser Ser Leu Thr Ser His Phe Tyr Glu Lys Lys Leu Thr Phe Asp
 225 230 235 240
 Ser Leu Ser Arg Phe Phe Val Ser His Gln His Trp Thr Phe Tyr Pro
 245 250 255
 Val Met Cys Met Ala Arg Val Asn Met Phe Val Gln Ser Leu Ile Met
 260 265 270
 Leu Leu Thr Lys Arg Asn Val Phe Tyr Arg Ser Gln Glu Leu Leu Gly
 275 280 285
 Leu Val Val Phe Trp Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro
 290 295 300
 Asn Trp Gly Glu Arg Val Met Phe Val Val Ala Ser Leu Ser Val Thr
 305 310 315 320
 Gly Met Gln Gln Val Gln Phe Ser Leu Asn His Phe Ser Ser Ser Val
 325 330 335
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	Tyr	Val	Gly	Gln	Pro	Lys	Gly	Asn	Asp	Trp	Phe	Glu	Lys	Gln	Thr	Cys
				340					345					350		
5	Gly	Thr	Leu	Asp	Ile	Ser	Cys	Pro	Ser	Trp	Met	Asp	Trp	Phe	His	Gly
			355					360					365			
	Gly	Leu	Gln	Phe	Gln	Val	Glu	His	His	Leu	Phe	Pro	Lys	Leu	Pro	Arg
		370					375					380				
10	Cys	His	Leu	Arg	Lys	Ile	Ser	Pro	Phe	Val	Met	Glu	Leu	Cys	Lys	Lys
	385					390					395					400
	His	Asn	Leu	Ser	Tyr	Asn	Cys	Ala	Ser	Phe	Ser	Glu	Ala	Asn	Asn	Met
					405					410						415
15	Thr	Leu	Arg	Thr	Leu	Arg	Asp	Thr	Ala	Leu	Gln	Ala	Arg	Asp	Leu	Thr
				420					425					430		
	Lys	Pro	Leu	Pro	Lys	Asn	Leu	Val	Trp	Glu	Ala	Leu	Asn	Thr	His	Gly
			435					440					445			
20	<210> 11															
	<211> 448															
	<212> PRT															
	<213> Echium gentianoides															
25	<400> 11															
30																
35																
40																
45																
50																
55																

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Met Ala Asn Ala Ile Lys Lys Tyr Ile Thr Ala Glu Glu Leu Lys Lys
 1 5 10 15

His Asp Lys Glu Gly Asp Leu Trp Ile Ser Ile Gln Gly Lys Val Tyr
 5 20 25 30

Asp Val Ser Asp Trp Leu Lys Asp His Pro Gly Gly Lys Phe Pro Leu
 35 40 45

Leu Ser Leu Ala Gly Gln Glu Val Thr Asp Ala Phe Val Ala Phe His
 10 50 55 60

Ser Gly Ser Thr Trp Lys Phe Leu Asp Ser Phe Phe Thr Gly Tyr Tyr
 65 70 75 80

Leu Lys Asp Tyr Ser Val Ser Glu Val Ser Lys Asp Tyr Arg Lys Leu
 15 85 90 95

Val Phe Glu Phe Asn Lys Met Gly Leu Phe Asp Lys Lys Gly His Ile
 100 105 110

Val Leu Val Thr Val Leu Phe Ile Ala Met Met Phe Ala Met Ser Val
 20 115 120 125

Tyr Gly Val Leu Phe Cys Glu Gly Val Leu Val His Leu Leu Ala Gly
 130 135 140

Gly Leu Met Gly Phe Val Trp Ile Gln Ser Gly Trp Ile Gly His Asp
 25 145 150 155 160

Ala Gly His Tyr Ile Val Met Pro Asn Pro Arg Leu Asn Lys Leu Met
 165 170 175

Gly Ile Val Ala Gly Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp
 30 180 185 190

Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Asp Tyr
 195 200 205

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Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Leu
 210 215 220
 Phe Ser Ser Leu Thr Ser His Phe Tyr Glu Lys Lys Leu Thr Phe Asp
 225 230 235 240
 Ser Leu Ser Arg Phe Phe Val Ser His Gln His Trp Thr Phe Tyr Pro
 245 250 255
 Val Met Cys Ser Ala Arg Val Asn Met Phe Val Gln Ser Leu Ile Met
 260 265 270
 Leu Leu Thr Lys Arg Asn Val Phe Tyr Arg Ser Gln Glu Leu Leu Gly
 275 280 285
 Leu Val Val Phe Trp Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro
 290 295 300
 Asn Trp Gly Glu Arg Ile Met Phe Val Val Ala Ser Leu Ser Val Thr
 305 310 315 320
 Gly Met Gln Gln Val Gln Phe Ser Leu Asn His Phe Ser Ala Ser Val
 325 330 335
 Tyr Val Gly Gln Pro Lys Gly Asn Asp Trp Phe Glu Lys Gln Thr Cys
 340 345 350
 Gly Thr Leu Asp Ile Ser Cys Pro Ser Trp Met Asp Trp Phe His Gly
 355 360 365
 Gly Leu Gln Phe Gln Val Glu His His Leu Phe Pro Lys Leu Pro Arg
 370 375 380
 Cys His Leu Arg Lys Ile Ser Pro Phe Val Met Glu Leu Cys Lys Lys
 385 390 395 400
 His Asn Leu Ser Tyr Asn Cys Ala Ser Phe Ser Glu Ala Asn Glu Met
 405 410 415
 Thr Leu Arg Thr Leu Arg Asp Thr Ala Leu Gln Ala Arg Asp Leu Thr
 420 425 430
 Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu Asn Thr His Gly
 435 440 445

40 <210> 12
 <211> 448
 <212> PRT
 <213> Echium pitardii

45 <400> 12

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EP 1 965 658 B1

	Met	Ala	Asn	Ala	Ile	Lys	Lys	Tyr	Ile	Thr	Ala	Glu	Glu	Leu	Lys	Lys
	1				5					10					15	
5	His	Asp	Lys	Glu	Gly	Asp	Leu	Trp	Ile	Ser	Ile	Gln	Gly	Lys	Val	Tyr
				20					25					30		
	Asp	Val	Ser	Asp	Trp	Leu	Lys	Asp	His	Pro	Gly	Gly	Lys	Phe	Pro	Leu
			35					40					45			
10	Leu	Ser	Leu	Ala	Gly	Gln	Glu	Val	Thr	Asp	Ala	Phe	Val	Ala	Phe	His
		50					55					60				
	Ser	Gly	Ser	Thr	Trp	Lys	Leu	Leu	Asp	Ser	Phe	Phe	Thr	Gly	Tyr	Tyr
	65					70					75					80
15																
20																
25																
30																
35																
40																
45																
50																
55																

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Leu Lys Asp Tyr Ser Val Ser Glu Val Ser Lys Asp Tyr Arg Lys Leu
 85 90 95
 Val Phe Glu Phe Asn Lys Met Gly Leu Phe Asp Lys Lys Gly His Ile
 100 105 110
 Val Leu Val Thr Val Phe Phe Ile Ala Met Met Phe Ala Met Ser Val
 115 120 125
 Tyr Gly Val Leu Phe Cys Glu Gly Val Leu Val His Leu Leu Ala Gly
 130 135 140
 Gly Leu Met Gly Phe Val Trp Ile Gln Ser Gly Trp Ile Gly His Asp
 145 150 155 160
 Ala Gly His Tyr Ile Val Met Pro Asn Pro Lys Leu Asn Lys Leu Met
 165 170 175
 Gly Ile Val Ala Ser Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp
 180 185 190
 Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Asp Tyr
 195 200 205
 Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Leu
 210 215 220
 Phe Ser Ser Leu Thr Ser His Phe Tyr Glu Lys Lys Leu Thr Phe Asp
 225 230 235 240
 Ser Leu Ser Arg Phe Phe Val Ser His Gln His Trp Thr Phe Tyr Pro
 245 250 255
 Val Met Cys Ser Ala Arg Val Asn Met Phe Val Gln Ser Leu Ile Met
 260 265 270
 Leu Leu Thr Lys Arg Asn Val Phe Tyr Arg Ser Gln Glu Leu Leu Gly
 275 280 285
 Leu Val Val Phe Trp Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro
 290 295 300
 Asn Trp Gly Glu Arg Ile Met Phe Val Val Ala Ser Leu Ser Val Thr
 305 310 315 320
 Gly Leu Gln Gln Val Gln Phe Ser Leu Asn His Phe Ala Ala Ser Val
 325 330 335
 Tyr Val Gly Gln Pro Lys Gly Ile Asp Trp Phe Glu Lys Gln Thr Cys
 340 345 350
 Gly Thr Leu Asp Ile Ser Cys Pro Ser Trp Met Asp Trp Phe His Gly
 355 360 365
 Gly Leu Gln Phe Gln Val Glu His His Leu Phe Pro Lys Leu Pro Arg
 370 375 380
 Cys His Leu Arg Lys Ile Ser Pro Phe Val Met Glu Leu Cys Lys Lys
 385 390 395 400
 His Asn Leu Ser Tyr Asn Cys Ala Ser Phe Ser Gln Ala Asn Glu Met
 405 410 415
 Thr Leu Arg Thr Leu Arg Asp Thr Ala Leu Gln Ala Arg Asp Leu Thr
 420 425 430
 Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu Asn Thr His Gly
 435 440 445

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<210> 13
 <211> 444
 <212> PRT
 <213> Danio rerio

5

<400> 13

Met Gly Gly Gly Gly Gln Gln Thr Asp Arg Ile Thr Asp Thr Asn Gly
 1 5 10 15

Arg Phe Ser Ser Tyr Thr Trp Glu Glu Val Gln Lys His Thr Lys His
 20 25 30

Gly Asp Gln Trp Val Val Val Glu Arg Lys Val Tyr Asn Val Ser Gln
 35 40 45

Trp Val Lys Arg His Pro Gly Gly Leu Arg Ile Leu Gly His Tyr Ala
 50 55 60

Gly Glu Asp Ala Thr Glu Ala Phe Thr Ala Phe His Pro Asn Leu Gln
 65 70 75 80

Leu Val Arg Lys Tyr Leu Lys Pro Leu Leu Ile Gly Glu Leu Glu Ala
 85 90 95

Ser Glu Pro Ser Gln Asp Arg Gln Lys Asn Ala Ala Leu Val Glu Asp
 100 105 110

Phe Arg Ala Leu Arg Glu Arg Leu Glu Ala Glu Gly Cys Phe Lys Thr
 115 120 125

Gln Pro Leu Phe Phe Ala Leu His Leu Gly His Ile Leu Leu Leu Glu
 130 135 140

Ala Ile Ala Phe Met Met Val Trp Tyr Phe Gly Thr Gly Trp Ile Asn
 145 150 155 160

Thr Leu Ile Val Ala Val Ile Leu Ala Thr Ala Gln Ser Gln Ala Gly
 165 170 175

Trp Leu Gln His Asp Phe Gly His Leu Ser Val Phe Lys Thr Ser Gly
 180 185 190

Met Asn His Leu Val His Lys Phe Val Ile Gly His Leu Lys Gly Ala
 195 200 205

Ser Ala Gly Trp Trp Asn His Arg His Phe Gln His His Ala Lys Pro
 210 215 220

Asn Ile Phe Lys Lys Asp Pro Asp Val Asn Met Leu Asn Ala Phe Val
 225 230 235 240

Val Gly Asn Val Gln Pro Val Glu Tyr Gly Val Lys Lys Ile Lys His
 245 250 255

Leu Pro Tyr Asn His Gln His Lys Tyr Phe Phe Phe Ile Gly Pro Pro
 260 265 270

Leu Leu Ile Pro Val Tyr Phe Gln Phe Gln Ile Phe His Asn Met Ile
 275 280 285

Ser His Gly Met Trp Val Asp Leu Leu Trp Cys Ile Ser Tyr Tyr Val
 290 295 300

Arg Tyr Phe Leu Cys Tyr Thr Gln Phe Tyr Gly Val Phe Trp Ala Ile
 305 310 315 320

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Ile Leu Phe Asn Phe Val Arg Phe Met Glu Ser His Trp Phe Val Trp
 325 330 335

5 Val Thr Gln Met Ser His Ile Pro Met Asn Ile Asp Tyr Glu Lys Asn
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Gln Asp Trp Leu Ser Met Gln Leu Val Ala Thr Cys Asn Ile Glu Gln
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10 Ser Ala Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe Gln Ile Glu
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His His Leu Phe Pro Thr Val Pro Arg His Asn Tyr Trp Arg Ala Ala
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15 Pro Arg Val Arg Ala Leu Cys Glu Lys Tyr Gly Val Lys Tyr Gln Glu
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Lys Thr Leu Tyr Gly Ala Phe Ala Asp Ile Ile Arg Ser Leu Glu Lys
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20 Ser Gly Glu Leu Trp Leu Asp Ala Tyr Leu Asn Lys
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 50 taatcaatac tcaactgaag tttgtgcagt taattgaagg gattaacggc caaaatgcac 1740

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25 ttaaatacatg aattttaaat ttataaaaat aatctccaat cggaagaaca acattccata 240
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30 tcctgaaatg attoctatta actacaagcc ttgttgccgg cggagaagtg atcgccgagg 420
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 40 tatgacactc agaacattaa gggacacagc attgcaagct cgcgatttaa ccaagccgct 1380
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45 <210> 26
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Glu Val Phe Asp Gly Gln Gln Gln Tyr Glu Gly Ile Gly Lys Arg Ala
 5 20 25 30

Ala Asp Lys Phe Asp Pro Ala Ala Pro Pro Pro Phe Lys Ile Ala Asp
 35 40 45

Ile Arg Ala Ala Ile Pro Ala His Cys Trp Val Lys Ser Pro Trp Arg
 10 50 55 60

Ser Leu Ser Tyr Val Val Trp Asp Val Ala Ala Val Ser Ala Arg Pro
 65 70 75 80

Ala Ala Val Tyr Ile Asn Ser Trp Ala Phe Trp Pro Val Tyr Trp Ile
 15 85 90 95

Ala Gln Gly Thr Met Phe Trp Ala Leu Phe Val Leu Gly His Asp Cys
 100 105 110

Gly His Gly Ser Phe Ser Asp Asn Thr Thr Leu Asn Asn Val Val Gly
 20 115 120 125

His Val Leu His Ser Ser Ile Leu Val Pro Tyr His Gly Trp Arg Ile
 130 135 140

Ser His Arg Thr His His Gln Asn His Gly His Val Glu Lys Asp Glu
 25 145 150 155 160

Ser Trp Val Pro Leu Pro Glu Asn Leu Tyr Lys Lys Leu Asp Phe Ser
 165 170 175

Thr Lys Phe Leu Arg Tyr Lys Ile Pro Phe Pro Met Phe Ala Tyr Pro
 30 180 185 190

Leu Tyr Leu Trp Tyr Arg Ser Pro Gly Lys Thr Gly Ser His Phe Asn
 195 200 205

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Pro Tyr Ser Asp Leu Phe Lys Pro Asn Glu Arg Gly Leu Ile Val Thr
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5 Ser Thr Met Cys Trp Ala Ala Met Gly Val Phe Leu His Tyr Ala Thr
 225 230 235 240

Thr Ile Val Gly Pro Asn Met Met Phe Lys Leu Tyr Gly Val Pro Tyr
 245 250 255

10 Leu Ile Phe Val Met Trp Leu Asp Thr Val Thr Tyr Leu His His His
 260 265 270

Gly Tyr Asp Lys Lys Leu Pro Trp Tyr Arg Ser Lys Glu Trp Ile Tyr
 275 280 285

15 Leu Arg Gly Gly Leu Thr Thr Val Asp Gln Asp Tyr Gly Phe Phe Asn
 290 295 300

Lys Ile His His Asp Ile Gly Thr His Val Ile His His Leu Phe Pro
 305 310 315 320

20 Gln Ile Pro His Tyr His Leu Val Glu Ala Thr Arg Glu Ala Lys Arg
 325 330 335

Val Leu Gly Asn Tyr Tyr Arg Glu Pro Arg Lys Ser Gly Pro Val Pro
 340 345 350

25 Leu His Leu Ile Pro Ala Leu Leu Lys Ser Leu Gly Arg Asp His Tyr
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Val Ser Asp Asn Gly Asp Ile Val Tyr Tyr His Thr Val Asp Glu Leu
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30 Phe Pro Ser Lys Lys Ile
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<210> 27

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35 <212> PRT

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	Ile	Gly	Asp	Ile	Arg	Ala	Ala	Ile	Pro	Lys	His	Cys	Trp	Val	Lys	Ser
			35					40					45			
10	Pro	Leu	Arg	Ser	Met	Ser	Tyr	Val	Thr	Arg	Asp	Ile	Phe	Ala	Val	Ala
		50					55					60				
	Ala	Leu	Ala	Met	Ala	Ala	Val	Tyr	Phe	Asp	Ser	Trp	Phe	Leu	Trp	Pro
	65					70				75						80
15	Leu	Tyr	Trp	Val	Ala	Gln	Gly	Thr	Leu	Phe	Trp	Ala	Ile	Phe	Val	Leu
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	Gly	His	Asp	Cys	Gly	His	Gly	Ser	Phe	Ser	Asp	Ile	Pro	Leu	Leu	Asn
				100					105					110		
20	Ser	Val	Val	Gly	His	Ile	Leu	His	Ser	Phe	Ile	Leu	Val	Pro	Tyr	His
			115					120					125			
25																
30																
35																
40																
45																
50																
55																

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Gly Trp Arg Ile Ser His Arg Thr His His Gln Asn His Gly His Val
 130 135 140
 5 Glu Asn Asp Glu Ser Trp Val Pro Leu Pro Glu Lys Leu Tyr Lys Asn
 145 150 155 160
 Leu Pro His Ser Thr Arg Met Leu Arg Tyr Thr Val Pro Leu Pro Met
 165 170 175
 10 Leu Ala Tyr Pro Ile Tyr Leu Trp Tyr Arg Ser Pro Gly Lys Glu Gly
 180 185 190
 Ser His Phe Asn Pro Tyr Ser Ser Leu Phe Ala Pro Ser Glu Arg Lys
 195 200 205
 15 Leu Ile Ala Thr Ser Thr Thr Cys Trp Ser Ile Met Leu Ala Thr Leu
 210 215 220
 Val Tyr Leu Ser Phe Leu Val Asp Pro Val Thr Val Leu Lys Val Tyr
 225 230 235 240
 20 Gly Val Pro Tyr Ile Ile Phe Val Met Trp Leu Asp Ala Val Thr Tyr
 245 250 255
 Leu His His His Gly His Asp Glu Lys Leu Pro Trp Tyr Arg Gly Lys
 260 265 270
 25 Glu Trp Ser Tyr Leu Arg Gly Gly Leu Thr Thr Ile Asp Arg Asp Tyr
 275 280 285
 Gly Ile Phe Asn Asn Ile His His Asp Ile Gly Thr His Val Ile His
 290 295 300
 30 His Leu Phe Pro Gln Ile Pro His Tyr His Leu Val Asp Ala Thr Arg
 305 310 315 320
 Ala Ala Lys His Val Leu Gly Arg Tyr Tyr Arg Glu Pro Lys Thr Ser
 325 330 335
 35 Gly Ala Ile Pro Ile His Leu Val Glu Ser Leu Val Ala Ser Ile Lys
 340 345 350
 Lys Asp His Tyr Val Ser Asp Thr Gly Asp Ile Val Phe Tyr Glu Thr
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 370 375 380

<210> 28

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45 <212> PRT

<213> Betula pendula

<400> 28

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Asn Gly Phe His Gly Val Val Glu Lys Asp Asp Phe Asp Pro Ser Ala
5 20 25 30

Pro Pro Pro Phe Lys Ile Ala Glu Ile Arg Ala Ala Ile Pro Lys His
35 40 45

Cys Trp Ala Lys Asn Pro Trp Arg Ser Leu Ser Tyr Ala Leu Arg Asp
10 50 55 60

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	Ala	Leu	Phe	Val	Leu	Gly	His	Asp	Cys	Gly	His	Gly	Ser	Phe	Ser	Asp
				100					105					110		
10	Asn	Pro	Glu	Leu	Asn	Asn	Leu	Val	Gly	His	Val	Leu	His	Ser	Ala	Ile
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	Leu	Val	Pro	Tyr	His	Gly	Trp	Arg	Ile	Ser	His	Arg	Thr	His	His	Gln
		130					135						140			
15	Asn	His	Gly	Asn	Val	Glu	Asn	Asp	Glu	Ser	Trp	Val	Pro	Leu	Thr	Glu
		145				150					155					160
	Lys	Leu	Tyr	Lys	Ser	Leu	Gly	Tyr	Ser	Thr	Arg	Leu	Leu	Arg	Phe	Thr
				165						170					175	
20	Val	Pro	Phe	Pro	Leu	Phe	Ala	Tyr	Pro	Ile	Tyr	Leu	Trp	Ser	Arg	Ser
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	Pro	Gly	Lys	Glu	Gly	Ser	His	Phe	Asn	Pro	Tyr	Ser	Asn	Leu	Phe	Ser
			195					200					205			
25	Pro	Asn	Glu	Arg	Lys	Asp	Val	Ile	Thr	Ser	Thr	Leu	Cys	Trp	Ser	Leu
		210					215						220			
	Met	Ala	Ala	Leu	Leu	Ile	Tyr	Ser	Ser	Cys	Ala	Ile	Gly	Pro	Ile	Gln
		225				230					235					240
30	Met	Leu	Lys	Leu	Tyr	Gly	Val	Pro	His	Leu	Ile	Phe	Val	Met	Trp	Leu
				245						250					255	
	Asp	Leu	Val	Thr	Tyr	Leu	His	His	His	Gly	Tyr	Glu	Gln	Lys	Leu	Pro
			260						265					270		
35	Trp	Tyr	Arg	Gly	Lys	Glu	Trp	Ser	Tyr	Leu	Arg	Gly	Gly	Leu	Thr	Thr
			275					280					285			
	Val	Asp	Arg	Asp	Tyr	Gly	Trp	Phe	Asn	Asn	Ile	His	His	Asp	Ile	Gly
		290					295					300				
40	Thr	His	Val	Ile	His	His	Leu	Phe	Pro	Gln	Ile	Pro	His	Tyr	His	Leu
		305				310					315					320
	Val	Glu	Ala	Thr	Asn	Ala	Ala	Lys	Pro	Val	Leu	Gly	Lys	Tyr	Tyr	Arg
				325						330					335	
45	Glu	Pro	Lys	Arg	Ser	Gly	Pro	Phe	Pro	Ile	His	Leu	Ile	Lys	Asn	Leu
			340						345					350		
	Val	Arg	Ser	Ile	Ser	Glu	Asp	His	Tyr	Val	Asn	Asp	Asn	Gly	Asp	Ile
			355					360					365			
50	Val	Tyr	Tyr	Gln	Thr	Asp	Pro	Glu	Leu	Tyr	Lys	Ser	Ser	Asn	Thr	Lys
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<212> PRT

<213> *Arabidopsis thaliana*

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				20					25					30		
	Pro	Phe	Lys	Ile	Gly	Asp	Ile	Arg	Ala	Ala	Ile	Pro	Lys	His	Cys	Trp
			35					40					45			
10	Val	Lys	Ser	Pro	Leu	Arg	Ser	Met	Ser	Tyr	Val	Val	Arg	Asp	Ile	Ile
		50					55					60				
	Ala	Val	Ala	Ala	Leu	Ala	Ile	Ala	Ala	Val	Tyr	Val	Asp	Ser	Trp	Phe
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15	Leu	Trp	Pro	Leu	Tyr	Trp	Ala	Ala	Gln	Gly	Thr	Leu	Phe	Trp	Ala	Ile
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30	Leu	Pro	Met	Leu	Ala	Tyr	Pro	Leu	Tyr	Leu	Cys	Tyr	Arg	Ser	Pro	Gly
				180					185					190		
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			195					200					205			
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	225					230					235					240
40	Lys	Val	Tyr	Gly	Val	Pro	Tyr	Ile	Ile	Phe	Val	Met	Trp	Leu	Asp	Ala
					245					250					255	
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				260					265					270		
45	Arg	Gly	Lys	Glu	Trp	Ser	Tyr	Leu	Arg	Gly	Gly	Leu	Thr	Thr	Ile	Asp
			275					280					285			
	Arg	Asp	Tyr	Gly	Ile	Phe	Asn	Asn	Ile	His	His	Asp	Ile	Gly	Thr	His
		290					295					300				
50	Val	Ile	His	His	Leu	Phe	Pro	Gln	Ile	Pro	His	Tyr	His	Leu	Val	Asp
	305					310					315					320
	Ala	Thr	Lys	Ala	Ala	Lys	His	Val	Leu	Gly	Arg	Tyr	Tyr	Arg	Glu	Pro
					325					330					335	
55																

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Lys Thr Ser Gly Ala Ile Pro Ile His Leu Val Glu Ser Leu Val Ala
340 345 350

5 Ser Ile Lys Lys Asp His Tyr Val Ser Asp Thr Gly Asp Ile Val Phe
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Tyr Glu Thr Asp Pro Asp Leu Tyr Val Tyr Ala Ser Asp Lys Ser Lys
370 375 380

10 Ile Asn
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<211> 46

<212> DNA

<213> Artificial

<220>

<223> Oligonucleotide primer

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<400> 30

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<210> 31

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<223> Oligonucleotide primer

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<400> 31

tggagctca accatgagta ttaagagctt c 31

35

Claims

1. A method of rearing a salmon which is a *Salmo sp.* or *Oncorhynchus sp.*, the method comprising feeding the salmon a feedstuff comprising lipid, the free and esterified fatty acids of the total lipid of the feedstuff comprising at least 5.5% (w/w) stearidonic acid (SDA), wherein the salmon, after having been fed the feedstuff for at least 6 weeks, has higher SDA and eicosatetraenoic acid (ETA) levels in muscle tissue when compared with a salmon fed the same feedstuff but which substantially lacks SDA.
2. The method of claim 1 wherein the free and esterified fatty acids of said total lipid comprise at least 11% (w/w) SDA.
3. The method of claim 1 or 2, wherein the lipid of the feedstuff comprises a plant lipid.
4. The method of claim 3, wherein the plant is a transgenic plant comprising a $\Delta 6$ desaturase comprising an amino acid sequence which is at least 75% identical to the amino acid sequence set forth in GenBank accession number AY234127.
5. The method of any one of claims 1 to 4 wherein the free and esterified fatty acids of the total lipid of the white muscle of the salmon obtained thereby comprise less than 29.6% saturated fatty acid (SFA), at least 18.3% docosahexaenoic acid (DHA), and at least 2.1% SDA.
6. The method of claim 5, wherein the free and esterified fatty acids of the total lipid of the white muscle of said salmon comprise at least 2.7% SDA.

- 5 7. A feedstuff suitable for a salmon which is a *Salmo sp.* or *Oncorhynchus sp.*, the feedstuff comprising lipid, the free and esterified fatty acids of the lipid in the feedstuff comprising at least 11% (w/w) stearidonic acid (SDA), wherein the feedstuff comprises one or more of fishmeal in an amount of 20 to 700g per kg dry weight, a protein source other than fishmeal, and starch at 10-150 g/kg, and wherein the protein source comprises wheat or other cereal gluten, soymeal, meal from other legumes, casein, blood or feathers.
8. The feedstuff of claim 7, wherein the lipid comprises a plant lipid.
- 10 9. The feedstuff of claim 8, wherein the plant is a transgenic plant comprising a $\Delta 6$ desaturase comprising an amino acid sequence which is at least 75% identical to the amino acid sequence set forth as GenBank accession number AY234127.
- 15 10. A salmon which is a *Salmo sp.* or *Oncorhynchus sp.*, wherein the free and esterified fatty acids of the total lipid of the white muscle of the salmon is less than 29.6% SFA, at least 18.3% docosahexaenoic acid (DHA), and at least 2.7% stearidonic acid (SDA).
11. The salmon according to claim 10, wherein the free and esterified fatty acids of the total lipid of the white muscle of said salmon comprise at least 3.5% SDA.
- 20 12. A method for producing a feedstuff for salmon which is a *Salmo sp.* or *Oncorhynchus sp.*, the method comprising admixing oil obtained from a plant with at least one other ingredient selected from fishmeal in an amount of 20 to 700g per kg dry weight, a protein source other than fishmeal, and starch at 10-150 g/kg, to produce a feedstuff comprising lipid and said other ingredient, the free and esterified fatty acids of the lipid of the feedstuff comprising at least 11% (w/w) stearidonic acid (SDA), wherein the protein source comprises wheat or other cereal gluten, soymeal, meal from other legumes, casein, blood or feathers.
- 25 13. The method according to claim 12, wherein said plant is a transgenic plant comprising a $\Delta 6$ desaturase comprising an amino acid sequence which is at least 75% identical to the amino acid sequence set forth as GenBank accession number AY234127.
- 30 14. A method for producing a fish oil comprising stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA) or any combination thereof, said method comprising extracting oil from a salmon according to claim 10 or 11.

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Patentansprüche

- 40 1. Verfahren zur Zucht eines Lachses, der *Salmo sp.* oder *Oncorhynchus sp.* angehört, wobei das Verfahren das Füttern des Lachses mit einem Futter umfasst, das Lipid umfasst, wobei die freien und veresterten Fettsäuren des Gesamtlipids des Futters zumindest 5,5 Gew.-% Stearidonsäure (SDA) umfassen, wobei der Lachs, nachdem er mit dem Futter zumindest sechs Wochen lang gefüttert wurde, höhere SDA- und Eicosatetraensäure- (ETA-) Spiegel in Muskelgewebe aufweist als ein Lachs, der mit demselben Futter gefüttert wurde, das allerdings im Wesentlichen keine SDA aufweist.
- 45 2. Verfahren nach Anspruch 1, wobei die freien und veresterten Fettsäuren des Gesamtlipids des Futters zumindest 11 Gew.-% SDA aufweisen.
3. Verfahren nach Anspruch 1 oder 2, wobei das Lipid des Futters ein pflanzliches Lipid umfasst.
- 50 4. Verfahren nach Anspruch 3, wobei die Pflanze eine transgene Pflanze ist, die eine $\Delta 6$ -Desaturase umfasst, die eine Aminosäuresequenz umfasst, die zumindest zu 75 Prozent ident in Bezug auf die unter GenBank-Zugriffsnr. AY234127 hinterlegte Aminosäuresequenz ist.
- 55 5. Verfahren nach einem der Ansprüche 1 bis 4, wobei die dadurch erhaltenen freien und veresterten Fettsäuren des Gesamtlipids des weißen Muskels des Lachses weniger als 29,6 % gesättigte Fettsäure (SFA), zumindest 18,3 % Docosahexaensäure (DHA) und zumindest 2,1 % SDA umfassen.
6. Verfahren nach Anspruch 5, wobei die freien und veresterten Fettsäuren des Gesamtlipids des weißen Muskels

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des Lachses zumindest 2,7 % SDA umfassen.

- 5 7. Futter, das für einen Lachs, der *Salmo sp.* oder *Oncorhynchus sp.* angehört, geeignet ist, wobei das Futter Lipid umfasst, wobei die freien und veresterten Fettsäuren des Gesamtlipids des Futters zumindest 11 Gew.-% Stearidonsäure (SDA) umfassen, wobei das Futter ein oder mehrere von Fischmehl in einer Menge von 20 bis 700 g pro kg Trockengewicht, eine andere Proteinquelle als Fischmehl und Stärke in einer Menge von 10 bis 150 g/kg umfasst und wobei die Proteinquelle Weizen- oder anderes Getreidegluten, Sojamehl, Mehl von anderen Hülsenfrüchten, Casein, Blut oder Federn umfasst.
- 10 8. Futter nach Anspruch 7, wobei das Lipid ein pflanzliches Lipid umfasst.
9. Futter nach Anspruch 8, wobei die Pflanze eine transgene Pflanze ist, die eine $\Delta 6$ -Desaturase umfasst, die eine Aminosäuresequenz umfasst, die zumindest zu 75 Prozent ident in Bezug auf die unter GenBank-Zugriffsnr. AY234127 hinterlegte Aminosäuresequenz ist.
- 15 10. Lachs, der *Salmo sp.* oder *Oncorhynchus sp.* angehört, wobei die freien und veresterten Fettsäuren des Gesamtlipids des weißen Muskels des Lachses weniger als 29,6 % SFA, zumindest 18,3 % Docosahexaensäure (DHA) und zumindest 2,7 % Stearidonsäure (SDA) umfassen.
- 20 11. Lachs nach Anspruch 10, wobei die freien und veresterten Fettsäuren des Gesamtlipids des weißen Muskels des Lachses zumindest 3,5 % SDA umfassen.
- 25 12. Verfahren zur Herstellung eines Futters für Lachs, der *Salmo sp.* oder *Oncorhynchus sp.* angehört, wobei das Verfahren das Mischen eines aus einer Pflanze erhaltenen Öls mit zumindest einer weiteren Zutat umfasst, die aus Fischmehl in einer Menge von 20 bis 700 g pro kg Trockengewicht, einer anderen Proteinquelle als Fischmehl und Stärke in einer Menge von 10 bis 150 g/kg ausgewählt ist, um ein Futter herzustellen, das Lipid und die andere Zutat umfasst, wobei die freien und veresterten Fettsäuren des Gesamtlipids des Futters zumindest 11 Gew.-% Stearidonsäure (SDA) umfassen, wobei die Proteinquelle Weizen- oder anderes Getreidegluten, Sojamehl, Mehl von anderen Hülsenfrüchten, Casein, Blut oder Federn umfasst.
- 30 13. Verfahren nach Anspruch 12, wobei die Pflanze eine transgene Pflanze ist, die eine $\Delta 6$ -Desaturase umfasst, die eine Aminosäuresequenz umfasst, die zumindest zu 75 Prozent ident in Bezug auf die unter GenBank-Zugriffsnr. AY234127 hinterlegte Aminosäuresequenz ist.
- 35 14. Verfahren zur Herstellung eines Fischöls, das Stearidonsäure (SDA), Eicosapentaensäure (EPA), Docosapentaensäure (DPA), Docosahexaensäure (DHA) oder eine beliebige Kombination davon umfasst, wobei das Verfahren das Extrahieren von Öl aus einem Lachs nach einem der Ansprüche 10 oder 11 umfasst.

40 Revendications

- 45 1. Procédé d'élevage d'un saumon qui est un *Salmo sp.* ou *Oncorhynchus sp.*, le procédé comprenant le fait de nourrir le saumon avec un aliment comprenant un lipide, les acides gras libres et estérifiés du lipide total de l'aliment comprenant au moins 5,5 % (p/p) d'acide stéaridonique (SDA), dans lequel le saumon, après avoir été nourri avec l'aliment pendant au moins 6 semaines, présente des taux de SDA et d'acide eicosatétraénoïque (ETA) plus élevés dans le tissu musculaire par comparaison à un saumon nourri avec le même aliment mais qui est essentiellement dépourvu de SDA.
- 50 2. Procédé selon la revendication 1, dans lequel les acides gras libres et estérifiés dudit lipide total comprennent au moins 11 % (p/p) de SDA.
3. Procédé selon la revendication 1 ou 2, dans lequel le lipide de l'aliment comprend un lipide végétal.
- 55 4. Procédé selon la revendication 3, dans lequel la plante est une plante transgénique comprenant une $\Delta 6$ désaturase comprenant une séquence d'acides aminés qui est au moins 75 % identique à la séquence d'acides aminés décrite dans le numéro d'accès GenBank numéro AY234127.
5. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel les acides gras libres et estérifiés dudit lipide

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total du muscle blanc du saumon ainsi obtenus comprennent moins de 29,6 % d'acides gras saturés (SFA), au moins 18,3 % d'acide docosahexaénoïque (DHA), et au moins 2,1 % de SDA.

- 5
6. Procédé selon la revendication 5, dans lequel les acides gras libres et estérifiés dudit lipide total du muscle blanc dudit saumon comprennent au moins 2,7 % de SDA.
- 10
7. Aliment approprié pour un saumon qui est un *Salmo sp.* ou *Oncorhynchus sp.*, l'aliment comprenant un lipide, les acides gras libres et estérifiés du lipide total dans l'aliment comprenant au moins 11 % (p/p) d'acide stéaridonique (SDA), où l'aliment comprend un ou plusieurs farines de poisson en une quantité de 20 à 700 g par kg de poids sec, une source de protéine autre qu'une farine de poisson, et de l'amidon à 10-150 g/kg, et où la source de protéine comprend du blé ou du gluten d'une autre céréale, de la farine de soja, une farine d'autres légumes, de la caséine, du sang ou des plumes.
- 15
8. Aliment selon la revendication 7, dans lequel le lipide comprend un lipide végétal.
9. Aliment selon la revendication 8, dans lequel la plante est une plante transgénique comprenant une $\Delta 6$ désaturase comprenant une séquence d'acides aminés qui est au moins 75 % identique à la séquence d'acides aminés décrite en tant que numéro d'accès GenBank numéro AY234127.
- 20
10. Saumon qui est un *Salmo sp.* ou *Oncorhynchus sp.*, dans lequel les acides gras libres et estérifiés du lipide total du muscle blanc du saumon sont moins de 29,6 % de SFA, au moins 18,3 % d'acide docosahexaénoïque (DHA), et au moins 2,7 % d'acide stéaridonique (SDA).
- 25
11. Saumon selon la revendication 10, dans lequel les acides gras libres et estérifiés dudit lipide total du muscle blanc dudit saumon comprennent au moins 3,5 % de SDA.
- 30
12. Procédé pour produire un aliment pour saumon qui est un *Salmo sp.* ou *Oncorhynchus sp.*, le procédé comprenant le mélange d'une huile obtenue à partir d'une plante avec au moins un autre ingrédient sélectionné parmi une farine de poisson en une quantité de 20 à 700 g par kg de poids sec, une source de protéine autre qu'une farine de poisson, et de l'amidon à 10-150 g/kg, afin de produire un aliment comprenant un lipide et ledit autre ingrédient, les acides gras libres et estérifiés du lipide de l'aliment comprenant au moins 11 % d'acide stéaridonique (SDA), où la source de protéine comprend du blé ou du gluten d'une autre céréale, de la farine de soja, une farine d'autres légumes, de la caséine, du sang ou des plumes.
- 35
13. Procédé selon la revendication 12, dans lequel ladite plante est une plante transgénique comprenant une $\Delta 6$ désaturase comprenant une séquence d'acides aminés qui est au moins 75 % identique à la séquence d'acides aminés décrite en tant que numéro d'accès GenBank numéro AY234127.
- 40
14. Procédé pour produire une huile de poisson comprenant de l'acide stéaridonique (SDA), de l'acide éicosapentaénoïque (EPA), de l'acide docosapentaénoïque (DPA), de l'acide docosahexaénoïque (DHA) ou une quelconque combinaison de ceux-ci, ledit procédé comprenant l'extraction d'huile à partir d'un saumon selon la revendication 10 ou 11.
- 45
- 50
- 55

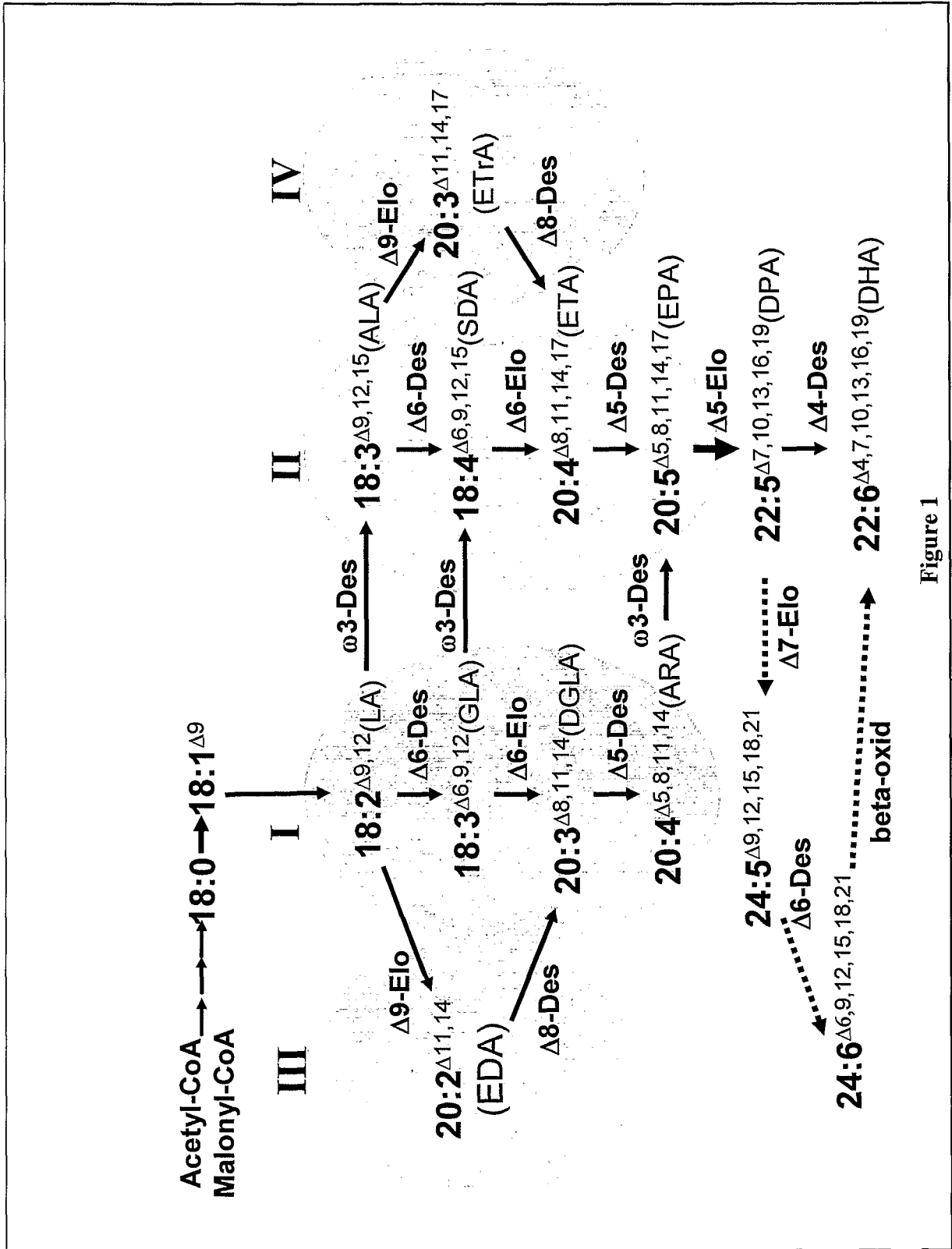


Figure 1

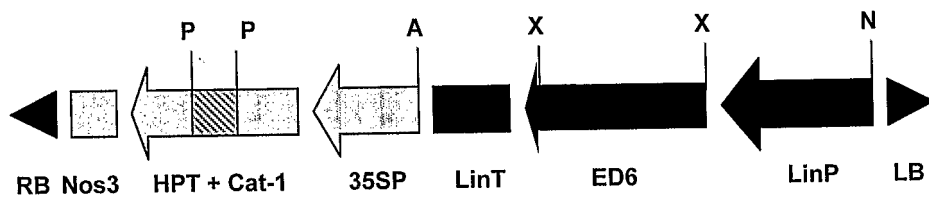


Figure 2

REFERENCES CITED IN THE DESCRIPTION

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