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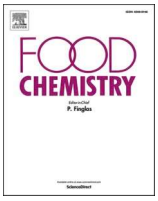

Inclusion of oil from transgenic *Camelina sativa* in feed effectively supplies EPA and DHA to Atlantic salmon (*Salmo salar*) grown to market size in seawater pens

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ABSTRACT

Atlantic salmon were fed either a diet reflecting current commercial feeds with added oil supplied by a blend of fish oil and rapeseed oil (COM), or a diet formulated with oil from transgenic *Camelina sativa* containing 20% EPA + DHA (TCO). Salmon were grown from smolt to market size (>3 kg) in sea pens under semi-commercial conditions. There were no differences in growth, feed efficiency or survival between fish fed the TCO or COM diets at the end of the trial. Levels of EPA + DHA in flesh of salmon fed TCO were significantly higher than in fish fed COM. A 140 g fillet from TCO-fed salmon delivered 2.3 g of EPA + DHA, 67% of the weekly requirement level recommended by many health agencies, and 1.5-fold more than the 1.5 g of EPA + DHA for COM-fed fish. Oil from transgenic *Camelina* supported growth and improved the nutritional quality of farmed salmon in terms of increased "omega-3" supply for human consumers.

1. Introduction

It is well established and widely accepted that the omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA), have health-promoting effects in the human diet (Calder, 2018; Innes & Calder, 2020). Based on these health benefits, many national and international agencies across the world have set levels for recommended EPA and DHA intake with 250–500 mg per day being reported commonly as required to maintain and support cardiac health (*e.g.* EFSA, 2010; ISSFAL, 2004; Richter, Skulas-Ray, & Kris-Etherton, 2016). While fish and seafood are the main sources of these important nutrients, the capture fisheries that traditionally supplied them are, at best, stagnant or at worst, in decline, and so over 50% of all fish and seafood are now farmed (FAO, 2022). Paradoxically, while the growth of aquaculture has ensured that the demand for fish and seafood from the increasing human population can be met, it has not been able to ensure the supply of EPA and DHA (Tocher, 2015; Tocher, Betancor, Sprague, Olsen, & Napier, 2019). This is because many farmed fish like Atlantic salmon (*Salmo salar*) themselves also require a dietary supply of EPA and DHA to ensure maximum growth and optimum health (NRC, 2011; Tocher, 2010, 2015). This was historically supplied in feeds by the inclusion of fishmeal (FM) and fish oil (FO), also derived from feed fisheries that are similarly at their sustainable limit and unable to supply the increasing demand from aquaculture (Cottrell, Blanchard, Halpern, Metian, & Froehlich, 2020; Naylor et al., 2021; Tacon, 2020; Tacon, Metian, & McNevin, 2022). The high use of FM and FO was an unsustainable practice, which prompted the development of more sustainable feeds based on raw materials such as plant meals and vegetable oils (Turchini et al., 2022; Turchini, Ng, & Tocher, 2011). However, these ingredients derived from terrestrial agriculture are devoid of n-3 LC-PUFA and, therefore, their increased use resulted in reduced levels of EPA and DHA in farmed fish, as has been well documented in salmon (Reksten et al., 2022; Sprague, Dick, & Tocher, 2016). Lower levels of dietary EPA and DHA not only impacts human consumers, but also has potential consequences for the health of the farmed fish themselves (Lutfi et al., 2022; Tocher & Glencross, 2015).

While the gap between the demand for n-3 LC-PUFA to satisfy human

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dietary requirements and the available supply from all sources is clearly a global issue (Salem Jr. & Eggersdorfer, 2015), it was felt particularly acutely in fish farming, and the constantly increasing demand from the aquaculture industry was key in prompting the search for alternative sources of EPA and DHA (Tocher et al., 2019). Two main research directions were developed, both based on the fact that marine microalgae are the main organisms responsible for the primary production of EPA and DHA. While one line of research focused on mass cultivation of microalgae, particularly heterotrophic species (Sprague, Betancor, & Tocher, 2017), another line of research utilised transgenic approaches to combine the trait for n-3 LC-PUFA production found in microalgae with the trait for the production and accumulation of oil in large quantities found in oilseed crops (Napier & Betancor, 2023; Napier, Olsen, & Tocher, 2019; Napier, Usher, Haslam, Ruiz-Lopez, & Sayanova, 2015; Petrie et al., 2020). The transgenic approach came with the benefits that oilseeds bring as major agricultural commodity products, with well-established and highly organised infrastructure that supports the cultivation, harvest, and processing of oilseeds, along with distribution, marketing and utilisation of the resultant vegetable oils (VO) (Salunkhe, Adsule, Chavan, & Kadam, 1992). Furthermore, VO had been the main alternatives to dietary FO as primary lipid sources in sustainable fish feed formulations (Aas, Åsgård, & Ytrestøyl, 2022; Aas, Ytrestøyl, & Åsgård, 2019; Turchini et al., 2011; Ytrestøyl, Aas, & Åsgård, 2015). Finally, while no VO contains LC-PUFA, several such as false flax *Camelina sativa*, a member of the Brassicaceae family, can be rich in α-linolenic acid (18:3n-3) and, thus, potentially suited for genetic modification to promote the production of EPA and DHA from the precursor form (18:3n-3) (Napier et al., 2015; Napier, Haslam, Olsen, Tocher, & Betancor, 2020).

Consequently, in recent years, *C. sativa* crops genetically-modified to produce EPA or EPA and DHA in their seeds were developed (Ruiz-Lopez, Haslam, Napier, & Sayanova, 2014; Usher et al., 2017), and have been evaluated extensively as replacements for dietary FO in feeds for Atlantic salmon (Betancor, Sprague, Usher, et al., 2015, Betancor, Sprague, Sayanova, et al., 2015, Betancor, Sprague, Sayanova, et al., 2016, Betancor et al., 2017), gilthead sea bream (*Sparus aurata*) (Betancor, Sprague, Montero et al., 2016), European sea bass (*Dicentrarchus labrax*) (Betancor et al., 2021), rainbow trout (*Oncorhynchus mykiss*) (Osmond et al., 2021) and Atlantic bluefin tuna (*Thunnus thynnus*) (Betancor et al., 2022). Specifically, in previous studies in Atlantic salmon, oils from 1st and 2nd iterations of transgenic *Camelina* supplying either 20% EPA or 6% each of EPA and DHA, respectively, were compared initially with "gold standard" feeds formulated with high FM and FO (Betancor, Sprague, Sayanova, et al., 2015; Betancor, Sprague, Sayanova, et al., 2016; Betancor, Sprague, Usher, et al., 2015), and the 2nd iteration oil (EPA + DHA) was tested subsequently in comparison with more commercially-representative feeds formulated with lower levels of both FM and FO (Betancor et al., 2017). All the above studies in salmonids and marine fish species showed highly encouraging results, with the oils from transgenic *Camelina* supporting good fish growth and enabling the deposition and accumulation of n-3 LC-PUFA in flesh.

The success of the transgenic *Camelina* oils in the above-mentioned trials prompted the development of a third-generation oil that contained almost 28% of total fatty acids as n-3 LC-PUFA including of 10.5% EPA and 9% DHA, levels greater than those found in many FO (Betancor et al., 2018). This oil was tested in salmon using feeds that more closely reflected commercial feeds for salmon, with even lower levels of FM and FO (Betancor et al., 2018). The diet formulated with the oil from transgenic *Camelina* showed no negative effects on growth, survival or health of the salmon, and flesh n-3 LC-PUFA levels were >2-fold higher compared with those of fish fed the diet with a commercial-like formulation containing 30% FM and 5% FO (Betancor et al., 2018). The data demonstrated that the oil from the 3rd-generation transgenic *Camelina* crop could efficiently supply EPA and DHA to salmon resulting in flesh n-3 LC-PUFA levels that were similar to those found routinely in farmed salmon prior to the large-scale replacement of dietary FM and FO (Sprague et al., 2016). However, all the above trials in salmon were carried out in land-based seawater tanks in experimental research facilities with smolts grown over a period of up to 12-weeks and to a maximum size of 500 g.

The aim of the present study was to further validate the efficacy of the 3rd-generation transgenic *Camelina* oil as a dietary oil for farmed Atlantic salmon in a trial carried out in seawater pens and growing fish over a period of 9 months to a market size of >3 kg. Triplicate groups of Atlantic salmon were fed experimental diets formulated with low FM that declined as dietary oil content increased as fish and corresponding pellet size increased during the trial. Two feeds were produced with added oil supplied either by a mixture of rapeseed oil and FO reflecting the current oil blend used in commercial salmon feeds in the northern hemisphere (Diet COM), or by 100% transgenic *Camelina* oil (Diet TCO). The impacts of diet on survival, growth performance, feed efficiency, tissue fatty acid contents and compositions, and flesh quality were assessed.

2. Materials and methods

2.1. Ethics statement

All experimental procedures associated with the Atlantic salmon feeding trial were conducted in compliance with the Animals Scientific Procedures Act 1986 (Home Office Code of Practice. HMSO: London January 1997) under project licence PPL7007916 "Environmental Regulation of Fish Physiology" and personal licence number PIL107216B95, and in accordance with EU regulation (EC Directive 86/609/EEC). In addition, all experimentation performed by the University of Stirling is subjected to a thorough ethical review process carried out by the Animal Welfare and Ethical Review Board (AWERB) prior to any work being approved. This involves all projects, irrespective of where they are carried out, to be submitted to AWERB for approval using detailed Ethical Approval forms that require all aspects of the experimentation to be described including all animal health and welfare issues as well as other ethical considerations. The present research was assessed by the AWERB and passed the ethical review process (Ethical Approval No. AWERB/16–17/83/New ASPA].

2.2. Production of oil from transgenic Camelina sativa

Seeds for the third iteration (identified as DHA2015.1, event #39) were grown under Canadian Food Inspection Agency (CFIA) permit 17-AGQ1–406-CAM at a site in Elm Creek, Manitoba, Canada. This was managed by AgQuest LLC, as described previously (Han et al., 2020). Seed was harvested and transferred to an approved facility (POS Bio-Sciences, Saskatoon, Canada) where the oil was extracted by cold-pressing and solvent (hexane) extraction. The resulting oil was then provided to BioMar AS for the production of experimental feed.

2.3. Experimental feeds

Two isonitrogenous and isolipidic feeds were formulated to satisfy the known requirements of Atlantic salmon, and produced by vacuum coating extruded base pellets with either a blend of FO and rapeseed oil (Control/reference, Diet COM) or the high n-3 LC-PUFA *Camelina* oil (Diet TCO) (Table 1). The initial formulation (fed to 187 g smolt) provided 44% protein, 28% lipid and 24 MJ.kg^{-1} of energy, and changed as the fish grew to supply 36% protein, 36% lipid and 26 MJ.kg^{-1} of energy to fish growing from 1.5 kg to market size. Initially the base pellet contained around 50% plant protein sources and 10% land animal proteins, and low FM that declined from 15% to 7.5% as the proportion of added oil increased from around 24% to 32% as the fish and corresponding pellet size increased during the trial. In addition, the ratio of rapeseed oil to FO in the COM diet varied from 0.75:1 in the smallest pellet (5 mm, weeks 1–11), to 2:1 in the larger pellet sizes (7 mm, weeks

Table 1
Formulations of experimental diets fed for the initial (250–800 g), intermediate (800–1500 g) and final pellet sizes (1500 g – harvest).

	Initial (5 mm)		Intermediate (7 mm)		Final (10 mm)	
Ingredient (g.kg^{-1})	COM	TCO	COM	TCO	COM	TCO
Fishmeal	150	150	75	75	75	75
Soy protein concentrate	244	244	101	101	80	80
Maize gluten	50	50	50	50	0	0
Pea protein	74	74	54	54	121	121
Guar meal	0	0	150	150	150	150
Wheat	113	113	109	109	110	110
Land animal products	100	100	100	100	100	100
Fish oil	136	0	108	0	116	0
Rapeseed oil	102	0	208	0	205	0
Camelina oil (GM)	0	238	0	316	0	320
Premixes	32	32	36	36	35	35
Yttrium oxide	0.5	0.5	0.5	0.5	0.5	0.5

COM, control/reference feed reflecting current commercial practices; TCO, feed with all added oil supplied by the oil from transgenic Camelina.

12–23 and 10 mm, weeks 24 to 37). The changes in the protein and oil contents of the feeds, and the oil blend ratio of the COM diet, as the fish grew reflected the commercial practices current in most salmon farming, globally. The fatty acid profiles of the diets showed that the total replacement of the commercial-type dietary oil blend with the transgenic *Camelina* oil resulted in higher percentages of all n-3 LC-PUFA, including EPA and DHA, in diet TCO compared to diet COM (Table 2). The proportions of both linoleic acid (18:2n-6) and 18:3n-3 were also higher in the TCO diet compared with the COM diet, with the overall higher proportions of PUFA in the TCO feed being balanced by lower proportions of monoenes (Table 2). The feeds were manufactured at the BioMar Tech Centre (Brande, Denmark).

2.4. Feeding trial

The nutritional feeding trial was carried out at the facilities of the Mowi Feeds Trial Unit (FTU), Ardnish, Lochailort, Scotland from May 2018 to March 2019. Smolts of the Mowi strain of Atlantic salmon were transferred to the Ardnish FTU in May 2018 and fed standard commercial transfer feed from then until initiation of the feeding trial in June. A total of 900 well-adapted post-smolt Atlantic salmon (initial weight ~ 187 g) were distributed randomly into six 5 × 5 m square seawater pens (150 fish per pen) fitted with automatic feeders (Arvo-Tec Oy, Huutokoski, Finland) and uneaten feed collection systems. The fish were fed with one of the two feeds in triplicate for a total period of 37 weeks starting on 20 June 2018 with the trial terminated on 6 March 2019. During the experiment, feeds were provided by the automatic feeders at a ration based on size of the fish and water temperature as per standard feeding tables for the Ardnish FTU. The actual feed supplied was the ration +5%, to ensure feeding to satiation. Feeds were distributed to the fish twice daily (8.15–9.15 am and 2.00–3.00 pm) with uneaten feed collected 30 min later and accurate feed intake calculated.

Table 2
Fatty acid compositions (% total fatty acids, and mg fatty acid.100 g^{-1}) of experimental feeds.

	Initial				Final			
	Percentage		mg.100 g^{-1}		Percentage		mg.100 g^{-1}	
Fatty acid	COM	TCO	COM	TCO	COM	TCO	COM	TCO
14:0	2.37	0.61	520.10	111.63	3.16	0.23	780.83	61.17
16:0	9.84	7.78	2160.92	1433.20	9.84	6.74	2434.57	1769.62
18:0	2.27	4.49	498.79	827.58	3.24	4.38	801.67	1150.21
Total saturated[1]	15.34	15.63	3367.64	2878.28	18.13	14.73	4484.79	3865.06
16:1n-7	4.14	1.00	908.05	184.82	4.43	0.40	1094.80	105.62
18:1n-9	32.87	9.65	7217.02	1776.93	39.29	10.01	9717.74	2625.34
18:1n-7	3.72	1.65	816.77	304.43	3.09	1.39	763.09	365.80
20:1n-9	6.27	6.46	1377.50	1189.01	1.30	7.87	322.41	2064.11
22:1n-11	4.76	1.08	1044.97	198.42	0.11	0.00	27.88	0.00
Total monoenes[2]	54.05	21.75	11,866.86	4006.63	49.14	22.23	12,153.44	5833.39
18:2n-6	11.60	20.40	2546.36	3757.90	15.20	19.62	3759.71	5147.45
18:3n-6	0.08	1.86	16.55	342.43	0.10	1.48	25.62	387.88
20:2n-6	0.17	1.65	37.37	303.35	0.10	1.58	25.34	414.43
20:3n-6	0.07	0.83	15.80	153.08	0.08	0.47	19.71	124.12
20:4n-6	0.31	2.59	68.21	477.38	0.40	1.68	98.27	439.79
22:4n-6	0.00	0.67	0.00	123.50	0.00	0.37	0.00	96.37
22:5n-6	0.08	0.09	16.55	16.19	0.09	0.05	22.25	12.83
Total n-6 PUFA	12.30	28.09	2700.83	5173.83	15.97	25.24	3950.90	6622.88
18:3n-3	4.92	9.96	1079.33	1833.93	5.81	19.10	1436.36	5011.39
18:4n-3	1.13	1.34	249.02	247.22	0.68	1.21	167.26	317.76
20:3n-3	0.07	0.97	15.30	177.91	0.00	1.15	0.00	301.35
20:4n-3	0.36	1.99	77.99	366.61	0.37	1.48	91.23	388.18
20:5n-3	4.81	8.47	1056.26	1560.59	4.85	5.70	1198.98	1494.53
22:5n-3	0.64	4.40	141.19	810.52	0.74	3.85	182.75	1009.08
22:6n-3	5.55	7.40	1217.51	1362.82	2.43	5.27	600.06	1382.04
Total n-3 PUFA	17.48	34.53	3892.01	6359.60	15.08	37.75	3730.70	9904.33
Total n-3 LC-PUFA	11.35	22.26	2492.94	4100.55	8.39	16.30	2073.02	4273.83
n-3 PUFA/n-6 PUFA	1.42	1.23	1.42	1.23	0.94	1.50	0.94	1.50

Values are means of duplicate assays. [1], includes 15:0, 20:0, 22:0 and 24:0, present at up to 0.3%. [2], includes 16:1n-9, 17:1, 20:1n-11, 20:1n-7, 22:1n-9 and 24:1n-9, present at up to 0.8%. COM, control/reference feed reflecting current commercial practices; LC-PUFA, long-chain PUFA; PUFA, polyunsaturated fatty acids; TCO, feed with all added oil supplied by the oil from transgenic Camelina.

Fish were monitored at feeding to ensure normal feeding behaviour. Growth was determined by weighing all the fish in the trial pens as appropriate time points including changes in pellet size. Mortalities were collected daily and examined for any signs of ill health. In the initial 2 weeks after stocking, mortalities were replaced from the same stock fish to maintain numbers at 150/pen but, after 2 weeks, mortalities were not replaced. Water temperature, salinity, clarity and dissolved oxygen were monitored daily for the duration of the experiment and can be found in Supplementary Fig. 1.

2.5. Sample collection

At the termination of the nutritional trial, fish were starved for 24 h prior to sampling. All fish were measured (wet weight and fork length) after anaesthesia with tricaine methanesulphonate (MS222 compound; Merck, Darmstadt, Germany) as per the standard protocol at the Ardnish FTU. A total of twelve fish per pen were killed by an overdose of MS222 (> 150 mg.l^{-1}). Four whole fish per pen were collected onto dry ice and frozen immediately as two pooled samples of two fish per pen ($n = 6$ per diet) for biochemical analysis (proximate and fatty acid compositions). A second batch of four fish from each pen were specifically selected to be most representative of harvest-size (~3.5 kg) and immediately filleted with the fillets from the right side labelled, bagged and taken immediately on ice for flesh quality analyses (Xelect, St. Andrews, Scotland). A further batch of four fish per pen were used for tissue biochemical analyses with the tissues collected being flesh (Norwegian Quality Cut, NQC), liver, intestine (pyloric caeca), gills, eyes and brain. The tissue samples were collected as two pools of two fish per pen (n = 6 per diet), with samples placed in 10 ml plastic tubes and immediately frozen in liquid nitrogen.

2.6. Calculations

Biometric parameters were calculated using the following equations:

Feed conversion ratio (FCR) = feed (dry weight) consumed /weight gain (wet weight).

Fulton's condition factor (k) = $100W/L^3$), where W is the fish weight (g) and L is the total length (cm).

Hepato – somatic index (HSI) = (LW/W)* 100, where LW is the liver weight and W is the somatic weight.

Specific growth rate (SGR) = 100* (lnWt–lnWo)* D^{-1}, where Wo and Wt are the initial and end weights (tanks means, $n = 3$) of the fish in a specific period, respectively, and D represents the number of feeding days.

Thermal growth coefficient (TGC) = 1000* [(Wt(1/3)–Wo(1/3)) /°D], where Wo and Wt are the initial and end weights (tanks means, n = 3) of the fish in a specific period , respectively, and °D represents degree – days, the sum of daily temperatures in °C in the specific period (or duration in days × average temperature in period).

Viscero – somatic index (VSI) = (VW/W)* 100, where VW is the weight of the viscera (without liver) and W is the somatic weight.

Weight gain (WG, g) = Wt–Wo, where Wo and Wt are the initial and end weights (tanks means, n = 3) of the fish in a specific period.

2.7. Proximate compositions of whole fish

Pooled whole fish (Robot Coupe R23 Vertical Food Processor; Robot-Coupe, Vincennes, France) and salmon flesh samples (NQC) (Robot Coupe Blixer® 4 V.V.) were homogenised before determination of proximate composition in samples of the resultant pates according to standard procedures (AOAC, 2000). Protein contents were determined by measuring nitrogen content (N × 6.25) using automated Kjeldahl analysis (Tecator Kjeltec Auto 1030 Analyzer, Foss, Warrington, UK), while lipid contents were determined gravimetrically after extraction using the Soxhlet method (Tecator Soxtec system 2050 Auto Extraction apparatus). Moisture contents were obtained after drying in an oven at 110 °C for 24 h, while ash contents were determined by incinerating the samples in a muffle furnace at 600 °C for 20 h.

2.8. Lipid content and fatty acid composition

Total lipid was extracted from ground feeds, homogenised whole fish and flesh (NQC), and homogenates of liver, intestine (pyloric caeca), gill, brain and eye (MX blender; Waring, USA) prepared from the two pools of two fish per tank ($n = 6$ per diet) according to the method of Folch, Lees, and Sloane-Stanley (1957). Briefly, approximately 1 g samples of experimental material were homogenised in 10 volumes of ice-cold chloroform/methanol (2:1, *v*/v) containing 0.01% (*w*/*v*) butylated hydroxytoluene (BHT) as antioxidant using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK), with content determined gravimetrically. Acid-catalysed transesterification at 50 °C for 16 h was used to prepare fatty acid methyl esters (FAME) from total lipid (Christie, 2003). The FAME were extracted and purified as described previously (Tocher & Harvie, 1988) and quantified by gas chromatography in a Fisons GC-8160 (Thermo Scientific, Hemel Hempstead, UK) equipped with a 30 m × 0·32 mm internal diameter × 0·25 μm ZB-wax column (Phenomenex, Macclesfield, UK), on-column injector, and a flame ionisation detector. Hydrogen was used as carrier gas with an initial oven thermal gradient from 50 °C to 150 °C at 40 °C. min^{-1}, and to a final temperature of 230 °C at 2 °C.min^{-1}. Individual FAME were identified by comparison with a standard mixture (Restek 20-FAME Marine Oil Standard; Thames Restek UK Ltd.) and by reference to published data (Tocher & Harvie, 1988). The GC data were collected and processed using Chromcard for Windows (version 1.19; Thermoquest Italia S.p.A.).

2.9. Flesh quality analyses

Fillets for flesh quality analyses were delivered on ice on the day of slaughter. At day three post-slaughter all fillets ($n = 24$, 12 per diet) were analysed for colour and gaping, then deboned and analysed for texture. To determine flesh colour, all fillets were photographed with a 10-megapixel camera (Canon PowerShot G12) together with a SalmoFan colour scale ruler (Roche, Welwyn Garden City, UK) and a white reference as a white-colour balance for analysis in ImageJ. Colour was determined by comparing with the SalmoFan Lineal colour scale ruler (Roche) in three regions of the fillet above the lateral line: anterior (A, anterior to the dorsal fin), middle (B, below the dorsal fin), and posterior (C, tail area). For each area, a colour- numbered score from the SalmoFan was assigned. The scores from the SalmoFan ruler ranged from 20 to 34. Gaping was assessed on a 5-point scale, but was found to be minimal with only one individual showing very minor gapes. All measurements were assessed independently by two people and the mean score calculated.

Mechanical texture analysis was performed using a TA.XTplus texture analyzer (Stable Micro Systems, Godalming, UK). Firmness measurements were made using a Warner Bratzler blade, which is a blunt blade of 3 mm thickness with a V-shape notch in the cutting surface. Tensile strength was measured by mounting the sample on a Pizza Tensile rig and using the skin to maintain good grip. The skin was cut with scissors between the mounts and the sample was then pulled apart while measuring the force required to do so. All test samples were cut from standardised locations on the fillet and were cut and trimmed using measured moulds to ensure maximum sample accuracy (4 × 4 × 2 cm blocks for firmness, and 4 × 8 × 2 cm for tensile strength). Both measurements were performed in duplicate and were analysed by calculating the area under the force/distance curve generated. The resulting value was expressed as mJ of work required to perform the standardised test movement. Full technical details of the procedure are reported in Ashton, Michie, and Johnston (2010).

2.10. Carotenoid analysis

Carotenoid contents of flesh (NQC) were determined using a modification of the method of Barua, Kostic, and Olsen (1993). Briefly, samples of approximately 1 g of homogenised NQC (see above) were added to 10 ml ethanol/ethyl acetate (1:1, by volume) and thoroughly blended (Ultra-Turrax tissue disrupter; Fisher Scientific) before being centrifuged at 1000 x*g* for 5 min. The supernatant was collected into a clean glass tube and the pellet homogenised and centrifuged twice more, firstly in 5 ml ethyl acetate then 5 ml isohexane. The combined supernatants were dried at room temperature under a stream of nitrogen and desiccated overnight *in vacuo* before being resuspended in 2 ml isohexane. Samples were analysed by HPLC on an Ultimate 300 UHPLC system (Thermo Scientific) equipped with a 50 × 3 mm, 1.7 μ silica column (Synchronis; Thermo Scientific), using an isocratic solvent system consisting of isohexane/acetone/isopropanol (82:16:2, by volume) at a flow rate of 0.5 ml.min^{-1} with detection at a wavelength of 470 nm. Astaxanthin and other carotenoids were quantified using an external standard of astaxanthin obtained from DSM (Heerlen, Netherlands).

2.11. Statistical analysis

Data were presented as means ± SD with $n = 3$ for fish performance data (Table 3), or $n = 6$ for biochemical analyses data (Tables 4–6), while flesh quality data were presented as means ± SEM with $n = 12$. Percentage data were subjected to arcsin square-root transformation prior to statistical analyses, and data were tested for normality and homogeneity of variances with Levene's test prior to nested one-way analysis of variance (ANOVA) with the factor "pen" nested into "treatment" followed by a Tukey and post-hoc test to determine significant differences for multiple comparisons. All statistical analyses were performed using SPSS software (IBM SPSS Statistics 23; SPSS Inc., Chicago, IL, USA) except for the flesh quality analyses, including Pearson Correlation, that were performed in R. For all data, a *P*-value <0.05 was considered significant.

3. Results

3.1. Fish growth performance and feed efficiency

There were almost no significant differences observed in any of the growth, biometric or feed efficiency parameters evaluated at the end of the feeding trial between the fish fed the COM and TCO diets (Table 3). Overall mortality during the trial was low at around 5% and not related to the feeds. While the average size of fish fed the TCO diet was just over 3.1 kg compared to 3.6 kg for the average size of fish fed the COM diet, the range of fish sizes obtained, especially in pens fed the COM diet, meant that this difference was not statistically significant ($P = 0.0555$) (Table 3). Furthermore, there were no differences in weight gain and TGC at the end of the trial. While there was also no difference in VSI, HSI was slightly, but significantly, higher in the fish fed the TCO diet at the end of the trial.

In contrast to the overall trial results, significant differences in final weights, weight gain, SGR and TGC between fish fed the COM and TCO diets were observed in the intermediate phase of the trial from approximately 850 g up to around 2 kg (Table 3). However, other than condition factor (k) that was significantly higher in the COM fish, there were no significant differences in any measured parameter between fish fed the COM and TCO diets in the latter phase of the trial. In addition, there were no significant differences in feed intake or feed efficiency as measured by FCR between the fish fed the two diets at any stage of the trial.

Table 3
Effects of diet on survival, growth performance, biometric parameters, feed intake and feed efficiency.

Parameter	COM			TCO			
OVERALL							
Initial Weight (g)	186.6	±	2.5	187.7	±	1.3	
Final Weight (g)	3601.9	±	307.6	3108.3	±	87.0	
Final Length (cm)	63.1	±	1.8	60.6	±	0.8	
Weight gain (g)	3414.2	±	307.2	2921.7	±	85.6	
SGR	1.1	±	0.0	1.1	±	0.0	
TGC	3.3	±	0.1	3.1	±	0.0	
HSI	1.1	±	0.0	1.2	±	0.0	*
VSI	10.1	±	0.3	10.0	±	0.6	
FI (g/fish/day)	16.1	±	0.9	14.8	±	1.6	
FCR	1.3	±	0.1	1.3	±	0.1	
Survival (%)	95.8	±	2.0	94.6	±	1.4	
WEEKS 0–11							
Initial Weight (g)	186.7	±	13.7	188.0	±	13.7	
Final Weight (g)	855.0	±	24.1	813.0	±	11.3	
Length (cm)	40.8	±	1.1	39.6	±	0.4	
Weight gain (g)	668.0	±	24.1	626.0	±	11.3	
SGR	2.0	±	0.0	1.9	±	0.0	
TGC	3.2	±	0.1	3.1	±	0.0	
FI (g/fish/day)	8.4	±	1.0	7.9	±	0.5	
FCR	1.0	±	0.1	1.0	±	0.0	
Condition (k)	1.0	±	0.0	1.0	±	0.0	
WEEKS 12–23							
Initial Weight (g)	855.0	±	24.1	813.0	±	11.3	
Final Weight (g)	2071.2	±	97.3	1812.4	±	37.0	*
Length (cm)	51.6	±	0.7	49.4	±	0.3	*
Weight gain (g)	1216.2	±	75.7	999.4	±	36.3	*
SGR	1.2	±	0.1	1.0	±	0.0	*
TGC	3.3	±	0.1	2.9	±	0.1	*
FI (g/fish/day)	17.6	±	0.9	15.4	±	1.8	
FCR	1.1	±	0.0	1.2	±	0.1	
Condition (k)	0.9	±	0.0	0.8	±	0.0	
WEEKS 24–37							
Initial Weight (g)	2071.2	±	97.3	1812.4	±	37.0	*
Final Weight (g)	3601.9	±	307.6	3108.3	±	87.0	
Length (cm)	63.1	±	1.8	60.6	±	0.8	
Weight gain (g)	1530.7	±	97.3	1295.9	±	37.0	
SGR	0.6	±	0.1	0.6	±	0.0	
TGC	3.6	±	0.4	3.4	±	0.1	
FI (g/fish/day)	19.3	±	1.4	18.3	±	3.2	
FCR	1.2	±	0.1	1.4	±	0.3	
Condition (k)	1.4	±	0.0	1.3	±	0.0	*

Values are means ± SD (n = 3). An asterisk denotes a significant difference ($P < 0.05$) between mean values for fish fed the COM and TCO diets.
COM, control/reference feed reflecting current commercial practices;
FCR, feed conversion ratio; FI, feed intake; SGR, specific growth rate;
TCO, feed with all added oil supplied by the oil from transgenic Camelina;
TGC, thermal growth coefficient.

Table 4
Effects of diet on proximate compositions (percentage) and fatty acid compositions (percentage and mg.100 g^{-1}) of total lipid of whole fish.

	Percentage							mg. 100 g^{-1}						
	COM			TCO				COM			TCO			
Proximate composition														
Lipid	21.11	±	1.75	20.08	±	1.20			–			–		
Protein	15.65	±	0.55	15.79	±	0.30			–			–		
Ash	1.68	±	0.18	1.65	±	0.09			–			–		
Moisture	59.42	±	1.28	59.71	±	0.70			–			–		
Fatty acid														
14:0	1.94	±	0.05	0.42	±	0.06	*	364.4	±	24.4	72.9	±	10.0	*
16:0	9.28	±	0.08	7.58	±	0.21	*	1747.5	±	119.9	1310.4	±	90.2	*
18:0	2.64	±	0.08	4.07	±	0.10	*	496.4	±	43.7	703.1	±	54.0	*
Total saturated[1]	14.47	±	0.10	13.64	±	0.23	*	2722.9	±	193.4	2357.5	±	153.7	*
16:1n-7	3.23	±	0.12	0.67	±	0.09	*	606.8	±	36.9	115.7	±	17.8	*
18:1n-9	39.12	±	0.69	12.57	±	0.79	*	7360.2	±	466.6	2179.0	±	176.9	*
18:1n-7	4.23	±	0.16	1.66	±	0.15	*	795.7	±	43.6	286.4	±	29.1	*
20:1n-9	4.18	±	0.08	6.61	±	0.09	*	786.1	±	59.9	1142.5	±	84.1	*
22:1n-11	1.69	±	0.15	0.32	±	0.17	*	316.7	±	29.3	53.7	±	28.0	*
Total monoenes[2]	54.27	±	0.98	23.65	±	1.11	*	10,208.8	±	633.4	4086.0	±	291.2	*
18:2n-6	13.99	±	0.25	19.72	±	0.04	*	2633.5	±	182.4	3410.5	±	246.0	*
18:3n-6	0.13	±	0.03	1.06	±	0.02	*	25.3	±	6.5	183.4	±	14.1	*
20:2n-6	0.94	±	0.04	2.04	±	0.28	*	177.8	±	15.8	354.4	±	61.2	*
20:3n-6	0.25	±	0.03	1.17	±	0.04	*	47.8	±	7.7	201.8	±	19.0	*
20:4n-6	0.30	±	0.05	1.88	±	0.06	*	57.0	±	12.3	324.9	±	26.1	*
22:4n-6	0.00	±	0.00	0.64	±	0.02	*	0.0	±	0.0	110.7	±	9.8	*
22:5n-6	0.00	±	0.00	0.08	±	0.02	*	0.0	±	0.0	14.4	±	4.2	*
Total n-6 PUFA	15.63	±	0.33	26.60	±	0.37	*	2941.3	±	217.0	4600.2	±	357.4	*
18:3n-3	5.53	±	0.40	14.26	±	0.16	*	1042.3	±	115.8	2466.0	±	181.5	*
18:4n-3	0.60	±	0.03	1.03	±	0.01	*	113.3	±	8.8	178.2	±	12.6	*
20:3n-3	0.41	±	0.04	1.59	±	0.06	*	78.0	±	11.1	275.1	±	24.7	*
20:4n-3	0.74	±	0.05	2.17	±	0.07	*	140.2	±	13.7	375.4	±	35.2	*
20:5n-3	2.69	±	0.12	5.56	±	0.13	*	507.3	±	51.5	961.6	±	80.7	*
22:5n-3	1.13	±	0.08	4.58	±	0.29	*	213.5	±	26.2	792.0	±	83.1	*
22:6n-3	3.86	±	0.18	6.85	±	0.38	*	726.8	±	70.8	1185.3	±	121.9	*
Total n-3 PUFA	15.12	±	0.65	36.08	±	0.87	*	2850.1	±	274.3	6241.3	±	522.3	*
Total PUFA	31.27	±	0.91	62.71	±	1.24	*	5888.9	±	497.9	10,847.9	±	880.6	*
Total n-3 LC-PUFA	8.84	±	0.33	20.74	±	0.90	*	1665.7	±	161.5	3589.4	±	340.4	*
EPA:DHA	0.70	±	0.05	0.81	±	0.03	*		–			–		
n-3PUFA:n-6PUFA	0.97	±	0.03	1.36	±	0.01	*		–			–		
Content (g.100 g^{-1})														
EPA		–			–			0.51	±	0.05	0.96	±	0.08	*
DHA		–			–			0.73	±	0.07	1.19	±	0.12	*
EPA + DHA		–			–			1.23	±	0.12	2.15	±	0.20	*
n-3LC-PUFA		–			–			1.66	±	0.14	3.59	±	0.28	*

Values are means ± SD (n = 6). An asterisk denotes a significant difference ($P < 0.05$) between mean values for fish fed the COM and TCO diets. [1], includes 15:0, 20:0, 22:0 and 24:0, present at up to 0.3%. [2], includes 16:1n-9, 17:1, 20:1n-11, 20:1n-7, 22:1n-9 and 24:1n-9, present at up to 0.8%. COM, control/reference feed reflecting current commercial practices; DHA, docosahexaenoic acid (22:6n-3); EPA, eicosapentaenoic acid (20:5n-3); LC-PUFA, long-chain PUFA; PUFA, polyunsaturated fatty acids; TCO, feed with all added oil supplied by the oil from transgenic Camelina.

3.2. Proximate compositions of whole fish and muscle/flesh

There were no significant differences in the protein, lipid, ash and moisture contents of whole fish between salmon fed the COM and TCO diets (Table 4). However, the proportion of total lipid in flesh of salmon fed the TCO diet was slightly, but significantly, lower than the proportion of lipid in flesh of fish fed the COM diet, while moisture contents were higher (Table 5). Diet had no effect the proportions of protein or ash in the salmon flesh.

3.3. Fatty acid compositions of whole fish and muscle/flesh

In percentage terms, total lipid of whole fish of salmon fed the TCO diet showed significantly increased proportions of both n-3 and n-6 PUFA and lower proportions of saturates and, especially, monoenes compared to fish fed the COM diet (Table 4). Percentages of all individual saturated and monounsaturated fatty acids fatty acids were reduced, other than 20:1n-9, while percentages of all individual n-6 PUFA were increased. Total n-3 LC-PUFA were increased by almost 2.3-fold, with EPA, DHA, 20:4n-3 and 22:5n-3 increased by 2.1-, 1.8-, 2.9- and 4.1-fold, respectively. In addition, the proportions of 18:3n-3, 18:2n-6 and arachidonic acid (20:4n-6) were increased 2.6-. 1.4- and 6.3-fold in fish fed the TCO diet compared to fish fed the COM diet. The same significant trends in fatty acid contents were also apparent when reported in mg fatty acids per 100 g of fish, absolute terms that also reflected lipid content. These data showed that fish fed the TCO diet contained almost 3.6 g and 2.2 g of total n-3 LC-PUFA and EPA + DHA, respectively, compared to just under 1.7 g and 1.2 g of total n-3 LC-PUFA

Table 5
Effects of diet on proximate compositions (percentage) and fatty acid compositions (percentage and mg.100 g^{-1}) of total lipid of muscle/flesh (NQC).

	Percentage							mg. 100 g^{-1}						
	COM			TCO				COM			TCO			
Proximate composition														
Lipid	16.43	±	1.50	14.50	±	0.90	*		–			–		
Protein	18.85	±	0.75	19.64	±	0.44			–			–		
Ash	1.64	±	0.15	1.75	±	0.13			–			–		
Moisture	62.88	±	1.07	64.42	±	0.42	*		–			–		
Fatty acid														
14:0	1.89	±	0.09	0.40	±	0.06	*	282.0	±	31.0	52.0	±	7.9	*
16:0	9.08	±	0.12	7.46	±	0.16	*	1353.8	±	107.1	962.9	±	49.2	*
18:0	2.62	±	0.11	3.85	±	0.11	*	391.7	±	39.0	497.0	±	29.9	*
Total saturated[1]	14.27	±	0.16	13.23	±	0.25	*	2128.5	±	182.2	1707.5	±	90.7	*
16:1n-7	3.15	±	0.10	0.63	±	0.12	*	470.7	±	45.6	81.5	±	14.8	*
18:1n-9	39.19	±	0.99	11.97	±	1.09	*	5842.9	±	489.4	1544.4	±	154.6	*
18:1n-7	3.49	±	0.07	1.60	±	0.09	*	520.9	±	43.1	207.1	±	15.7	*
20:1n-9	4.00	±	0.18	6.66	±	0.09	*	597.6	±	62.9	860.0	±	63.7	*
22:1n-11	1.54	±	0.08	0.30	±	0.03	*	228.5	±	17.4	38.3	±	5.9	*
Total monoenes[2]	53.06	±	0.95	23.27	±	1.22	*	7911.8	±	662.5	3004.4	±	232.4	*
18:2n-6	14.02	±	0.20	19.87	±	0.21	*	2092.2	±	201.0	2566.7	±	188.8	*
18:3n-6	0.14	±	0.03	1.03	±	0.04	*	21.6	±	5.9	133.7	±	10.3	*
20:2n-6	0.99	±	0.07	2.17	±	0.08	*	148.3	±	18.3	280.5	±	23.7	*
20:3n-6	0.27	±	0.04	1.16	±	0.06	*	40.6	±	8.2	150.1	±	15.3	*
20:4n-6	0.33	±	0.04	1.80	±	0.08	*	49.2	±	8.7	232.4	±	18.7	*
22:4n-6	0.06	±	0.02	0.63	±	0.05	*	9.5	±	2.9	81.8	±	9.8	*
22:5n-6	0.06	±	0.01	0.07	±	0.01		9.2	±	1.9	8.5	±	1.0	
Total n-6 PUFA	15.88	±	0.37	26.73	±	0.49	*	2370.7	±	237.6	3453.6	±	262.6	*
18:3n-3	5.75	±	0.41	14.74	±	0.24	*	859.1	±	116.2	1904.7	±	143.9	*
18:4n-3	0.61	±	0.04	0.99	±	0.02	*	91.6	±	11.6	127.9	±	8.4	*
20:3n-3	0.44	±	0.06	1.62	±	0.07	*	65.5	±	11.5	209.2	±	18.7	*
20:4n-3	0.78	±	0.06	2.17	±	0.08	*	116.9	±	15.4	280.7	±	26.4	*
20:5n-3	2.85	±	0.10	5.40	±	0.11	*	424.6	±	39.9	697.3	±	44.5	*
22:5n-3	1.29	±	0.11	4.68	±	0.21	*	193.4	±	30.4	605.3	±	58.3	*
22:6n-3	4.36	±	0.14	7.07	±	0.31	*	650.5	±	54.3	914.0	±	85.1	*
Total n-3 PUFA	16.25	±	0.68	36.73	±	0.80	*	2426.9	±	266.6	4745.8	±	376.1	*
Total PUFA	32.67	±	1.02	63.50	±	1.23	*	4878.2	±	510.9	8204.5	±	634.8	*
Total n-3 LC-PUFA	9.72	±	0.29	20.94	±	0.69	*	1450.9	±	142.2	2706.5	±	229.5	*
EPA:DHA	0.65	±	0.02	0.76	±	0.03	*		–			–		
n-3PUFA:n-6PUFA	1.02	±	0.02	1.37	±	0.01	*		–			–		
Content (g.100 g^{-1})														
EPA		–			–			0.42	±	0.04	0.70	±	0.04	*
DHA		–			–			0.65	±	0.05	0.91	±	0.09	*
EPA + DHA		–			–			1.08	±	0.09	1.61	±	0.13	*
n-3LC-PUFA		–			–			1.45	±	0.12	2.71	±	0.19	*

Values are means ± SD (n = 6). An asterisk denotes a significant difference ($P < 0.05$) between mean values for fish fed the COM and TCO diets. [1], includes 15:0, 20:0, 22:0 and 24:0, present at up to 0.3%. [2], includes 16:1n-9, 17:1, 20:1n-11, 20:1n-7, 22:1n-9 and 24:1n-9, present at up to 0.8%. COM, control/reference feed reflecting current commercial practices; DHA, docosahexaenoic acid (22:6n-3); EPA, eicosapentaenoic acid (20:5n-3); LC-PUFA, long-chain PUFA; PUFA, polyunsaturated fatty acids; TCO, feed with all added oil supplied by the oil from transgenic Camelina.

and EPA + DHA, respectively, in fish fed the COM diet (Table 4).

Similarly, in percentage terms, total lipid of flesh of fish fed diet TCO also showed significantly increased proportions of n-3 and n-6 PUFA and lower proportions of monoenes compared to fish fed the COM diet (Table 5). More specifically, total n-3 LC-PUFA were increased 2.1-fold, with EPA, DHA, 20:4n-3 and 22:5n-3 increased by 1.9-, 1.6-, 3.7- and 3.6-fold, respectively, while proportions of 18:3n-3, 18:2n-6 and 20:4n-6 increased 2.6-, 1.4- and 5.5-fold in flesh of salmon fed the TCO diet compared to fish fed the COM diet. Both the EPA:DHA and n-3:n-6 PUFA ratios increased in flesh of salmon fed TCO compared to fish fed COM. The key data with respect to human consumers showed that, in absolute terms (g fatty acids per 100 g of flesh), the flesh of salmon fed the TCO diet contained 2.7 g of n-3 LC-PUFA including 0.7 g of EPA, 0.9 g of DHA and 1.6 g of EPA + DHA. These data were all significantly higher than the equivalent data in flesh of fish fed the COM diet that delivered 1.45 g of n-3 LC-PUFA including 0.4, 0.65 and just under 1.1 g of EPA, DHA and EPA + DHA, respectively (Table 5).

3.4. Lipid contents and fatty acid compositions of tissues

The lipid content of liver of salmon fed the TCO diet was significantly higher than the liver lipid content of fish fed the COM diet (Supplementary Table 1). In contrast, diet had no significant effects on the lipid contents of intestine (pyloric caeca) and gill (Supplementary Table 1), or brain and eye (Supplementary Table 2).

As reported above for whole fish and flesh, the proportions of total n-3 and total n-6 PUFA in almost all the tissues (liver, intestine, gill, and eye) were higher in fish fed the TCO diet compared to fish fed the COM

Table 6
Summary of flesh quality measurements for each dietary treatment.

	COM			TCO		
Firmness (mJ)	525.3	±	26.3	544.2	±	34.3
Tensile strength (mJ)	267.7	±	14.1	282.2	±	15.7
Gaping		nd			nd	
Roche colour score A	26.8	±	0.4	27.1	±	0.6
Roche colour score B	26.7	±	0.5	27.9	±	0.6
Roche colour score C	28.7	±	0.3	28.9	±	0.3
Average colour ABC	27.2	±	0.4	28.0	±	0.4*
Total carotenoids (mg.kg^{-1})	2.65	±	0.63	2.20	±	0.42

Values are means ± SEM (n = 12) except for carotenoid content of NQC, which was $n = 3$. An asterisk denotes a significant difference ($P < 0.05$) between mean values for fish fed the COM and TCO diets.COM, control/reference feed reflecting current commercial practices; nd, no noteworthy gaping detected; TCO, feed with all added oil supplied by the oil from transgenic Camelina.

diet, while the proportions of monoenes were significantly lower, and diet had no effect on the proportions of total saturated fatty acids (Supplementary Tables 1 and 2). It was clear that, of all the tissues, the fatty acid composition of brain was least affected by diet (Supplementary Table 2). However, while total n-3 LC-PUFA was significantly higher in salmon fed diet TCO compared to fish fed diet COM in all tissues except brain, this was largely due to increased proportions of EPA, 20:4n-3 and 22:5n-3 while the proportions of DHA were only higher in gill, but unaffected by diet in intestine, brain and eye (tissues with the highest DHA contents) or even lower as in liver (Supplementary Tables 1 and 2).

3.5. Flesh quality

A summary of the trait values across all flesh quality comparisons are presented in Table 6. On average, measurements for both firmness and tensile strength were numerically higher for the flesh of salmon fed the TCO diet in comparison to flesh of fish fed the COM diet 1 (Table 6). However, these differences in firmness and tensile strength were not statistically significant (ANOVA: F = 0.206, $p = 0.66$ and F = 0.617, $P = 0.44$, respectively). Interestingly, a positive significant correlation was found between tensile and firmness measurements (Pearson correlation: $r = 0.47$, $P = 0.02$). While there was no noteworthy gaping observed among fillets, flesh colour of salmon fed the COM diet was significantly paler that those fed the TCO diet (ANOVA: F = 4.286, $P = 0.05$). However, analyses of flesh (NQC) showed that there were no significant differences in total contents or compositions (> 95% astaxanthin, 3% astacene and < 2% lutein) of carotenoids between fish fed the two diets.

4. Discussion

It is over 50 years since the pioneering work of Dyerberg and Bang first reported on the effects of a marine-based diet (Bang, Dyerberg, & Nielsen, 1971), which subsequently led to the discovery of the importance of the omega-3 LC-PUFA in human nutrition (Dyerberg, Bang, Stoffersen, Moncada, & Vane, 1978). Therefore, the role of a marine diet and fish in providing the human population with the health-beneficial fatty acids, EPA and DHA, was known from the very beginning of "omega-3" research. However, it is now well established that there is a large gap between the demand for EPA and DHA required for human health and their supply from both traditional (capture fisheries) and modern (aquaculture) sources (Tocher et al., 2019). Bridging this gap is a very real problem with many human populations around the globe shown to have very low levels of EPA and DHA in the blood (Stark, Van Elswyk, Higgins, Weatherford, & Salem Jr, 2016). It is within this context that present study is placed.

In the present trial, salmon were grown in seawater pens for 9 months during which time they grew from around 180 g to >3 kg. There were no significant differences in final average weight, weight gain, SGR or TGC between fish fed the COM and TCO diets. However, it is clear that salmon fed the TCO diet were on average smaller than fish fed the COM diet, and this was only not significant because of the large range in weights obtained in these ungraded populations. The range in fish size was >3-fold greater in fish fed the COM diet and due to the presence of some very large fish in that group, rather than the presence of smaller fish in the TCO group. This size difference between the dietary treatments stemmed from lower growth rate in fish fed the TCO diet during the second/intermediate phase of the trial, when significantly lower final weights, weight gain, SGR and TGC were recorded. Although the difference in final weight increased in the final phase of the trial, growth in that phase was not significantly different between the dietary treatments. The difference in final weights in the present trial was not observed in the previous trial where salmon were fed the same oil used in the TCO diet with fish grown from 130 g to 400 g (Betancor et al., 2018), or in any of the earlier trials feeding salmon the oils obtained from previous iterations of GM *Camelina* and growing fish to 200 g (Betancor, Sprague, Usher, et al., 2015), 400 g (Betancor et al., 2017), or 500 g (Betancor, Sprague, Sayanova, et al., 2016).

Fish fed the TCO diet showed numerically lower FI (in g/fish/day) throughout the trial, being 5–6% lower in the first and last phases of the trial, but over 12% lower in the intermediate phase, and 8% lower overall. Although none of these differences in FI were statistically significant, it is highly likely that lower FI was the reason for differences observed in final weights and weight gains of fish fed the TCO diet compared to fish fed the COM diet. Supporting this conclusion, FCR was not significantly different between fish fed the COM and TCO diets at any point in the trial suggesting that there were no differences in intermediary metabolism and/or metabolic performance of the diets, and that both feeds were utilised with the same efficiency. However, in a trial in European sea bass, FI and SGR were significantly lower in the first month of the 4-month trial in fish fed the TCO oil compared to fish fed a control FO diet (Betancor et al., 2021). It was speculated that the initially lower FI, which affected growth, was possibly due to reduced palatability, as it was overcome in the later phases of the trial. *Camelina sativa* is a Brassicaceae and, as such, contains glucosinolates (Berhow et al., 2013) that are known to cause the bitter/sharp taste of many cruciferous vegetables (Clarke, 2010), and studies investigating feed ingredients rich in glucosinolates have shown they negatively affect palatability and reduce growth of fish (Francis, Makkar, & Becker, 2001), which may be exacerbated in feeds with limited inclusion of fishmeal. The Camelina oil used in the present study was equivalent to a "virgin" oil and received no processing post-extraction, which could cause palatability issues, but also suggests that these could be alleviated by reducing the glucosinolate level and/or the inclusion of feed additives or palatants including palatability enhancers and feed attractants (Pilmer, Woolley, Lymbery, Salini, & Partridge, 2022). This would be part of the normal process of commercial optimisation of feed formulations containing the Camelina oil, securing the best inclusion levels to achieve optimal diet performance. The reduction of glucosinolates in *C. sativa* using a biotechnological approach would be one option and a potential future target (Nour-Eldin et al., 2017). Overall, therefore, the difference in final weights reported in the present trial was likely due to the crude nature of the oil used in the TCO diet, which impacted the palatability and intake of the feed, particularly in the first part of the trial. Several options are available to mitigate this issue since it is commonly encountered in the replacement of marine ingredients with plant ingredients (Francis et al., 2001; Nagel et al., 2012).

While diet had no impact on the biochemical composition of whole fish, the flesh of salmon fed the COM diet had a lipid content of almost 16.5% whereas the lipid content of flesh of fish fed the TCO diet TCO was significantly lower at 14.5% of wet weight. As would be expected, the lower lipid content of flesh of salmon fed the TCO diet was accompanied by increased moisture content. The "target" value for flesh lipid content of farmed salmon in Scotland is around 16–17% based on

retailer and quality label specifications (*e.g.* Label Rouge, ≤ 16%), so the COM fish were perfectly in this range. As the tissue that arguably represents the largest fat store in salmon, the lower flesh lipid level in fish the salmon fed TCO may reflect the lower FI and, consequently, energy intake of fish fed this diet resulting in lower lipid deposition and accumulation in flesh and, possibly, lower body weight. Lower lipid contents in whole fish and flesh of smaller (400 g) Atlantic salmon fed the TCO diet compared to fish fed a COM diet were reported previously (Betancor et al., 2018). In that study, it was suggested that the lower body and flesh lipid contents could be associated with the higher EPA and DHA contents of the TCO diet compared to the COM diet as these n-3 LC-PUFA are known to have anti-adipogenic effects in mammals (Dentin et al., 2005). In addition, microarray analysis revealed that the lipogenic gene, *acsl1* (acyl-CoA synthetase long chain family member 1) was down-regulated in fish fed TCO compared to fish fed COM, possibly indicating reduced lipogenesis, and the *lpl* (lipoprotein lipase) gene was also downregulated in TCO-fed fish, which could be considered consistent with lower flesh lipid levels (Betancor et al., 2018). However, in trials in similarly smaller salmon fed the oils obtained from earlier iterations of GM *Camelina* including an EPA-only oil (Betancor, Sprague, Usher, et al., 2015) or an oil with 6% each of EPA + DHA (Betancor et al., 2017; Betancor, Sprague, Sayanova, et al., 2016), no significant impacts on flesh lipid contents were observed.

The small, but significant, difference in lipid content in flesh discussed above was generally not reflected in any of the flesh quality parameters measured, which showed no difference in firmness, tensile strength or gaping between dietary treatments. However, there was a significant difference in flesh colour with fish fed the TCO diet showing higher average colour in the Roche SalmoFan Lineal colour scale. The effects of the TCO diet on flesh colour and carotenoid content had not been measured previously in our earlier trials as they were performed in land-based tanks and fish were still too small at the end of the feeding trials for impacts on pigmentation to be meaningful (Betancor, Sprague, Sayanova et al., 2016; Betancor et al., 2018; Betancor et al., 2018). While this result was interesting, the underpinning reason was unclear as the flesh carotenoid content (mg.kg^{-1}) was not significantly different between fish fed the two diets, and the amount of carotenoid relative to flesh lipid level was also very similar. However, a similar result was reported in salmon fed diets containing oil ("Aquaterra") from GM Canola and grown in sea pens to 1.5 kg (Ruyter, Bou, TuridSissener, Monica Lutfi, & Østbye, 2022). In that study, red colour intensity was significantly higher in flesh of salmon fed a diet with 50% of oil supplied by the GM Canola (replacing the VO components of the diet) compared to control fish without GM Canola, while flesh astaxanthin levels were not significantly different between dietary groups (Ruyter et al., 2022). Similarly, in another study with salmon grown from 700 g to over 4.5 kg on feeds containing increasing levels of GM Canola, no differences were reported in flesh astaxanthin and total carotenoid concentrations (Hatlen et al., 2022).

In contrast to flesh, lipid contents were unaffected by diet in most tissues other than liver where lipid content was over 50% higher in salmon fed the TCO diet compared to fish fed the COM diet. This was consistent with the slightly, but significantly, higher HSI of fish fed TCO. Increased liver lipid levels are often regarded as reflecting a metabolic disturbance, potentially as a result of some lipid or nutrient imbalance. The high proportion of 18:3n-3 that would, arguably, be more likely to be esterified into tissue lipids, combined with lower proportions of monoenoic fatty acids that would be more likely to promote fatty acid oxidation, may represent a metabolic imbalance that could lead to accumulation of lipid in liver of fish fed TCO compared to fish fed COM. In our previous trial with smaller fish, liver lipid contents were not significantly different between fish fed the TCO and COM diets (Betancor et al., 2018) and, similarly, the oil from GM *Camelina* containing 6% each of EPA + DHA had no impact on liver lipid contents (Betancor et al., 2017, Betancor, Sprague, Sayanova, et al., 2016). In contrast, salmon fed the EPA-only oil (Betancor, Sprague, Usher, et al., 2015) showed higher whole body and liver lipid contents compared to salmon fed the control FO diet (Betancor, Sprague, Usher, et al., 2015).

The main driver for the development of new sources of EPA and DHA was to increase the availability of these critical EFA to the human population and, therefore, arguably, the most important data in the present study are those showing the impact of the TCO diet on the fatty acid compositions of the salmon. Thus, it was noteworthy that the levels of all n-3 LC-PUFA in whole fish increased considerably, and total n-3 LC-PUFA were over 2-fold greater in salmon fed the TCO diet compared to fish fed the COM diet. However, nutritional quality of the salmon in terms of EPA and DHA is based on the composition of the edible portion, flesh/muscle, and the present study showed that, in relative terms, the proportion of total n-3 LC-PUFA of flesh also more than doubled from 9.7% of total fatty acids (TFA) in fish fed COM to 20.9% in fish fed the TCO diet. In absolute terms, EPA, DHA, EPA + DHA and total n-3 LC-PUFA increased from 0.42, 0.65, 1.08 and 1.45 g/100 g^{-1} flesh, respectively, in fish fed the COM diet to 0.70, 0.91, 1.61 and 2.71 g/100 g^{-1} flesh, respectively, in salmon fed the TCO diet. In consequence, a standard 140 g portion of flesh of salmon fed the TCO diet would deliver almost 2.3 g of EPA + DHA and 3.8 g of total n-3 LC-PUFA (EPA, DHA, 22:5n-3, 20:4–3 and 20:3n-3) and, therefore, a single 140 g portion of salmon fed TCO would deliver 67% of the weekly requirement level of EPA and DHA (3.5 g; 500 mg daily) recommended by many health agencies (ISSFAL, 2004; EFSA, 2010). In contrast, a 140 g portion of the salmon fed the COM diet, reflecting current farming practices, would deliver 1.5 g EPA+ DHA, similar to the level reported for commercial Scottish salmon in 2016 (Sprague et al., 2016), and 0.8 g lower than a portion of salmon fed TCO, and less than half the recommended weekly intake (ISSFAL, 2004; EFSA, 2010). Salmon fed the TCO diet, and with a similar flesh lipid content (16.5%) to the COM fish, could arguably have an even higher EPA + DHA content at around 2.6 g per 140 g portion, representing 75% of the recommended weekly intake.

Therefore, replacing entirely (100%) the current added oil, blends of FO and rapeseed oil, used in commercial salmon farming with oil from transgenic *Camelina* in feed for salmon during the seawater growth phase to market size had a major beneficial impact on the nutritional quality of the flesh for human consumers in terms of substantially increased n-3 LC-PUFA including, importantly, both EPA and DHA. Two other GM crops have been developed, both from rapeseed/Canola, producing oils that are either relatively rich in DHA ("Aquaterra®", ~ 9% DHA and 0.5% EPA; Davis & Devine, 2023) or EPA ("Latitude", ~ 7% EPA and 1% DHA). In consequence, incorporating Aquaterra into feed increased predominantly DHA levels in juvenile (Ruyter et al., 2019), on-growing (Ruyter et al., 2022) and harvest-size (Hatlen et al., 2022) Atlantic salmon reared in seawater, while incorporation of Latitude into feed increased predominantly EPA level in rainbow trout reared to market size in freshwater (Hong et al., 2022). In addition to the oils from GM crops, the microalgal oil, "Veramaris" that has high levels of both DHA and EPA (almost 40% and 16% of TFA, respectively), has also been used to replace the FO component of FO/VO blends to successfully maintain EPA and increase DHA levels in flesh of Atlantic salmon grown to market size (3 kg) (Santigosa, Olsen, Madaro, Trichet, & Carr, 2023). Incorporating Veramaris into feeds also improved flesh DHA levels in trials where it was used to replace the FO component of FO/VO blends in both rainbow trout (Santigosa, Constant, Prudence, Wahli, & Verlhac-Trichet, 2020) and gilthead seabream (Santigosa, Brambilla, & Milanese, 2021). Although Veramaris has the highest levels of EPA + DHA of all the new sources, it depends on fermentation, which currently limits supply and is also costly, and so the algal oil is expensive and likely to have a much higher cost per percentage point of EPA + DHA than GM crops. Thus, while not containing as high levels as Veramaris, the oil from transgenic *Camelina* used in the TCO feed was designed to have an EPA + DHA content and composition similar to the FO traditionally used in salmon farming, with higher levels of EPA + DHA (~ 20%) and a better ratio of EPA and DHA (~ 1: 1) than the GM Canola oils and, therefore, it perhaps represents a unique balanced

solution among all the alternatives. However, it is important to stress that all the oils from GM crops, as well as algal oils, have key roles to play in improving the health and nutritional quality of farmed salmon (Tocher et al., 2019), ensuring they can again supply the high levels of EPA and DHA farmed salmon once did before large-scale replacement of marine ingredients (Sprague et al., 2016; Reksten et al., 2022). Reflecting the current very high interest in new sources of omega-3 LC-PUFA, the use of oils from GM crops in aquafeeds received a boost recently when the Aquaterra® GM Canola oil was approved by the Norwegian Food Safety Authority for use in fish feed applications in Norway (Aquaterra, 2023). Furthermore, the fact that the beneficial impacts of oils from GM *Camelina* in increasing n-3 LC-PUFA levels in flesh observed in earlier trials in smaller salmon (Betancor et al., 2016, 2018) translated to market size fish, argues that this would likely extend to other farmed fish species such as gilthead seabream (Betancor, Sprague, Montero, et al., 2016), European sea bass (Betancor et al., 2021), rainbow trout (Osmond et al., 2021) and Atlantic bluefin tuna (Betancor et al., 2022), where oils from GM *Camelina* increased flesh n-3 LC-PUFA levels in trials with small/less than market size fish. This suggested that similar benefits would accrue in these species if fed oil from GM *Camelina* during grow out to market size, providing farmed fish in general with the levels of EPA + DHA expected traditionally of wild capture fish and seafood (Tocher et al., 2019).

Increasing the dietary levels of EPA and DHA in feeds for farmed fish not only benefits human consumers, but also the health and welfare of the farmed fish themselves. Recent studies have shown that the dietary level of EPA + DHA to support optimal health in salmon is much higher than the level of 0.5% of feed reported commonly (Tocher, 2010; NRC, 2011). One study suggested that salmon in seawater required a minimum level of EPA + DHA of at least 2.7% of TFA (~ 1% of diet) based largely on growth (Rosenlund, Torstensen, Stubhaug, Usman, & Sissener, 2016), and other studies suggested that a level of EPA + DHA of at least 1.6% of diet was required to ensure growth and maintain robustness of farmed salmon in seawater (Bou et al., 2017; Sissener et al., 2016). Most recently, however, a further trial indicated that a level of EPA + DHA of 3.5% of diet improved health and welfare of salmon in challenging, but essentially normal, farming conditions (Lutfi et al., 2022). Current levels of EPA + DHA used in salmon farming vary from <2% (Chile) to 3.5% of diet (Faroes), with Norway possibly transitioning between 2.0 and 2.5%. In the present study, the COM diet was formulated to supply EPA + DHA at 2.5% of diet (almost 7% of TFA), which, as indicated earlier, was the standard level in feeds for Scottish salmon at the time of the trial, while the TCO diet supplied EPA + DHA at 4% of diet (11% of TFA), above the highest levels tested in salmon in any of the earlier studies.

The above highlights the importance of EPA and DHA to both fish health and nutritional quality of farmed products in not only salmon but, likely, all farmed fish. While finding alternatives to traditional fish meals as protein sources remains a major driver of research into the feed resources required to support sustainable salmon farming (Albrektsen, Kortet, Skov, et al., 2022), the development of entirely new, sustainable, and economically-viable sources of EPA and DHA is a challenge that has been, at least partly, solved. While recovery and recycling of EPA and DHA from fisheries and aquaculture by-products has increased in recent years, and opportunities likely exist for increased by-product utilisation and waste prevention, various economic, cultural and technical challenges remain to be overcome (Hamilton, Newton, Auchterlonie, & Müller, 2020).

In conclusion, the current study represents an important step in the validation of oil from an oilseed crop, *Camelina sativa*, genetically engineered to produce high levels of EPA and DHA in seeds, as an entirely new, *de novo* source of these health-critical omega-3 LC-PUFA. The present study was performed in semi-commercial conditions in sea pens in salmon grown for 9 months from new smolt (~ 180 g) to market size (> 3 kg). Although there was a size difference at harvest, there were no differences in SGR, FCR or survival between fish fed the TCO or COM diets over the whole growth period. Nutritional quality in terms of "omega-3" content was substantially improved in fish fed the TCO, diet with total n-3 LC-PUFA level of flesh more than double that in fish fed the COM diet. Consequently, a standard 140 g portion of flesh of salmon fed a diet formulated with oil from transgenic *Camelina* would deliver a dose of EPA + DHA sufficient to cover at least two-thirds of the weekly requirement level recommended by many health agencies, and over one and a half times more than the level supplied by fish fed the current commercial dietary regime.

CRediT authorship contribution statement

Douglas R. Tocher: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Matthew Sprague:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Lihua Han:** Writing – review & editing, Resources. **Olga Sayanova:** Writing – review & editing, Resources. **Fernando Norambuena:** Writing – review & editing, Resources. **Johnathan A. Napier:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition, Conceptualization. **Mónica B. Betancor:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest exist.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139414.

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