Received: 18 August 2022 | Revised: 4 January 2023 | Accepted: 11 January 2023

DOI: 10.1111/jwas.12947

APPLIED RESEARCH

Journal of the World Aquaculture Society Open Access | WORLD AQUACULTURE Society | WILEY

Algal oil gives control of long-chain omega-3 levels in full-cycle production of Atlantic salmon, without detriment to zootechnical performance and sensory characteristics

Ester Santigosa[1] | Rolf Erik Olsen[2,3] | Angelico Madaro[2] | Viviane Verlhac Trichet[1] | Ian Carr[4]

[1]DSM Nutritional Products, Basel, Switzerland

[2]Animal Welfare Science Group, Institute of Marine Research, Matredal, Norway

[3]Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

[4]Veramaris V.O.F., Delft, The Netherlands

Correspondence
Ester Santigosa, DSM Nutritional Products France, Research Centre for Animal Nutrition & Health, Saint-Louis Cedex BP1970, France.
Email: ester.santigosa@dsm.com

Abstract

The levels of eicosapentaenoic (EPA) and docosahexaenoic (DHA) in salmon fillets have decreased because of the progressive replacement of fish oil (FO). This study contributes to enabling the sustainable growth of aquaculture by confirming the effects of partially or fully replacing FO with microalgal oil (AO) on growth, muscle fatty acid profiles, and muscle quality of farmed Atlantic salmon. Crucially, this is now done throughout the entire post-smolt production cycle and up to a harvest weight of 3 kg. Three experiments were performed using fish ranging from 145 g to 3 kg and testing different diets, replacing FO up to 100%. Zootechnical performance was similar among treatments in all experiments. Changing the lipid source in the diet resulted in EPA and DHA digestibility of greater than 96%. Sensory characteristics of raw fish fillets were similar among treatments, supporting a similar sensorial experience with the replacement of FO with no impact on consumers. Overall, results confirm that the AO tested here enables the sustainable growth of Atlantic salmon aquaculture by helping to maintain a level of EPA and DHA in the fish fillets,

without detriment to zootechnical performance and sensory characteristics, while simultaneously contributing to a reduced marine footprint for aquafeeds.

KEYWORDS
algal oil, EPA + DHA, FFDRoil, human health, sustainable

1 | INTRODUCTION

Omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) are essential for human nutrition and health. Not only are they precursors of other fatty acids (FA), but they also participate in the activation of metabolic pathways that inhibit the synthesis of proinflammatory compounds (Oliver et al., 2020). Of particular relevance are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whose consumption is strongly recommended because of their well-documented abilities to prevent the onset of cardiovascular diseases (Bernasconi et al., 2021; Mozaffarian & Wu, 2011), optimize neuronal development (Martins et al., 2020), and control inflammatory conditions (Calder, 2017). High levels of EPA and DHA are also essential for fish health and welfare, as increasing levels of these LC-PUFA improve growth performance and intestinal barrier functions in response to chronic stress (Løvmo et al., 2021), immune function (Thompson et al., 1996), and tissue integrity (Bou et al., 2017). Fish products are usually the selected nutritional source of the LC-PUFA necessary for a well-balanced human diet (Tocher et al., 2019). In particular, Atlantic salmon has traditionally been recognized as very rich in both EPA and DHA and thus as a valuable source of these FA for humans (Horn et al., 2019). As such, the Atlantic salmon industry has potential to further expand from the current 2.44 million tonnes, and supply growing global demand for salmonids as a healthy food (FAO, 2020). However, supply limitations and sustainability concerns related to the overuse of marine resources to produce fish oil (FO), the traditional primary lipid sources of salmon aquafeeds because of its excellent nutritional properties (Ytrestøyl et al., 2015), have led to the innovation of new ingredients rich in omega-3 FA (Sprague et al., 2017).

Viable alternative ingredients for salmon aquafeeds have included cost-effective vegetable-based oils (Bell et al., 2005; Bell et al., 2004; Nasopoulou & Zabetakis, 2012). However, these sources often contain high quantities of omega-6 LC-PUFA, such as linoleic acid, and are deficient in omega-3 LC-PUFA, such as EPA and DHA (Jobling, 2011). Therefore, in recent decades, EPA and DHA levels in salmon fillets have decreased by more than half (Nichols et al., 2014; Sprague et al., 2016; Sprague et al., 2020), which is disadvantageous for the health of both farmed salmon (Bou et al., 2017; Horn et al., 2019) and humans (Calder, 2017). The low contribution of omega-3 LC-PUFA, together with an excess of omega-6 LC-PUFA, may unbalance the optimal omega-3/omega-6 ratios for fish (Karalazos et al., 2007; Sissener et al., 2020) and humans (Simopoulos, 2011). As such, with the growing demand for aquaculture products, it is imperative that the industry addresses this significant reduction in total EPA and DHA (Nichols et al., 2014; Sprague et al., 2016; Sprague et al., 2020). Further study on how changes in the ratio of these FA in the diet can improve the health and therefore the welfare of fish is needed (Lutfi et al., 2022; Martinez-Rubio et al., 2012). Such changes can at the same time increase the nutritional quality of fillets for the consumer (Glencross et al., 2022; Mock et al., 2020). Most experts advise an adequate intake (AI) of 250–500 mg of EPA and DHA per week (Nøstbakken et al., 2021; Richter et al., 2016; Sprague et al., 2020). Based on the current levels of EPA and DHA in farmed fish muscle, being around 1 g/100 g of fillet (Sprague et al., 2020; Nifes database), any further reduction in the content of these FA would increase the number of fish servings per week needed to achieve the AI for humans, conflicting with the recommendations from various national health authorities (GOED, 2014; USDA, 2015) regarding the consumption of oily fish, such as salmon. As production volumes continue to rise, it is therefore critical

to deploy innovative and affordable sources of EPA and DHA to restore the levels of these FA in farmed salmon to levels that are consistent with nutritional guidelines and National Health Authority AI recommendations.

Within the last decade, a variety of omega-3-rich ingredients have been developed to address the disparity between EPA and DHA supply and demand in aquaculture (Tocher et al., 2019). The majority of potential products available originate from genetically modified crops (Betancor et al., 2018) or microalgae biomass (Kousoulaki et al., 2016). A good alternative is microalgae oil (AO). In addition to being easily incorporated into fish feed formulations (Tocher et al., 2019), it also has the advantage of containing a combination of EPA, DHA, and arachidonic acid (ARA)—as found in marine ingredients—while minimizing omega-6 LC-PUFA inclusion (Santigosa et al., 2021). Indeed, previous studies have shown the successful replacement of FO and vegetable oil (VO) with AO in rainbow trout, gilthead seabream, and Atlantic salmon diets without any negative effects on fish health and growth (Carvalho et al., 2020; Carvalho et al., 2022; Miller et al., 2007; Santigosa et al., 2020; Santigosa et al., 2021). As aquafeeds formulated from microalgae show less contamination by heavy metals, dioxins, or dioxin-like PCBs (Ratledge, 2010; Santigosa et al., 2021), AO also offers enhanced food safety.

The slow uptake of alternative feed ingredients (Aas et al., 2022) and the call to solve remaining challenges to successfully implement new ingredients in the aquaculture industry (Albrektsen et al., 2022) support the fact that the effects of using AO on Atlantic salmon (*Salmo salar* L.) farmed in seawater warrants further study. Therefore, the novelty of this work lies in clearly documenting the suitability of AO as an alternative salmon feed ingredient throughout the entire post-smolt production cycle and up to a harvest weight of 3 kg, while maintaining the level of EPA and DHA in the fish fillets, without detriment to zootechnical performance and sensory characteristics, while simultaneously contributing to reduce the marine footprint of aquafeeds. To our knowledge, the present study is the first of its kind in providing such a rounded package of documentation in support of AO as a suitable salmon feed ingredient.

2 | MATERIALS AND METHODS

Three separate but consecutive experiments were set to assess the impact of substituting FO with AO as a source of EPA + DHA in Atlantic salmon feeds. These experiments were conducted on fish ranging from 145 g to 3 kg in body weight (BW) to understand the oil inclusion effects during the post-smolt production cycle.

2.1 | Fish

The Atlantic salmon used in all three experiments were from the NLA strain (Norwegian breeding program). Fish were reared from the egg stage at the Matre Research Station of the Institute of Marine Research (IMR, Bergen, Norway), smoltified, and kept in 3-m tanks (12 m^3 volume) until they reached the desired sizes of 143 g (Exp 1) and 413 g for (Exp 2). For Exp 3, the fish were transferred to 7-m tanks (38 m^3) after smoltification and kept there until reaching 1.3 kg. The trials were abiding to Norwegian laws and regulations approved through the Norwegian Food Safety Authority (license 8224, 16,615).

2.2 | Diets and experimental design

Feeds were produced by SPAROS (Olhão, Portugal) for Experiment 1 (Exp 1) and by Nofima AS (Tromsø, Norway) for Experiments 2 and 3 (Exp 2 and Exp 3) (Table 1). A diet containing FO as the main source of omega-3 LC-PUFA was used as the control (FO Ctrl) in Exp1, Exp2, and Exp3. Test diets were formulated using different inclusion levels of AO (oil FA profile is described in Santigosa et al., 2020, Table S1).

TABLE 1 Formulation of the experimental diets.

	Exp 1					Exp 2					Exp 3		
Ingredients (%)	FO ctrl	25%RFA	50%RFA	100%RFA	50%RFO	FO ctrl	AO2.5%	AO5.0%	AO7.5%	AO10%	FO ctrl	Diet50RFA	Diet100RFA
Fish oil[a]	10	7.5	5	0	5	10.0	7.5	5.0	2.5	0.0	13.7	6.85	0
Micro algal oil	0	0.88	1.76	3.52	5	0.0	2.5	5.0	7.5	10.0	0	1.73	3.46
Rapeseed oil	14	15.62	17.24	20.98	14	10.0	10.0	10.0	10.0	10.0	17.5	22.62	3.46
Soybean oil	-	-	-	-	-	-	-	-	-	-	0	0	5.12
Fish meal	10	10	10	10	10	10.0	10.0	10.0	10.0	10.0	15	15	15
Wheat	9.52	9.27	9.27	9.27	9.45	7.42	7.42	7.42	7.42	7.42	12.5	12.5	12.5
Wheat gluten	16	16	16	16	16	16	16	16	16	16	12.5	12.5	12.5
SPC	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	13.6	13.6	13.6
Corn gluten	10	10	10	10	10	10	10	10	10	10	6	6	6
Horse beans	6	6	6	6	6	6	6	6	6	6			
Soya lecithin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix[b]	-	-	-	-	-	0.52	0.52	0.52	0.52	0.52	0.5	0.5	0.5
Vitamin mix[c]	-	-	-	-	-	2	2	2	2	2	2	2	2
Vit and Min Premix[d]	0.200	0.200	0.200	0.200	0.200								
MCP 24%/MSP 26%	1	1	1	1	1	1	1	1	1	1	2.5	2.5	2.5
Betaine as Betafine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5			
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.5	0.5	0.5
Astaxanthin as CAROPHYLL®Pink 10% CWS	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Yttrium oxide	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	-	-	-
L-Lysine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	0.75	0.75	0.75
DL-Methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.39	0.39	0.39
L-Threonine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.47	0.47	0.47

TABLE 1 (Continued)

	Exp 1					Exp 2					Exp 3		
Ingredients (%)	**FO ctrl**	**25%RFA**	**50%RFA**	**100%RFA**	**50%RFO**	**FO ctrl**	**AO2.5%**	**AO5.0%**	**AO7.5%**	**AO10%**	**FO ctrl**	**Diet50RFA**	**Diet100RFA**
L-Histidine	-	-	-	-	-	-	-	-	-	-	0.25	0.25	0.25
Pellet size (mm)	4	4	4	4	4	4	4	4	4	4	7 & 9	7 & 9	7 & 9

Note: Vitamins (IU or mg/kg diet): sodium menadione bisulfate, 10 mg; retinyl acetate, 8000 IU; DL-cholecalciferol, 1700 IU; thiamine, 12 mg; riboflavin, 20 mg; pyridoxine, 10 mg; cyanocobalamin, 0.06 mg; nicotinic acid, 30 mg; folic acid, 6 mg; inositol, 300 mg; biotin, 0.7 mg; calcium pantothenate, 70 mg; Minerals (g or mg/kg diet): copper sulfate, 5 mg; ferric sulfate, 60 mg; potassium iodide, 3.5 mg; manganese oxide, 20 mg; sodium selenite, 0.25 mg; zinc oxide, 30 g; sodium chloride, 80 mg; calcium carbonate, 37 2 mg; Excipient: wheat middlings.

Abbreviations: MCP, monocalcium phosphate; MSP, monosodium phosphate; SPC, soy protein concentrate.

[a]In Exp 1 fish oil from by-products; in Exp 2 and 3 North Hemisphere fish oil.

[b]Individual minerals purchased from Vilomix and mixed by Nofima.

[c]Vilomix.

[d]PREMIX Lda, Portugal.

All diets within the same experiment were iso-proteic, iso-lipidic, and iso-energetic (Table 2). Diet crude protein (CP) was determined by a nitrogen (N) analyzer (FP 528, LECO, St. Joseph, MO, USA) using the Dumas method (CP = N*6.25). Gross energy measurements were performed using an adiabatic bomb calorimeter (C 2000 basic, IKA, Staufen, Germany), and then used to calculate lipid levels. Experimental diets had average CP (48.78 ± 0.6; 42.0 ± 0.2; 36.0 ± 0.0) and average lipid levels (23.01 ± 0.2; 25.78 ± 0.3; 34.0 ± 0.0) for Exp 1, Exp 2, and Exp 3, respectively.

Yttrium oxide, an inert marker, was incorporated at 100 ppm in Exp 1 and Exp 2 to calculate the apparent digestibility coefficient (ADC) of EPA and DHA. Each diet was fed to fish in triplicate tanks.

Experiment 1. The aim of this experiment was to provide comparable levels of EPA + DHA across four treatment groups, based on the level of these LC-PUFA in the FO Ctrl diet. As such, the 10% FO in the control diet was progressively reduced to 7.5, 5, and 0% in the test diets and combined with 0.88, 1.76, and 3.52 AO in diets 25%RFA, 50%RFA, and 100%RFA, respectively. A fifth treatment group (50%RFO), aiming to increase the EPA + DHA level, provided FO and AO at equal inclusion levels, that is, 5%. The analyzed contents of EPA + DHA were 1.37, 1.45, 1.73, 1.78, and 3.44% in FO Ctrl, 25%RFA, 50%RFA, 100%RFA, and 50%RFO diets, respectively (Table 2). Rapeseed oil was added to maintain the same energy level across all diets.

Two weeks before the start of the trial, 525 Atlantic salmon post-smolts (average BW of 143 g) were randomly distributed into 15 standard fiberglass tanks (1 m^3), each holding 35 fish. The tanks were equipped with feed collectors and programmable feeders (Arvo-Tec T drum 2000, Arvo-Tec Oy, Huutokoski, Finland). Aerated seawater at 28 ppt (8.0 ± 0.5°C) was supplied at 15 L/min. The lid of each tank contained fluorescent light tubes that provided 24 h of light. Once transferred to these tanks, the fish were adapted to the experimental FO Ctrl diet for 1 week. Three days prior to the start of the trial, the fish were anesthetized with 0.4% benzocaine. At this stage, weight (to the nearest 0.1 g) and length (to the nearest 5 mm) were determined. The fish were returned to the tanks, fasted overnight, and fed the experimental diets twice a day, from 08:00 to 10:00 and from 12:00 to 14:00. The fish were overfed at 20%, adjusted once every 3 days. The surplus of feed pellets was collected for feed conversion ratio (FCR) assessment. The trial lasted 132 days until the final sampling, and the duration of the experimental feeding period was 117 days.

Experiment 2. This relatively short experiment aimed mainly to get data on the digestibility of EPA and DHA provided by new omega-3 sources to complement the digestibility data obtained from smaller fish (Exp 1). Five treatment groups were set up to test different inclusion levels of AO as an alternative to FO, allowing for different levels of EPA + DHA. As such, AO inclusion level was 2.5%, 5.0%, 7.5%, and 10%, the latter corresponding to the total replacement of FO by AO (Table 1). The levels of EPA + DHA analyzed ranged from 1.54% in the FO Ctrl diet to 5.59% in the diet AO10% diet (Table 2).

Atlantic salmon previously held in 12-m^3 tanks (average weight 413 g) were lightly anesthetized in 0.15% benzocaine and distributed into 15 square indoor tanks (400 L), each holding 20 fish. The tanks were supplied with aerated seawater (35 ppt; 12.0°C) at a rate of 15 L/min. The tanks were equipped with lids containing fluorescent light tubes and automatic feeders (Arvo-Tec T drum 2000). Fish were fed ad libitum (1.5% BW, at 08:00, 12:00, and 16:00) and they received the experimental FO Ctrl diet during the acclimation period. After 2 weeks, all fish were anesthetized in 0.4% benzocaine, and length and weight were determined as described for Exp 1. The fish were returned to the tanks and the trial began after a 2-day fasting period. The experimental feeding lasted for 35 days until the final sampling.

Experiment 3. Here, fish with higher initial body weight (IBW) were used to understand the effects of AO inclusion on performance, muscle FA profile, and sensory characteristics of the fillet. In this experiment, three treatment groups were a FO Ctrl, and two test groups, where the level of EPA + DHA in the FO Ctrl diet was substituted at 50% (Diet 50RFA) and 100% (Diet 100RFA), with AO aiming to

TABLE 2 Diet proximate and dietary LC-PUFA analyses.

	Exp 1					Exp 2					Exp 3[a]		
Ingredients (%)	FO ctrl	25%RFA	50%RFA	100%RFA	50%RFO	FO ctrl	AO2.5%	AO5.0%	AO7.5%	AO10%	FO ctrl	Diet50RFA	Diet100RFA
Protein	48.09	49.32	48.92	49.44	48.15	43.1	43.1	42.9	42.7	43.2	36.0	36.0	36.0
Lipid	22.95	22.89	22.96	23.41	22.84	26.04	26.03	25.82	25.57	25.44	34.0	34.0	34.0
Energy (MJ/kg)	24.03	23.77	23.88	24.07	24.0	23.78	24.08	23.84	23.92	23.98	-	-	-
FFDRoil[b]	1.86	1.39	0.93	0.00	0.93	1.86	1.39	0.93	0.46	0.00	2.54	1.27	0.00
Sum EPA	1.228	1.078	0.949	0.616	1.44	0.69	1.00	1.28	1.49	1.80	0.78	0.84	0.78
Sum DHA	0.743	0.882	1.035	1.274	2.11	0.85	1.67	2.43	3.00	3.78	1.05	1.37	1.52
Sum EPA + DHA	1.971	1.96	1.983	1.89	3.55	1.54	2.67	3.71	4.50	5.59	1.82	2.21	2.30
EPA:DHA	1.653	1.222	0.917	0.484	0.682	0.81	0.60	0.53	0.50	0.48	0.61	0.51	0.67

[a]Protein and Lipid levels for Exp3 are theoretical because of a technical error and sample limitation in the proximal analysis.

[b]FFDRoil describes the quantity of wild fish meal or wild FO used in feeds in relation to the farmed fish produced. FFDRoil is calculated following the ASC Salmon Standard (https://www.asc-aqua.org/): FFDRoil = % of fish oil in feed from forage fisheries/fish oil yield (5.0 or 7.0 depending on source of wild fish) × eFCR. Calculations are based on diets formulated using FO from North Atlantic origin, so 7.0% yield of fish oil is used to calculate FFDRoil. eFCR is 1.3 (Naylor et al., 2021) to allow for standard comparison between diets. For more information, refer to Naylor et al. (2021).

maintain a constant EPA + DHA level of 2.2%. The EPA + DHA contents of the analyzed diets were 1.82% in the FO Ctrl diet, and 2.21% and 2.30% in Diet50RFA and Diet100RFA, respectively (Table 2). Atlantic salmon (average weight 1.3 kg) were collected from 7-m tanks (35 m^3), anesthetized in 0.4% benzocaine, and transferred to 9 × 3 m (12 m^3) tanks, each containing 35 fish. The tanks were supplied with aerated seawater at constant temperature (35 ppt, 9.0°C). As large fish often have slow acclimation, they were left in the tanks until feeding had returned to normal (1% BW d^{-1}). When appetite was good, the fish were lightly anesthetized in 0.2% benzocaine, weighed in groups of five, and returned to the tanks. The salmon were then fed the experimental diets for 132 days.

2.3 | Sampling and analyses

Survival and growth parameters were monitored in all three experiments. IBW and final body weight (FBW) were determined and used to calculate specific growth rate (SGR, %BW d^{-1}) as follows: Specific growth rate (SGR; %/day) = (Ln FBW – Ln IBW) × 100/days, where d is the number of days between FBW and IBW. FCR was calculated as feed intake (g) × weight gain (g^{-1}) for Exp 1 only. The ADCs of EPA and DHA were calculated for Exp 1 and Exp 2. Dry matter, total lipids, total astaxanthin, and colorimetric scores were calculated for Exp 3 only.

2.3.1 | Sampling

At the end of Exp 1 and Exp 2, fish feces were collected by stripping, pooled per tank, and kept at −80°C until further analyses. To ensure a full gut and an improved feces collection, the daily ration was fed as a single meal 12 h before sampling. In addition, muscle samples (5 × 5 cm) from five fish per tank were obtained at the end of the experiment following anesthesia and frozen at −80°C until further analyses. Unused fish were discarded.

At the end of Exp 3, three fish per tank (nine fish per treatment) were examined for sensory characteristics. They were sacrificed by a blow to the head and then filleted on both right and left sides using the Norwegian Quality Cut (NQC). Fillets for organoleptic testing were immediately stored in vacuum bags at −20°C. Another 10 fish per tank were filleted on the right side only using the NQC. After visual and digital measurement of muscle color, the fillet was cut into two pieces, each individually vacuum packed for (1) pigment, dry matter, and total lipid analysis, and (2) for FA analysis. Unused fish were discarded.

2.3.2 | Apparent digestibility coefficient

The ADCs of selected FA were calculated as the fractional net absorption of nutrients from diets using yttrium oxide (Y_2O_3) as the nonabsorbable indicator. Yttrium concentration in dry matter feed and feces was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, 5100 Dual View, Agilent Technologies, Inc., Santa Clara, CA, USA) according to DIN EN ISO11885:1997 (DIN EN ISO1998; AOAC, 2006) after sulfuric acid mineralization. ADC (%) was calculated according to NRC (2011): ADC = [1-(% Y_2O_3 in feed × %nutrient in feces)/(% Y_2O_3 in feces × %nutrient in feed)] × 100.

2.3.3 | Muscle FA profile

The extraction and analysis of muscle lipid were performed with the chloroform–methanol method according to Folch et al. (1957). The data are given as percent of FA and as mg/g wet weight of the muscle. Data are given as

means of $n = 10$. In Exp 1 and Exp 2, the FA profiles of the different diets and pooled fish muscle tissues were analyzed at DSM Nutritional Products Dartmouth (Nova Scotia, Canada) by gas chromatography using a flame ionization detector (GC-FID) as described in Santigosa et al. (2021). Lipids were saponified in potassium hydroxide in methanol and then methylated with hydrochloride in methanol in a water bath at 72°C. FA methyl esters were extracted with hexane and then separated via GC (HP6890, Agilent Technologies Inc.) with a fused silica capillary column (007-CW, Hewlett Packard, Palo Alto, CA, USA). The column temperature was programmed to rise from 150°C to 200°C at 15°C min^{-1}, and then from 200°C to 250°C at 2°C min^{-1}. The injector and detector temperature was 250°C.

For analysis of FA in Exp 3, lipid samples were saponified with 0.5 M sodium hydroxide and methylated using 12% boron trifluoride in methanol at 100°C. After cooling the solution, the FA methyl esters were extracted with hexane. The FA composition was analyzed as previously described by Lie and Lambertsen (1991) and Torstensen et al. (2004). FA methyl esters were separated using a Perkin Elmer (Waltham, MA, USA) Auto System XL2000 GC ("cold on column" injection; 60°C for 1 min; 25°C min^{-1} to 160°C; hold for 25 min; 25°C min^{-1} to 190°C; hold for 17 min; 25°C min^{-1} to 220°C; hold for 6 min), equipped with a 50-m CP-sil 88 fused silica capillary column (id: 0.32 mm; Chromopack Ltd., Middelburg, The Netherlands). The FA methyl esters were detected on an FID (Perkin Elmer) and peaks were identified based on retention time using standard mixtures of FA methyl esters (Nu-Chek Prep Inc., Elysian, MN, USA), thus determining the FA composition (area %). All samples were integrated using Chromeleon™ (ThermoFisher Scientific, Waltham, MS, USA) connected to the gas liquid chromatography (GLC).

For all experiments, the amount of FA per gram of sample was calculated using 19:0 methyl ester as the internal standard.

2.3.4 | Muscle total lipids and dry matter

For muscle total lipid analysis, one of the individually packed NQC fillets was thawed and immediately homogenized following addition of butylated hydroxytoluene (BHT, 100 mg/L) to stabilize the lipids. After weighing, the tubes were added ethyl acetate and left overnight on a shaker. The ethyl acetate was then collected, evaporated, and the lipids were weighted gravimetrically. Means of the duplicate runs were used for further analysis. For dry matter analysis, duplicates of homogenized muscle (ca. 3 g) were dried in test tubes at 105°C for 24 h. Data are given as means of $n = 10$ fish per tank.

2.3.5 | Muscle color assessment

Muscle color was initially measured by both visual and digital fans (*Salmo*Fan®, DSM Nutritional Products, Heerlen, The Netherlands). Three sensor readings on each NQC were performed above the sideline and two readings below. The results are given as means of these five readings.

2.3.6 | Muscle astaxanthin content

For analysis of astaxanthin, one of the duplicated NQC fillets was thawed and immediately homogenized following addition of BHT (100 mg/L) to stabilize the pigments. Duplicate samples, each containing 1.5 g muscle, were loaded onto the extraction columns with 1.5 g water-free sodium sulfate. The astaxanthin was extracted with 5 mL of acetone with freshly made BHT/vitamin C (100/100 mg/L acetone). The sample was allowed to extract for 10 min before the supernatant was collected. The procedure was repeated three times. The combined phases were evaporated under nitrogen and dissolved in 10 mL mobile phase (heptane: acetone 86:14) and stored at −80°C until analysis. The analysis was performed at room temperature using a Hewlett Packard 110 series high-performance liquid

chromatography (HPLC) system equipped with a G1315A diode array detector set at 476 nm. The column was a C18 column (Lichrospher 5um C18) using heptane:acetone (86:14) as the mobile phase. Flow rate was 1.2 mL/min and 20 μL of each sample was injected twice. Astaxanthin was quantified using authentic standards. Data are the sum of isomers. Means of the duplicate runs were used for further analysis. Data are given as the means of $n = 10$ fish per tank.

2.3.7 | Muscle sensory characteristics

Samples for sensory analysis were shipped frozen and held at −18°C until assessment. The three salmon diets were assessed in three replicates. Samples were served in a randomized order in a plastic beaker marked with a random three-digit code covered with metal lid. The serving temperature of the samples was 17°C ± 1.

Analysis was performed by Nofima AS according to the "General guidance for establishing a sensory profile by a sensory panel consisting of trained assessors" (ISO 13299:2016 method). These assessors are selected based on their abilities to recognize smell and taste that meet the requirements of the "General guidelines for the selection, training, and monitoring of selected assessors and expert sensory assessors" (ISO 8586:2012 method). The sensory panel, consisting of 10 people, is trained, tested, and calibrated regularly. Twenty-six sensory attributes have been defined for raw salmon and scored using a 1–9 scale (1 = no intensity; 9 = strong intensity). Among them are odor (total intensity, sour, sea, cloying, fish, pungent, and rancid), appearance/color (color hue, color strength, and whiteness), taste (total intensity, sour, salty, acidic, bitter, sea, cucumber, metallic, cloying, fish, muddy, rancid, and aftertaste), and texture (hardness, juiciness, and tenderness) characteristics.

2.4 | Statistical analyses

Each tank was treated as an experimental unit. Sample size per treatment was determined based on previous growth trials, performed in the same facilities and with Atlantic salmon of similar initial BW. Statistical analyses were performed using Statgraphics Centurion XVII statistical software (Statpoint Technologies, Inc., Warrenton, VA, USA). One-way analysis of variance (ANOVA) was used to analyze data to determine whether statistical differences existed between treatment groups. Newman–Keuls multiple comparison tests were performed to compare treatment means. In addition, to analyze differences between diet groups, Student's *t*-tests were performed on the growth parameters measured at the end of each experiment. Data were arcsine transformed when necessary. Differences were considered significant at $p < 0.05$.

The statistical method for the analysis of sensory characteristics was as follows. ANOVA using F-tests was first performed to find significant differences ($p < 0.05$) between groups for each of the sensory attributes. When the F-test was significant, a Tukey's multiple comparison test was performed to determine which samples were different. If the difference between two means were larger than the critical value the test was calculating for, these two groups were significantly different. The means were the average of the assessors and two replicas. EyeQuestion and EyeOpenR software (Logic8 BV, Utrecht, Holland) and PanelCheck V.1.4.2 (Nofima AS) were used.

3 | RESULTS

3.1 | Survival, growth, and FCR

Table 3 shows the survival, growth, and FCR for Exp 1, Exp 2, and Exp 3 after 117, 35, and 216 days of experimental feeding, respectively. Recorded SGR was within the expected values in all groups, averaging 1.11 for Exp 1 and 1.01

TABLE 3 Survival and performance of Atlantic salmon fed in Experiment 1, Experiment 2, and Experiment 3 for 117, 35, and 216 days, respectively.

	Exp 1					
	FO ctrl	**25%RFA**	**50%RFA**	**100%RFA**	**50%RFO**	**Sign.**
Survival	100	100	100	100	100	
IBW	141.1 ± 1.34	140.3 ± 4.02	141.4 ± 1.43	139.6 ± 1.22	140.6 ± 4.82	$p > 0.05$
FBW	525.6 ± 28.01	526.2 ± 8.01	524.1 ± 17.95	485 ± 8.84	532.9 ± 70.47	$p > 0.05$
SGR	1.12 ± 0.051	1.13 ± 0.031	1.11 ± 0.03	1.06 ± 0.023	1.13 ± 0.089	$p > 0.05$
FCR	0.69 ± 0.044	0.70 ± 0.023	0.65 ± 0.034	0.71 ± 0.016	0.66 ± 0.026	$p > 0.05$
	Exp 2					
	FO Ctrl	**AO2.5%**	**AO5.0%**	**AO7.5%**	**AO10%**	**Sign.**
Survival	100	100	100	100	100	
IBW	401.6 ± 12.6	421.9 ± 15.3	408.2 ± 14.8	422.6 ± 17.2	418.0 ± 15.7	$p > 0.05$
FBW	552.5 ± 30.3	618.4 ± 31.0	589.3 ± 31.8	599.0 ± 38.1	595.2 ± 38.3	$p > 0.05$
SGR	0.95 ± 0.146	1.09 ± 0.043	1.04 ± 0.051	0.99 ± 0.099	1.00 ± 0.079	$p > 0.05$
	Exp 3					
	FO Ctrl	**Diet50RFA**	**Diet100RFA**			**Sign.**
Survival	100	100	100			
IBW	1299 ± 27	1282 ± 23	1289 ± 16			$p > 0.05$
FBW	3192 ± 218	3280 ± 114	3248 ± 200			$p > 0.05$
SGR	0.639 ± 0.0877	0.593 ± 0.0225	0.697 ± 0.0478			$p > 0.05$

Note: Data are shown as mean ± SD (standard deviation) ($n = 3$).
Abbreviations: AO, algal oil; FBW, final body weight; FCR, feed conversion ratio (feed intake/weight gain); FO, fish oil; IBW, initial body weight; SGR, specific growth rate (% body weight/day); Survival (%), survival percentage referred to the whole duration of the trial.

TABLE 4 Apparent digestibility coefficient of EPA and DHA of Atlantic salmon fed in Experiment 1 and Experiment 2.

	Exp 1					
	FO ctrl	**25%RFA**	**50%RFA**	**100%RFA**	**50%RFO**	**Sign.**
EPA (%)	98.82 ± 0.86	99.29 ± 0.03	99.52 ± 0.42	99.15 ± 0.03	100 ± 0.0	$p > 0.05$
DHA (%)	96.61 ± 1.23	97.15 ± 0.9	97.45 ± 1.0	98.3 ± 0.13	98.48 ± 0.3	$p > 0.05$
	Exp 2					
	FO Ctrl	**AO2.5%**	**AO5.0%**	**AO7.5%**	**AO10%**	**Sign.**
EPA (%)	98.59 ± 0.4	98.63 ± 0.4	99.13 ± 0.09	99.01 ± 0.17	99.12 ± 0.12	$p > 0.05$
DHA (%)	97.18 ± 0.58 b	97.75 ± 0.46 ab	98.53 ± 0.16 a	98.42 ± 0.27 a	98.56 ± 0.43 a	$p = 0.01$

Note: Different letters show significant differences ($p < 0.05$) between the experimental treatments.

for Exp 2. The average FCR was 0.68 for Exp 1. At the end of Exp 3, fish doubled their IBW. All diets appeared to be well suited for Atlantic salmon, and there were no significant differences in BW among treatment groups. SGR and FCR for Exp 3 averaged 0.64 and 0.68, respectively. Overall, statistical analyses showed no significant effect of diet on FBW, SGR, or FCR for any of the experiments (Table 3).

TABLE 5 Muscle FA profile (mg/g salmon muscle) in all three experiments.

Experiments		Exp 1					Exp 2					Exp 3		
Treatment groups		**FO ctrl**	**25%RFA**	**50%RFA**	**100%RFA**	**50%RFO**	**FO ctrl**	**AO2.5%**	**AO5.0%**	**AO7.5%**	**AO10%**	**FO ctrl**	**Diet50RFA**	**Diet100RFA**
Unsaturated fatty acids														
Lauric acid	C12:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.12 ± 0.04	0.15 ± 0.07	0.11 ± 0.06	0.12 ± 0.06	0.12 ± 0.04	0 ± 0	0 ± 0	0 ± 0
Tridecylic acid	C13:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.03 ± 0.02	0.05 ± 0.06	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0 ± 0	0 ± 0	0 ± 0
Mystric acid	C14:0	6.12 ± 0.77	4.37 ± 0.47	3.61 ± 0.21	1.74 ± 0.11	3.48 ± 0.10	5.61 ± 1.10	5.57 ± 1.51	5.16 ± 2.02	4.61 ± 0.42	4.20 ± 0.59	2.58 ± 1.15a	2.18 ± 0.06b	1.03 ± 0.07c
Palmitic acid	C16:0	7.88 ± 1.10	6.02 ± 0.55	4.54 ± 0.35	2.78 ± 0.79	4.10 ± 0.17	22.28 ± 3.72	24.72 ± 6.19	27.55 ± 8.71	26.21 ± 4.19	27.79 ± 5.06	12.13 ± 0.74	13.20 ± 0.91	12.79 ± 0.19
Margaric acid	C17:0	0.39 ± 0.04	0.08 ± 0.15	0.16 ± 0.14	0 ± 0	0.07 ± 0.13	0.34 ± 0.04	0.36 ± 0.08	0.36 ± 0.11	0.33 ± 0.03	0.39 ± 0.13	0.17 ± 0.00a	0.09 ± 0.01b	0.02 ± 0.01c
Stearic acid	C18:0	8.0 ± 1.11	6.53 ± 0.39	7.06 ± 0.46	6.07 ± 0.23	6.24 ± 0.23	5.12 ± 0.82	5.77 ± 1.61	6.05 ± 2.00	5.68 ± 0.74	6.15 ± 1.37	3.04 ± 0.22	3.26 ± 0.17	3.46 ± 0.08
Arachidic acid	C20:0	0.70 ± 0.09	0.43 ± 0.38	0.72 ± 0.05	0.81 ± 0.02	0.63 ± 0.03	0.50 ± 0.09	0.58 ± 0.17	0.72 ± 0.34	0.62 ± 0.12	0.73 ± 0.31	0.42 ± 0.02a	0.51 ± 0.04b	0.54 ± 0.00b
Heneicosylic acid	C21:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.46 ± 0.25	0.68 ± 0.21	0.69 ± 0.26	0.81 ± 0.34	0.99 ± 0.06	0 ± 0	0 ± 0	0 ± 0
Benhenic acid	C22:0	2.97 ± 2.36	2.12 ± 1.95	3.27 ± 0.55	1.20 ± 0.23	3.32 ± 0.17	0.27 ± 0.06	0.30 ± 0.07	0.29 ± 0.07	0.30 ± 0.06	0.31 ± 0.03	0.20 ± 0.00a	0.26 ± 0.02b	0.29 ± 0.00b
Lingoceric acid	C24:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.13 ± 0.04	0.16 ± 0.12	0.16 ± 0.08	0.11 ± 0.10	0.16 ± 0.05	0 ± 0	0 ± 0	0 ± 0
Monosaturated fatty acids														
Palmitioleic acid	C16:1 n-7	7.88 ± 1.10	6.02 ± 0.55	4.54 ± 0.35	2.78 ± 0.79	4.10 ± 0.17	3.27 ± 2.48	4.80 ± 1.48	4.44 ± 1.99	4.41 ± 1.17	2.79 ± 2.06	2.97 ± 0.24a	2.52 ± 0.05b	1.20 ± 0.10c
Oleic acid	C18:1 n-9	82.65 ± 10.82	78.35 ± 4.42	90.32 ± 3.82	96.68 ± 3.66	65.48 ± 2.88	68.89 ± 12.68	69.62 ± 13.89	75.33 ± 18.98	73.77 ± 10.92	73.30 ± 11.33	50.55 ± 3.35a	58.24 ± 3.71b	55.90 ± 1.31ab
Eicosenoic acid	C20:1 n-9	9.45 ± 1.04	8.13 ± 0.83	8.25 ± 0.54	8.19 ± 0.61	6.72 ± 0.50	10.39 ± 1.92	10.72 ± 2.60	10.24 ± 2.96	8.48 ± 0.84	7.38 ± 0.83	6.71 ± 0.46a	6.13 ± 0.42a	3.62 ± 0.10b
Euricic acid	C22:1 n-9	1.95 ± 0.49	0.99 ± 0.93	0.25 ± 0.43	1.59 ± 0.16	0 ± 0	0.93 ± 0.16	1.00 ± 0.24	0.96 ± 0.26	0.87 ± 0.08	0.75 ± 0.09	0.77 ± 0.05a	0.69 ± 0.04a	0.41 ± 0.01b
Nervonic acid	C24:1 n-9	1.15 ± 0.11	0.60 ± 0.52	0.26 ± 0.45	0.26 ± 0.46	0.24 ± 0.41	1.04 ± 0.15	1.12 ± 0.20	1.07 ± 0.22	0.99 ± 0.11	0.90 ± 0.07	0.57 ± 0.03a	0.55 ± 0.05b	0.38 ± 0.00b
Polyunsaturated fatty acids														
Linoleic acid (LA)	C18:2 n-6	35.63 ± 3.75	33.45 ± 3.05	36.45 ± 0.95	39.79 ± 1.60	30.12 ± 1.38	25.58 ± 4.91	27.88 ± 8.20	31.00 ± 10.09	30.21 ± 7.14	28.09 ± 5.04	17.51 ± 1.13a	20.19 ± 1.22b	27.55 ± 0.68c
α-Linolenic acid (ALA)	C18:3 n-3	11.22 ± 1.09	10.45 ± 01.25	11.08 ± 0.19	12.74 ± 0.40	9.38 ± 0.66	8.48 ± 1.79	9.65 ± 2.89	11.35 ± 3.93	10.12 ± 2.13	9.76 ± 2.0	7.15 ± 0.49a	8.42 ± 0.59b	9.07 ± 0.24b
Stearidonic acid	C18:4 n-3	2.09 ± 0.30	1.13 ± 0.98	1.34 ± 0.12	0.91 ± 0.12	1.02 ± 0.08	2.64 ± 0.66	2.37 ± 0.86	2.19 ± 0.75	1.72 ± 0.22	1.41 ± 0.25	0.75 ± 0.05a	0.64 ± 0.21b	0.38 ± 0.02c
Eicosatrienoic acid	C20:3 n-3	1.07 ± 0.10	0.68 ± 0.60	0.87 ± 0.30	1.32 ± 0.14	0.59 ± 0.31	0.63 ± 0.14	0.81 ± 0.19	0.95 ± 0.29	0.84 ± 0.19	0.78 ± 0.19	0 ± 0	0 ± 0	0 ± 0
Arachidonic acid	C20:4 n-6	2.10 ± 0.22	1.22 ± 1.05	1.56 ± 0.08	1.03 ± 0.23	1.42 ± 0.04	0.58 ± 0.08	0.66 ± 0.38	0.98 ± 0.26	0.94 ± 0.16	1.24 ± 0.46	0.83 ± 0.05a	1.04 ± 0.09b	1.10 ± 0.03b
Eicosapentaenoic acid (EPA)	C20:5 n-3	8.97 ± 0.86a	7.04 ± 0.32b	6.72 ± 0.14b	4.96 ± 0.03 c	9.44 ± 0.54a	5.82 ± 0.85	6.67 ± 1.25	7.51 ± 1.42	8.43 ± 1.29	8.87 ± 1.76	2.77 ± 0.14a	3.04 ± 0.10b	2.73 ± 0.07a
Heneicosapentaenoic acid	C21:5 n-3	0.52 ± 0.06	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.35 ± 0.19	0.31 ± 0.20	0.41 ± 0.22	0.37 ± 0.13	0.29 ± 0.14	0.83 ± 0.00a	0 ± 0b	0 ± 0b
Docosapentaenoic acid (DPA)	C22:5 n-3	3.38 ± 0.34	1.61 ± 1.40	2.52 ± 0.11	1.97 ± 0.44	3.99 ± 0.30	2.19 ± 0.31	2.84 ± 0.63	3.76 ± 1.11	4.03 ± 0.98	4.62 ± 1.08	1.10 ± 0.08a	1.35 ± 0.06b	1.32 ± 0.04b
Docosahexaenoic acid (DHA)	C22:6 n-3	17.03 ± 1.02c	17.97 ± 1.45c	18.40 ± 0.17c	20.77 ± 0.76b	29.37 ± 0.96a	16.59 ± 1.80	21.44 ± 3.43	24.15 ± 4.32	27.62 ± 4.45	29.28 ± 5.0	5.78 ± 0.28a	7.24 ± 0.19b	7.41 ± 0.24b
Calculated total EPA + DHA		25.97	24.94	25.12	25.66	38.74	22.32	28.07	31.61	36.03	38.07	8.55	10.28	10.14

Note: Different letters show significant differences ($p < 0.05$) between the experimental treatments. $N = 10$.

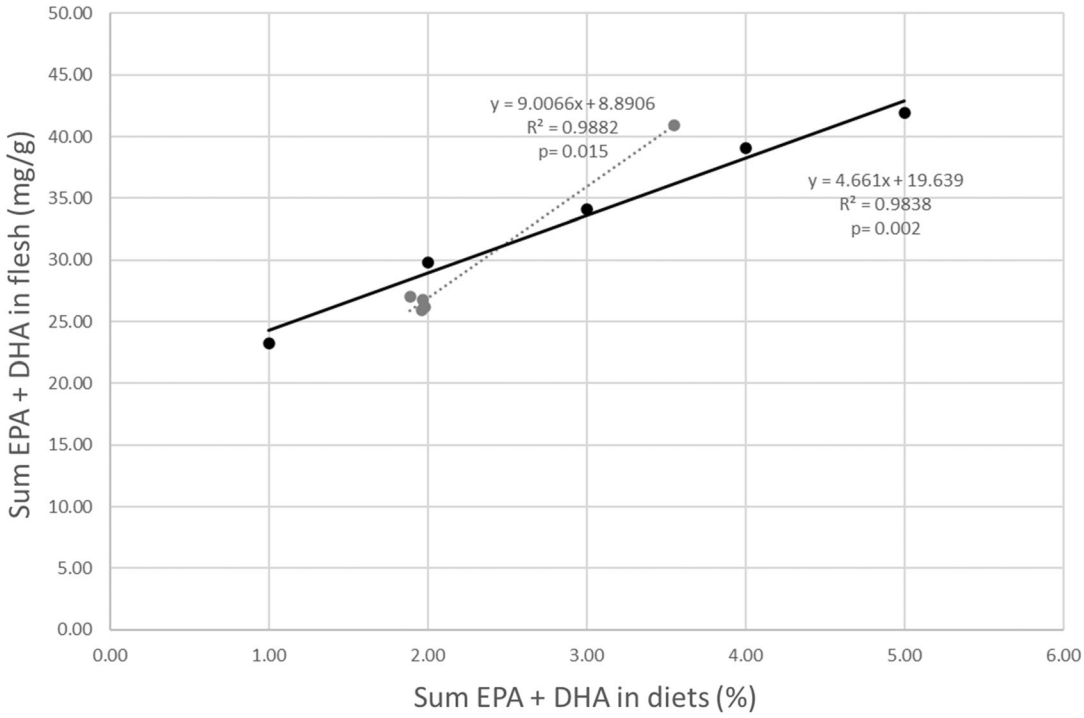

FIGURE 1 Muscle EPA + DHA levels in relation to dietary contents in 500-g Atlantic salmon post-smolts from Experiment 1 (dotted line) and Experiment 2 (plain line). $N = 10$.

TABLE 6 Dry matter (DM), total lipids (TL), total astaxanthin (Tot Ax), and colorimetric scores obtained for Atlantic salmon muscle after 132 days of feeding (Experiment 3).

	Initial	Control	Diet50RFA	Diet100RFA	Sign.
DM%	3.2 ± 1.17	34.1 ± 0.69	34.9 ± 0.46	34.4 ± 0.38	$p > 0.05$
TL%	10.3 ± 2.23	14.2 ± 1.07	14.6 ± 1.39	14.5 ± 0.02	$p > 0.05$
Tot Ax mg/kg	4.3 ± 1.09	3.46 ± 0.30	3.7 ± 0.11	3.6 ± 0.18	$p > 0.05$
Visual *Salmo*Fan		23.8 ± 0.15	24.2 ± 1.49	24.4 ± 0.48	$p > 0.05$
Digital *Salmo*Fan		24.3 ± 0.31	24.2 ± 0.55	24.2 ± 0.10	$p > 0.05$
CIE_L*		28.5 ± 0.92	28.7 ± 0.67	28.4 ± 0.65	$p > 0.05$
CIE_a*		9.88 ± 0.34	9.9 ± 0.70	9.8 ± 0.14	$p > 0.05$

Note: a, redness; CIE, commission internationale de l'éclairage; L, lightness.

3.2 | ADCs of EPA and DHA

ADCs of EPA and DHA were calculated for Exp 1 and Exp 2 (Table 4). In both experiments, the ADC of EPA was above 98.5% in all diets and at any level of AO replacement. The ADC of DHA was slightly lower than that of EPA, ranging from 96.61% in the FO Ctrl (Exp 1) to 98.56% in the AO10% diet in Exp 2. In this latter experiment, the ADC of DHA increased with the level of inclusion of AO in a dose-dependent manner, following a linear regression ($y = 0.1372x + 97.402$; $R^2 = 0.8019$).

TABLE 7 Sensory characteristics of 3 kg Atlantic salmon muscle in Experiment 3.

	Control	Diet50RFA	Diet100RFA	Sign.
Total odor intensity	4.09	4.46	4.10	$p > 0.05$
Sour odor	1.53	1.53	1.70	$p > 0.05$
Sea odor	1.36	1.38	1.23	$p > 0.05$
Cloying odor	3.25	3.60	3.57	$p > 0.05$
Fish odor	3.24	3.40	3.10	$p > 0.05$
Pungent odor	2.55	3.11	2.91	$p > 0.05$
Rancid odor	1.32	1.46	1.38	$p > 0.05$
Color hue	5.75	5.75	5.82	$p > 0.05$
Color strength	5.22	5.39	5.15	$p > 0.05$
Whiteness	4.98ab	4.71a	5.05b	$p < 0.05$
Total flavor intensity	5.10	5.20	5.00	$p > 0.05$
Sour flavor	1.75	1.55	1.67	$p > 0.05$
Salty taste	2.51	2.62	2.56	$p > 0.05$
Acidic taste	3.70	3.62	3.72	$p > 0.05$
Bitter taste	4.32	4.52	4.66	$p > 0.05$
Sea flavor	1.56	1.49	1.60	$p > 0.05$
Cucumber flavor	1.74	1.55	1.45	$p > 0.05$
Metallic flavor	4.19	4.32	4.30	$p > 0.05$
Cloying flavor	3.06	3.44	3.27	$p > 0.05$
Fish flavor	3.80	3.74	3.74	$p > 0.05$
Muddy flavor	3.43	3.42	3.57	$p > 0.05$
Rancid flavor	1.37	1.38	1.53	$p > 0.05$
Hardness	2.98	3.16	2.75	$p > 0.05$
Juiciness	6.26	6.34	6.46	$p > 0.05$
Tenderness	7.20	7.04	7.43	$p > 0.05$
Aftertaste	5.99	5.99	6.03	$p > 0.05$

Note: Scale 1–9, 1 = no intensity, 9 = strong intensity. Different letters within the same row show significant differences ($p < 0.05$) between the experimental treatments.

3.3 | Muscle FA profile and quality

3.3.1 | FA profile

The FA profile of Atlantic salmon muscle obtained for each experiment is detailed in Table 5. Figure 1 illustrates the positive correlation between the gradual increase in dietary levels of EPA + DHA and the muscle content of these FA in Exp 1 and Exp 2. When the EPA + DHA content in the diet was higher than that in the FO Ctrl diet (10% FO), a concomitant increase was observed in the fish muscle. In the larger fish from Exp 3, the FA compositions of the muscle mirrored that of the diets (Table 5). The EPA + DHA in the diets ranged from 1.82% in the FO Ctrl diet to 2.30% in Diet100RFA. These levels translated into 8.55 mg/g and 10.14 mg/g in salmon fed the FO Ctrl diet and Diet100RFA, respectively. Among PUFA, a significant increase in ALA, ARA, EPA, and DHA was observed in the two AO-supplied diets compared with the control diet (Table 6). LA and stearidonic acid levels also increased significantly in Diet50RFA and Diet100RFA and differed significantly between these two levels of AO supplementation.

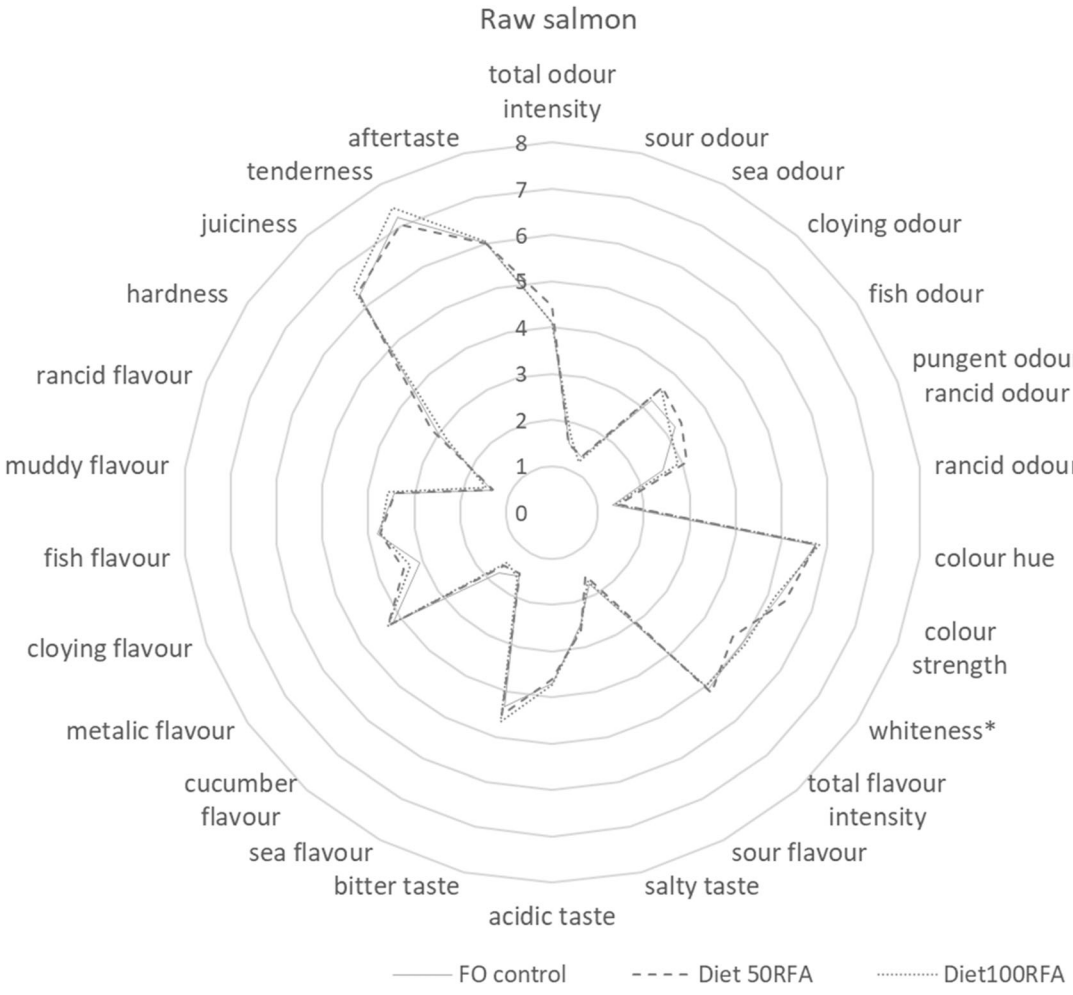

FIGURE 2 Overlap of sensory description of raw fillets from Atlantic salmon fed with the different diets in Experiment 3. Asterisk shows significant differences ($p < 0.05$) across treatments.

3.3.2 | Color, sensory characteristics, and astaxanthin content

Muscle quality was measured in Exp 3 through sensory characteristics, color attributes, and pigment deposition (Tables 7; Figure 2). The dry matter of muscle was approximately 34% across all tanks and diets. Total lipid content was approximately 14% and also similar between treatments. There was no difference in most of the sensory attributes between the three dietary groups (Tables 7 and 8). The only appearance/color attribute with significant difference among groups was whiteness (Table 7). However, the difference in the mean values was relatively small (4.98, 4.71, and 5.05 for diets FO Ctrl, Diet50RFA, and Diet100RFA, respectively), and there was no trend associated with the level of AO inclusion rate. All fish fillet sensorial quality parameters recorded in the present study, such as those related to odor, flavor, taste, and consistency, showed similar results among experimental groups (Table 7). The overlap of sensory characteristics for the three dietary groups clearly reflects the results of the statistical analysis, thus suggesting no difference between the muscle quality of Atlantic salmon fed the different diets (Figure 2).

No significant differences were observed in muscle color and astaxanthin content (Table 6). Total astaxanthin measured by HPLC ranged from 3.46 to 3.67 mg/kg of muscle and it was similar between all dietary groups. The initial content averaged 4.32 mg/kg of salmon muscle. The results of color assessment using the visual and digital fans followed the same trend, with no difference between the control fish and those fed the diets with AO inclusion. There was also no difference in color assessment between visual and digital fans with average treatment values of 24.1 ± 0.3 and 24.1 ± 0.1, respectively. Overall average CIE lightness (L*) and redness (a*) of the muscle were 28.5 ± 0.2 and 9.8 ± 0.1, respectively.

4 | DISCUSSION

EPA and DHA levels in seafood are critical for both fish and human health, with national health authorities recommending a weekly consumption of one portion of oily fish per week to attain their weekly AI of EPA and DHA Omega-3 (GOED, 2014; USDA, 2015). However, salmon flesh fed low amounts of FO and high levels of VO fails to meet the FA levels in fish fillet needed to address human requirements (Sprague et al., 2016). This gap can and should be closed. One option is to add the inclusion of AO as in salmon diets to restore EPA and DHA levels as recorded in the present study. For instance, the calculated total levels of EPA + DHA obtained here for the 3 kg salmon fed the FO control diet (1.11 g/130 g) suggests that 1.6 portions (130 g) of salmon would be required per week (Table S1) to meet the recommended daily intake of 250 mg EPA + DHA for humans (EFSA Panel on Dietetic Products Nutrition, 2010). With the higher EPA and DHA deposition levels recorded in this study driven by AO, it is thus possible to help human consumers attain the minimum recommended intake levels, consistently with the advice from national health authorities, and positively contribute to a healthier human population.

To this end, the use of AO in the aquafeed industry is a worthy alternative to FO, especially when compared with VO (Tocher et al., 2019), as they lack the omega-3 LC-PUFA, while having an excess of omega-6 PUFA. Moreover, the utilization of AO also minimizes dependency on marine resources as measured by the forage fish dependency ratio for fish oil (FFDRoil), thereby improving the sustainability of aquafeeds. FFDRoil is a metric describing the quantity of wild fish meal or wild FO used in feeds in relation to the farmed fish produced. FFDRoil is calculated following the ASC Salmon Standard (https://www.asc-aqua.org/). In Exp 3, the FFDRoil decreased from 2.54 to 0, demonstrating that the inclusion of AO instead of FO can improve the marine footprint during the grow-out phase. In addition, AO can be used to address disparities in the supply-to-demand gap for EPA + DHA (Panchal & Brown, 2021). The global demand for EPA + DHA has been estimated at 1.27 million tonnes, with a supply-to-demand gap between 0.4 and 1 million tonnes per year (Tocher et al., 2019). This is even more critical when considering the supply of EPA + DHA from Marine Stewardship Certified (MSC) sustainable small pelagic species, as 19% of global marine pelagic catches end up in fishmeal and FO, but only 20% of these are MSC certified (Marine Stewardship Council, 2021). Further increases in salmon production (FAO, 2020) would stretch current FO sources to an extent where farmed fish would likely reach deficient levels of omega-3 LC-PUFA, particularly EPA and DHA.

Importantly, the inclusion of high LC-PUFA sources in salmon feed ensures the requirements of these FA for optimum fish growth and health, as previously discussed by Bou et al. (2017) and Løvmo et al. (2021), and recently confirmed using high levels of FO to increase the amount of omega-3 FA in Atlantic salmon diets (Lutfi et al., 2022). However, few data exist on the benefits of high LC-PUFA diets in Atlantic salmon when omega-3 FA are provided by alternative sources such as microalgal oil. Previous studies incorporating *Schizochytrium* sp. oil into salmonid aquafeeds (Miller et al., 2007; Osmond et al., 2021; Rosenlund et al., 2018; Santigosa et al., 2018; Santigosa et al., 2020; Santigosa et al., 2021; Wei et al., 2021) showed no negative impact on the performance of Atlantic salmon and rainbow trout at a specific life stage. Results from the three experiments summarized here demonstrate that AO has no negative impact on Atlantic salmon growth performance at any stage (Table 3) and support that AO can be used throughout the entire production cycle of Atlantic salmon. Changing the lipid source from FO to AO maintained digestibilities of EPA and DHA above 96% in post-smolt salmon, which was comparable to those fed

FO only. This was within the range of previous reports for Atlantic salmon fed Camelina oil (Betancor et al., 2016) and rainbow trout fed AO (Santigosa et al., 2020). A dose-dependent and significant increase in DHA digestibility with increased inclusion of AO was also recorded, which can be associated with increasing unsaturation of the FA pool (Olsen & Ringø., 1998; Torstensen et al., 2000).

Importantly, sensory and quality characteristics of Atlantic salmon are also preserved when AO is used to increase the nutritional value of the aquafeed. Muscle pigmentation is a critical factor in the consumer's perception of quality in salmonid fillets (Amaya & Nickell, 2015). The AO used maintained the same muscle pigmentation across all 3-kg salmon (Table 6). These results are consistent with those previously obtained for similar-sized fish (Bente E. Torstensen et al., 2005), indicating that AO does not contain FA combinations or other components affecting the pigmentation of Atlantic salmon muscle. Recent studies have also addressed the effect of DHA-rich biomasses on Atlantic salmon pigmentation with positive results; however, data need to be carefully interpreted as EPA + DHA dietary levels were inadequate for the species (Kousulaki et al., 2020). In the future, higher LC-PUFA levels should be tested to reinforce data recently recorded on the benefits of high omega-3 diets on salmon fillet pigmentation, as recent studies suggest that increased levels of EPA + DHA can not only maintain but even improve fillet pigmentation (Hatlen et al., 2022; Lutfi et al., 2022; Ruyter et al., 2022). Interestingly, sensory characteristics of raw salmon muscle such as odor, appearance, taste, and texture did not differ between salmon-fed FO diets and those fed AO diets, supporting previous results obtained for other species (Meigs et al., 2020; Santigosa et al., 2021). Moreover, the muscle of salmon-fed AO diets was relished in the same way as that of salmon-fed FO diets, thus contributing to a similar sensorial experience with no impact for consumers.

The results obtained here also confirm in this species that the inclusion of this AO had no adverse effects on the performance at all tested life stages, while improving the FA profile of salmon muscle. Indeed, the restoration of LC-PUFA levels in salmon feed benefits not only the nutritional quality of the fish fillet but also the health, welfare, and product quality of the salmon when reared in sea cages (Ruyter et al., 2021). This is in agreement with research on other cultured species, such as rainbow trout (Santigosa et al., 2020), gilthead seabream (Santigosa et al., 2021), and yellowtail flounder (Stuart et al., 2021). As such, the use of LC-PUFA-rich alternatives throughout the full production cycle might enable the sustainable growth of the industry, further reducing its marine footprint to FFDRoil below 1, with benefits for the marine ecosystem without compromising fish health.

5 | CONCLUSION

Results obtained in this study confirm the use of the tested AO as an efficient source of omega-3 LC-PUFA throughout the full production cycle for both partial and full replacement of FO. Both strategies showed no difference in the zootechnical performance compared with the FO control treatment. Sensory and quality properties of fish fillets were also similar across experimental treatments. The use of high LC-PUFA in the diet improved the EPA and DHA profile of the muscle, thereby contributing to attaining the recommendations of global organization for the minimum intake of these omega-3 LC-PUFAs. Ultimately, the results from this study provide scientific evidence to support the salmon farming industry further decoupling from its reliance on marine FO.

ACKNOWLEDGMENTS

The authors would like to thank SPAROS and NOFIMA for their support on the feed production; the authors would like to thank Miguel Leal and Joana Marques for their constructive comments to improve the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors are employees of IMR, which provided the fish and experimental facilities; and DSM Nutritional Products and Veramaris V.O.F., which supported the experimental design and facilitated the delivery of the test product Veramaris oil®. There are no relevant financial or nonfinancial competing interests to report.

ORCID

Ester Santigosa https://orcid.org/0000-0002-9878-8283

REFERENCES

Aas, T. S., Åsgård, T., & Ytrestøyl, T. (2022). Utilization of feed resources in the production of Atlantic salmon (Salmo salar) in Norway: An update for 2020. *Aquaculture Reports*, *26*, 101316.

Albrektsen, S., Kortet, R., Skov, P. V., Ytteborg, E., Gitlesen, S., & Øverland, M. (2022). Future feed resources in sustainable salmonid production: A review. *Reviews in Aquaculture*, *14*, 1790–1812.

Amaya, E., & Nickell, D. (2015). Using feed to enhance the color quality of fish and crustaceans. In D. Allen Davis (Ed.), *Feed and feeding practices in aquaculture* (pp. 269–298). Elsevier.

Bell, G., Torstensen, B., & Sargent, J. (2005). Replacement of marine fish oils with vegetable oils in feeds for farmed salmon. *Lipid Technology*, *17*, 7–11.

Bell, J. G., Henderson, R. J., Tocher, D. R., & Sargent, J. R. (2004). Replacement of dietary fish oil with increasing levels of linseed oil: Modification of flesh fatty acid compositions in Atlantic salmon (Salmo salar) using a fish oil finishing diet. *Lipids*, *39*(3), 223–232.

Bernasconi, A. A., Wiest, M. M., Lavie, C. J., Milani, R. V., & Laukkanen, J. A. (2021). Effect of omega-3 dosage on cardiovascular outcomes: An updated meta-analysis and meta-regression of interventional trials. *Mayo Clinic Proceedings. Mayo Clinic*, *96*(2), 304–313.

Betancor, M. B., Li, K., Bucerzan, V. S., Sprague, M., Sayanova, O., Usher, S., Han, L., Norambuena, F., Torrissen, O., Napier, J. A., Tocher, D. R., & Olsen, R. E. (2018). Oil from transgenic Camelina sativa containing over 25% n-3 long-chain PUFA as the major lipid source in feed for Atlantic salmon (Salmo salar). *The British Journal of Nutrition*, *119*(12), 1378–1392.

Betancor, M. B., Sprague, M., Sayanova, O., Usher, S., Metochis, C., Campbell, P. J., Napier, J. A., & Tocher, D. R. (2016). Nutritional evaluation of an EPA-DHA oil from transgenic Camelina sativa in feeds for post-Smolt Atlantic Salmon (Salmo salar L.). *PLoS One*, *11*(7), e0159934.

Bou, M., Berge, G. M., Baeverfjord, G., Sigholt, T., Østbye, T.-K., Romarheim, O. H., Hatlen, B., Leeuwis, R., Venegas, C., & Ruyter, B. (2017). Requirements of n-3 very long-chain PUFA in Atlantic salmon (Salmo salar L): Effects of different dietary levels of EPA and DHA on fish performance and tissue composition and integrity. *The British Journal of Nutrition*, *117*(1), 30–47.

Calder, P. C. (2017). Omega-3 fatty acids and inflammatory processes: From molecules to man. *Biochemical Society Transactions*, *45*(5), 1105–1115.

Carvalho, M., Montero, D., Domenici, P., Afonso, J. M., & Izquierdo, M. (2022). Dietary novel oils modulate neural function and preserve locomotor response in gilthead sea bream (Sparus aurata) juveniles by regulating synthesis and contents of fatty acids in brain. *Aquaculture*, *550*, 737873.

Carvalho, M., Montero, D., Rosenlund, G., Fontanillas, R., Ginés, R., & Izquierdo, M. (2020). Effective complete replacement of fish oil by combining poultry and microalgae oils in practical diets for gilthead sea bream (Sparus aurata) fingerlings. *Aquaculture (Amsterdam, Netherlands)*, *529*(735696). https://www.sciencedirect.com/science/article/pii/S0044848620310942?via%3Dihub

EFSA Panel on Dietetic Products Nutrition. (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, *8*(3), 1461.

FAO. (2020). The state of world fisheries and aquaculture 2020: Sustainability in action. Food and Agriculture Organization of the United Nations.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, *226*(1), 497–509.

Glencross, B. D., Carr, I., & Santigosa, E. (2022). Distribution, deposition, and modelling of lipid and long-chain polyunsaturated fatty acids in Atlantic Salmon fillets. *Reviews in Fisheries Science & Aquaculture*, *31*, 119–140. https://doi.org/10.1080/23308249.2022.2090831

GOED. (2014). *Global recommendations for EPA and DHA intake. (Rev. November 19, 2014)*. Retrieved from: http://issfal.org/GlobalRecommendationsSummary19Nov2014Landscape_-3-.pdf

Hatlen, B., Larsson, T., Østbye, T.-K., Romarheim, O. H., Rubio, L. M., & Ruyter, B. (2022). Improved fillet quality in harvest-size Atlantic salmon fed high n-3 canola oil as a DHA-source. *Aquaculture*, *560*, 738555.

Horn, S. S., Sonesson, A. K., Krasnov, A., Moghadam, H., Hillestad, B., Meuwissen, T. H. E., & Ruyter, B. (2019). Individual differences in EPA and DHA content of Atlantic salmon are associated with gene expression of key metabolic processes. *Scientific Reports*, *9*(1), 3889.

Jobling, M. (2011). G.M. Turchini, W.-K. ng and D.R. Tocher (eds): Fish oil replacement and alternative lipid sources in aquaculture feeds. *Aquaculture International: Journal of the European Aquaculture Society*, *19*(3), 595–596.

Karalazos, V., Bendiksen, E. A., Dick, J. R., & Bell, J. G. (2007). Effects of dietary protein, and fat level and rapeseed oil on growth and tissue fatty acid composition and metabolism in Atlantic salmon (*Salmo salar* L.) reared at low water temperatures. *Aquaculture Nutrition*, *13*(4), 256–265.

Kousoulaki, K., Mørkøre, T., Nengas, I., Berge, R. K., & Sweetman, J. (2016). Microalgae and organic minerals enhance lipid retention efficiency and fillet quality in Atlantic salmon (Salmo salar L.). *Aquaculture (Amsterdam, Netherlands)*, *451*, 47–57.

Kousulaki, K., Berge, G. M., Mørkøre, T., Aleksei, K., Baeverfjor, G., Ytrestoyl, T., Carlehog, M., Sweetman, J., & Ruyter, B. (2020). Microalgal Schizochytrium limacinum biomass improves growth and filet quality when used long-term as a replacement for fish oil, in modern salmon diets. *Frontiers in Marine Science*, *7*, 57.

Lie, Ø., & Lambertsen, G. (1991). Fatty acid composition of glycerophospholipids in seven tissues of cod (Gadus morhua), determined by combined high-performance liquid chromatography and gas chromatography. *Journal of Chromatography*, *565*(1–2), 119–129.

Løvmo, S. D., Whatmore, P., Sundh, H., Sigholt, T., Madaro, A., Bardal, T., & Olsen, R. E. (2021). Effects of Atlantic salmon (Salmo salar) fed low- and high HUFA diets on growth and midgut intestinal health. *Aquaculture*, *539*, 736653.

Lutfi, E., Berge, G. M., Bæverfjord, G., Sigholt, T., Bou, M., Larsson, T., Mørkøre, T., Evensen, Ø., Sissener, N. H., Rosenlund, G., Sveen, L., Østbye, T. K., & Ruyter, B. (2022). Increasing dietary levels of the *n*-3 long-chain PUFA, EPA and DHA, improves the growth, welfare, robustness and fillet quality of Atlantic salmon in sea cages. *British Journal of Nutrition*, *129*, 1–19. https://doi.org/10.1017/s0007114522000642

Marine Stewardship Council. (2021). *Small pelagic fisheries* (Vol. 1). MSC.

Martinez-Rubio, L., Morais, S., Evensen, Ø., Wadsworth, S., Ruohonen, K., Vecino, J. L. G., Bell, G., & Tocher, D. R. (2012). Functional feeds reduce heart inflammation and pathology in Atlantic Salmon (*Salmo salar* L.) following experimental challenge with Atlantic salmon reovirus (ASRV). *PLoS One*, *7*(11), e40266.

Martins, B. P., Bandarra, N. M., & Figueiredo-Braga, M. (2020). The role of marine omega-3 in human neurodevelopment, including autism Spectrum disorders and attention-deficit/hyperactivity disorder - a review. *Critical Reviews in Food Science and Nutrition*, *60*(9), 1431–1446.

Meigs, H., Barrows, F., Sims, N. A., & Alfrey, K. (2020). *Testing diets without fishmeal and fish oil for kampachi*. Global Seafood Alliance.

Miller, M. R., Nichols, P. D., & Carter, C. G. (2007). Replacement of fish oil with thraustochytrid Schizochytrium sp. L oil in Atlantic salmon parr (Salmo salar L) diets. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *148*(2), 382–392.

Mock, T. S., Francis, D. S., Drumm, D. W., Versace, V. L., Glencross, B. D., Smullen, R. P., Jago, M. K., & Turchini, G. M. (2020). A systematic review and analysis of long-term growth trials on the effect of diet on omega-3 fatty acid levels in the fillet tissue of post-smolt Atlantic salmon. *Aquaculture*, *516*, 734643.

Mozaffarian, D., & Wu, J. H. Y. (2011). Omega-3 fatty acids and cardiovascular disease: Effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology*, *58*(20), 2047–2067.

Nasopoulou, C., & Zabetakis, I. (2012). Benefits of fish oil replacement by plant originated oils in compounded fish feeds. A review. *Lebensmittel-Wissenschaft Und Technologie [Food Science and Technology]*, *47*(2), 217–224.

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Luvchenco, J., Shumway, S. E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, *591*(7851), 551–563.

Nichols, P. D., Glencross, B., Petrie, J. R., & Singh, S. P. (2014). Readily available sources of long-chain omega-3 oils: Is farmed Australian seafood a better source of the good oil than wild-caught seafood? *Nutrients*, *6*(3), 1063–1079.

Nøstbakken, O. J., Rasinger, J. D., Hannisdal, R., Sanden, M., Frøyland, L., Duinker, A., Frantzen, S., Dahl, L. M., Lundebye, A. K., & Madsen, L. (2021). Levels of omega 3 fatty acids, vitamin D, dioxins and dioxin-like PCBs in oily fish; a new perspective on the reporting of nutrient and contaminant data for risk-benefit assessments of oily seafood. *Environment International*, *147*(106322), 106322.

Oliver, L., Dietrich, T., Marañón, I., Villarán, M. C., & Barrio, R. J. (2020). Producing Omega-3 polyunsaturated fatty acids: A review of sustainable sources and future trends for the EPA and DHA market. *Resources*, *9*(12), 148.

Olsen, H., Henderson, R. J., & Ringø, E. (1998). The digestion and selective absorption of dietary fatty acids in Arctic charr, Salvelinus Alpinus. *Aquaculture Nutrition*, *4*(1), 13–21.

Osmond, A. T. Y., Arts, M. T., Hall, J. R., Rise, M. L., Bazinet, R. P., Armenta, R. E., & Colombo, S. M. (2021). Schizochytrium sp. (T18) oil as a fish oil replacement in diets for juvenile rainbow trout (Oncorhynchus mykiss): Effects on growth performance, tissue fatty acid content, and lipid-related transcript expression. *Animals: An Open Access Journal from MDPI*, *11*(4), 1185.

Panchal, S. K., & Brown, L. (2021). Addressing the insufficient availability of EPA and DHA to meet current and future nutritional demands. *Nutrients*, *13*(8). https://doi.org/10.3390/nu13082855

Ratledge, C. (2010). Single cell oils for the 21st century. In Z. Cohen & C. Ratledge (Eds.), *Single Cell Oils* (pp. 3–26). Elsevier.

Richter, C. K., Skulas-Ray, A. C., & Kris-Etherton, P. M. (2016). Chapter 3 - recommended intake of fish and fish oils worldwide. In S. K. Raatz & D. M. Bibus (Eds.), *Fish and fish oil in health and disease prevention* (pp. 27–48). Academic Press.

Rosenlund, G., Baardsen, G. K., Stubhaug, I., & Holme, M. H. (2018). Sensory quality of Atlantic salmon (S. salar) fed no fish meal–no fish oil diets. Proceedings of the 18th international symposium on fish Nutrition & Feeding (ISFNF), 1–14. Presented at the 18th International Symposium on Fish Nutrition & Feeding (ISFNF), Las Palmas, Spain.

Ruyter, B., Bou, M. M., Berge, G. M., Mørkøre, T., Sissener, N. H., Sanden, M., Lutfi, E., Romarheim, O. H., Krasnov, A., & Østbye, T. K. (2022). A dose-response study with omega-3 rich canola oil as a novel source of docosahexaenoic acid (DHA) in feed for Atlantic salmon (*Salmo salar*) in seawater; effects on performance, tissue fatty acid composition, and fillet quality. *Aquaculture*, *561*, 738733.

Ruyter, B., Sissener, N., Lutfi Royo, E., Hundal, B. K., Berge, G. M., Glencross, B., Huyben, D., Gjøen, T., Andersen, A. M. S., Bæverfjord, B., Høglund, E., Powell, M. D., Bou, M., Chandrasekar, S., Østbye, T.-K. K., Liland, N. S., Rosenlund, G., Sigholt, T., Stabhaug, I., … Mørkøre, T. (2021). *Optimalisering av fett og fettsyrer i fôr til atlantisk laks for bedre helse og velferd gjennom ulike livsfaser og utfordrende miljøbetingelser* (Nofima Rapportserie 17/2021 978–82–8296-681–8). Retrieved from Nofima AS website: https://nofima.brage.unit.no/nofima-xmlui/handle/11250/2756520 (Accessed: July 16 2022)

Santigosa, E., Brambilla, F., & Milanese, L. (2021). Microalgae oil as an effective alternative source of EPA and DHA for gilthead seabream (Sparus aurata) aquaculture. *Animals: An Open Access Journal from MDPI*, *11*(4). https://doi.org/10.3390/ani11040971

Santigosa, E., Constant, D., Prudence, D., Wahli, T., & Verlhac-Trichet, V. (2020). A novel marine algal oil containing both EPA and DHA is an effective source of omega-3 fatty acids for rainbow trout (Oncorhynchus mykiss). *Journal of the World Aquaculture Society*, *51*(3), 649–665.

Santigosa, E., Verlhac-Trichet, V., Olsen, R. E., & Figuereido-Silva, C. (2018). A microalgal oil containing EPA+DHA can be an effective source of omega 3 for Atlantic salmon post- smolts. 3–20. Presented at the Proceedings of the 18th International Symposium on Fish Nutrition & Feeding (ISFNF), Las Palmas, Spain.

Simopoulos, A. P. (2011). Importance of the omega-6/omega-3 balance in health and disease: Evolutionary aspects of diet. *World Review of Nutrition and Dietetics*, *102*, 10–21.

Sissener, N. H., Araujo, P., Sæle, Ø., Rosenlund, G., Stubhaug, I., & Sanden, M. (2020). Dietary 18:2 n-6 affects EPA (20:5 n-3) and ARA (20:4 n-6) content in cell membranes and eicosanoid production in Atlantic salmon (*Salmo salar* L.). *Aquaculture*, *522*, 735098.

Sprague, M., Betancor, M. B., & Tocher, D. R. (2017). Microbial and genetically engineered oils as replacements for fish oil in aquaculture feeds. *Biotechnology Letters*, *39*(11), 1599–1609.

Sprague, M., Dick, J. R., & Tocher, D. R. (2016). Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006-2015. *Scientific Reports*, *6*(1), 21892.

Sprague, M., Fawcett, S., Betancor, M. B., Struthers, W., & Tocher, D. R. (2020). Variation in the nutritional composition of farmed Atlantic salmon (Salmo salar L.) fillets with emphasis on EPA and DHA contents. *Journal of Food Composition and Analysis: An Official Publication of the United Nations University, International Network of Food Data Systems*, *94*(103618), 103618.

Stuart, K. R., Barrows, F. T., Silbernagel, C., Alfrey, K., Rotstein, D., & Drawbridge, M. A. (2021). Complete replacement of fish oil and fish meal in the diet of juvenile California yellowtail Seriola dorsalis. *Aquaculture Research*, *52*(2), 655–665.

Thompson, K. D., Tatner, M. F., & Henderson, R. J. (1996). Effects of dietary (n-3) and (n-6) polyunsaturated fatty acid ratio on the immune response of Atlantic salmon, Salmo Salar L. *Aquaculture Nutrition*, *2*(1), 21–31.

Tocher, D. R., Betancor, M. B., Sprague, M., Olsen, R. E., & Napier, J. A. (2019). Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: Bridging the gap between supply and demand. *Nutrients*, *11*(1), 89.

Torstensen, B. E., Froyland, L., & Lie, O. (2004). Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil - effects on Atlantic salmon (Salmo salar L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities. *Aquaculture Nutrition*, *10*(3), 175–192.

Torstensen, B. E., Lie, O., & Frøyland, L. (2000). Lipid metabolism and tissue composition in Atlantic salmon (Salmo salar L.)—Effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. *Lipids*, *35*(6), 653–664.

Torstensen, B. E., Bell, J. G., Rosenlund, G., Henderson, R. J., Graff, I. E., Tocher, D. R., Lie, Ø., & Sargent, J. R. (2005). Tailoring of Atlantic salmon (Salmo salar L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. *Journal of Agricultural and Food Chemistry*, *53*(26), 10166–10178.

USDA. (2015). *United States Department of Agriculture and Department of Health and Human Services (USDA), Scientific Report of the 2015 Dietary Guidelines Advisory Committee*. Retrieved from Available at: http://health.gov/dietaryguidelines/2015-scientific-report/pdfs/scientific-report-of-the-2015-dietary-guidelines-advisory-committee.pdf (Accessed: November 2022)

Wei, M., Parrish, C. C., Guerra, N. I., Armenta, R. E., & Colombo, S. M. (2021). Extracted microbial oil from a novel Schizochytrium sp. (T18) as a sustainable high DHA source for Atlantic salmon feed: Impacts on growth and tissue lipids. *Aquaculture (Amsterdam, Netherlands)*, *534*(736249). https://doi.org/10.1016/j.aquaculture.2020.736249

Ytrestøyl, T., Aas, T. S., & Åsgård, T. (2015). Utilisation of feed resources in production of Atlantic salmon (Salmo salar) in Norway. *Aquaculture*, *448*, 365–374.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Santigosa, E., Olsen, R. E., Madaro, A., Trichet, V. V., & Carr, I. (2023). Algal oil gives control of long-chain omega-3 levels in full-cycle production of Atlantic salmon, without detriment to zootechnical performance and sensory characteristics. *Journal of the World Aquaculture Society*, *54*(4), 861–881. https://doi.org/10.1111/jwas.12947