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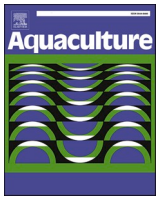

Improved fillet quality in harvest-size Atlantic salmon fed high n-3 canola oil as a DHA-source

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ABSTRACT

New sources of the very long-chain n-3 polyunsaturated fatty acids (n-3 VLC-PUFA) are needed for sustainable growth of salmon farming. In recent research high n-3 VLC-PUFA canola oil has shown promise as a safe and effective source of docosahexaenoic acid (DHA) and other n-3 PUFA in small Atlantic salmon. To study its long-term effects on performance and fillet quality under realistic farming conditions, a 12-month feeding experiment was carried out with Atlantic salmon growing to harvest weight (4.7 kg) in triplicate sea water cages. The fish were fed three diets containing graded inclusion levels of high n-3 canola oil resulting in levels of dietary DHA (22:6 n-3) of 4.0% (Standard; STD), 6.6% (Medium; MED) and 8.3% of total fatty acids (HIGH) and total EPA + DHA of 6.4, 9.1 and 11.0% of total fatty acids, respectively. The STD diet was considered to represent current commercial practice. There were no significant differences between the dietary groups in overall fish growth and weight at harvest, and no differences were seen in feed conversion ratio. Tissue FA-profiles mirrored the diets, giving increased DHA, EPA (20:5 n-3) and ALA (18:3 n-3) with increasing inclusion level of high n-3 LC-PUFA canola oil. Dietary inclusion of n-3 canola oil had no effect on muscle astaxanthin concentration, but instrumentally measured redness, yellowness and chroma increased with n-3 canola oil inclusion, suggesting a positive effect on colour via effects on muscle structure or composition. The prevalence and severity of dark melanin spots in the fillet was significantly reduced by increased inclusion level of n-3 canola oil. The prevalence of severe spots (score $\geq$ 2), average score and number of affected muscle segments were all lower in MED and HIGH, compared to STD.

In conclusion, high n-3 canola oil is an efficient source of DHA and other n-3 fatty acids for harvest-size Atlantic salmon. Increased dietary supply of DHA and other n-3 PUFA, above minimum requirement levels for DHA, improved fillet pigmentation and reduced fillet melanin spots, without compromising fish growth.

1. Introduction

The limited availability of marine oils rich in long-chain (LC) n-3 polyunsaturated fatty acids (PUFA), primarily eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), is a major constraint for further growth in the salmon aquaculture industry (Shepherd et al., 2017; Tocher, 2015; Tocher et al., 2019). To reduce the dependency on finite natural fish resources, fish oil in salmon feed has been increasingly replaced over the last 20 years with plant oils, predominately rapeseed oil, and the EPA and DHA available to the fish have been correspondingly diluted (Aas et al., 2019; Sprague et al., 2016; Ytrestøyl et al., 2015). Besides potentially reducing the health benefits of salmon consumption for humans, further replacement of dietary fish oil with ordinary plant oils would be expected to reduce the health and welfare of the farmed salmon (Bou et al., 2017a; Bou et al., 2017b; Lutfi et al., 2022).

Consequently, alternative sources have been sought, such as algae, microbes and genetically modified oil crops (Kousoulaki et al., 2020;

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Sprague et al., 2017; Tocher et al., 2019). Among these, long-chain n-3 PUFA rich oils from crops genetically modified with algal genes are promising new sources for the industry (Petrie et al., 2020). We have recently tested a high n-3 canola oil in diets of Atlantic salmon in the pre-smolt (freshwater) and post-smolt (seawater) life stages (Ruyter et al., 2022; Ruyter et al., 2019). The studies showed no negative effects on survival or growth, and EPA and DHA from the high n-3 canola oil were deposited with similar efficiency as from fish oil. No effects were seen on health markers, such as expression of genes and enzyme activities related to toxicity or stress.

In addition to optimal performance, fish health and welfare, the diet should also secure good quality of the final product. Fatty acid composition of salmon fillet, which itself is an important quality criterion, usually reflects the composition of the diet (Bou et al., 2017a; Grisdale-Helland et al., 2002; Sargent et al., 2003; Thomassen and Røsjø, 1989; Torstensen et al., 2005). There is also increasing evidence of additional effects of dietary EPA and DHA on other aspects of fillet quality, such as improved fillet colour (Kousoulaki et al., 2020; Lutfi et al., 2022; Ruyter et al., 2022) and reduced occurrence of black melanin spots in the fillet (Lutfi et al., 2022; Sissener et al., 2016).

The previous feeding experiments with high n-3 canola oil have been carried out under controlled and stable conditions of land-based tanks and with fish not reaching normal commercial slaughter size (Ruyter et al., 2022; Ruyter et al., 2019). It remains to be studied how different dietary levels of high n-3 canola oil will influence fish performance and fillet quality when salmon are reared from the post smolt stage until they reach a commercial slaughter size of approximately 5 kg under the challenging conditions in sea cages. The objective of this project was to elucidate possible effects of long-term feeding with high n-3 canola oil to Atlantic salmon in seawater on growth, feed utilisation, tissue fatty acid deposition and slaughter quality.

2. Materials and methods

2.1. Experimental feeds

Three feeds were produced at Nofima's Feed Technology Centre in Bergen, Norway. The feed manufacturing process was a pilot scale version of the process used industrially for production of salmonid feeds, and involved mixing of dry ingredients, extrusion, drying of the pellets and coating with the residual oil. The feeds were vacuum coated with different oil mixes, with increasing inclusion level of a high n-3 canola oil (Aquaterra® oil, Nuseed Nutritional US) to produce the three experimental diets. The diets were formulated targeting dietary total EPA + DHA levels of 6.5 ("STD"; representing current commercial practice), 9 ("MED"; medium) and 11% of total fatty acids ("HIGH"). The high n-3 canola oil was added as replacement for a standard rapeseed oil and small amounts of palm oil were added to balance the level of saturated fatty acids among the diets.

The feeds were produced in three production series, varying in composition and pellet size, to adapt to the increasing size of the fish: 7 mm (600-feed; for 0.6 to 1.2 kg), 9 mm (1200-feed; for 1.2–2.5 kg) and 9 mm with high lipid:protein ratio (2500-feed; for 2.5 kg to slaughter). The formulations of the feeds represented current commercial practice and are shown in Table 1. The feeds were shipped to Gildeskål Forskningsstasjon AS (Gifas, Inndyr, Norway) and stored indoors under ambient temperatures until feeding. The last batch of feed (2500 feed) was stored in a refrigerated storage room, and one large bag (500 kg) was taken out and stored under ambient temperature as needed.

Feed samples were taken shortly after production, and all feeds were analysed for moisture (drying loss at 103 °C to stable weight; ISO 6496: 1999), ash (combustion at 550 °C, ISO 5984: 2002), crude protein (Nx6.25, Kjeldahl), crude fat ("EU-method" with acid hydrolysis; EC 152/2009) and combustion energy (Parr 1271 adiabatic bomb calorimeter, Parr Instrument Company, Moline, IL, USA). Total fat was extracted from feeds according to (Bligh and Dyer, 1959) and fatty acid composition was determined according to AOCS Official Method Ce 1b-89. Astaxanthin content was analysed in the feeds by HPLC as described by (Schüep and Schierle, 1995). Minerals were analysed by inductively coupled plasma mass spectroscopy (ICP-MS) at Eurofins, Moss, Norway. The analysed chemical composition of the experimental diets is given in Table 2 and fatty acid composition in Table 3.

2.2. Fish trial

The feeding trial was carried out by Gildeskål Forskningsstasjon AS (Gifas), Inndyr, Norway at their site at Langholmen (N 67°, 3.47′; E 14°, 1.53′). Atlantic salmon (130 fish per cage, initial weight 704 g) were distributed in nine experimental-size (5 × 5 × 5 m) net-cages in seawater. The fish were weighed individually at the start of the trial and selected within a pre-defined size range of 600–800 g.

The fish were fed the experimental diets by handfeeding. The feeding frequency was adapted to daylength and was twice daily within the period 1 March to 31 October 2019 and once daily from 1 November 2019 to 29 February 2020. Adaptations in frequency and timing of meals were also made considering weather and light conditions; feeding was omitted on a few days because of bad weather conditions. The sequence of feeding of different pens was changed from day to day to avoid any systematic effects of feeding time.

Feed spill was collected daily to ensure satiation of the fish (slight

Table 1
Feed formulations.

	600-feed (ca 7 mm)			1200-feed (ca 9 mm)			2500-feed (ca 9 mm)		
	STD	MED	HIGH	STD	MED	HIGH	STD	MED	HIGH
Aquaterra oil	5.2	14.0	19.9	6.7	16.2	22.5	7.5	18.2	24.7
Rapeseed oil	16.8	8.1	2.3	17.1	7.8	1.6	17.5	6.9	0.0
Fish oil (South-Am)	5.4	5.4	5.4	5.7	5.7	5.7	5.9	5.9	5.9
Palm oil	2.2	2.1	2.0	2.4	2.3	2.2	2.6	2.5	2.3
Fish meal	10.0	10.0	10.0	10.0	10.0	10.0	10.1	10.1	10.1
Soy protein concentrate	24.0	24.0	24.0	21.2	21.2	21.2	21.7	21.7	18.1
Wheat gluten	14.0	14.0	14.0	8.8	8.8	8.8	14.3	14.3	16.3
Corn gluten	5.0	5.0	5.0	5.0	5.0	5.0	2.5	2.5	2.6
Guar meal				4.9	4.9	4.9			
Dehulled beans	8.0	8.0	8.0	10.0	10.0	10.0			
Wheat	2.7	2.7	2.7	2.9	2.9	2.9	11.8	11.7	13.8
Carophyll pink, 10% astaxanthin	0.059	0.059	0.059	0.054	0.054	0.054	0.048	0.048	0.048
Mineral premixes	2.7	2.7	2.7	2.3	2.3	2.3	2.2	2.2	2.3
Vitamin premixes	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Amino acids	1.6	1.6	1.6	1.2	1.2	1.2	0.7	0.7	0.9
Mannan oligosaccharide	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.4
Others/water balance	1.4	1.4	1.4	1.0	1.0	1.0	2.2	2.3	2.1

Table 2
Proximate composition, astaxanthin and mineral content of the experimental diets.

	600-feed			1200-feed			2500-feed		
	STD	MED	HIGH	STD	MED	HIGH	STD	MED	HIGH
Crude protein (N x 6.25), %	43.1	42.2	42.6	39.9	39.8	39.7	39.4	39.9	39.3
Dry matter, %	94.1	93.3	93.3	94.0	94.3	93.4	93.0	93.9	93.0
Ash, %	4.8	4.7	4.7	4.6	4.5	4.5	4.3	4.3	4.1
Crude fat, %	31.9	31.6	31.8	33.3	32.9	31.8	34.6	34.6	34.3
Gross energy, kJ/g	24.7	24.5	24.6	24.9	25.1	25.0	24.8	25.0	24.9
Astaxanthin, mg/kg	62	60	61	55	56	54	41	42	41
Astaxanthin (end), mg/kg							39	36	36
Minerals, mg/kg									
Ca	3400	3200	3200	4600	4500	4100	4100	3600	3200
P, %	1.1	1.0	1.0	1.0	1.0	0.98	0.94	0.95	0.90
Zn	180	180	180	150	150	140	150	170	150
Fe	250	220	220	230	240	210	220	210	210
Mg	1600	1400	1500	1500	1600	1500	1500	1400	1200
Mn	57	61	56	52	53	48	50	53	49

Table 3
Fatty acid composition of the experimental diets.

	600-feed			1200-feed			2500-feed		
	STD	MED	HIGH	STD	MED	HIGH	STD	MED	HIGH
Crude fat, % (Bligh and Dyer, 1959)	32.4	32.4	33.2	33.5	33.5	33.2	36.3	36.3	35.0
% of total FA									
14:0	1.5	1.4	1.4	1.3	1.3	1.3	1.3	1.3	1.2
16:0	10.3	9.9	10.3	10.0	10.1	10.0	9.6	9.3	9.1
18:0	2.3	2.4	2.6	2.3	2.5	2.6	2.2	2.3	2.3
Sum saturated FA[a]	*14.8*	*14.4*	*15.1*	*14.3*	*14.6*	*14.6*	*13.8*	*13.5*	*13.2*
16:1 n-7	1.5	1.4	1.5	1.4	1.4	1.4	1.3	1.3	1.3
18:1 (n-9) + (n-7) + (n-5)	45.5	39.8	38.0	45.0	40.6	37.3	43.3	36.4	32.1
20:1 (n-9) + (n-7)	1.5	1.3	1.4	1.4	1.3	1.2	1.4	1.3	1.2
Sum monoenoic FA[b]	*49.3*	*43.2*	*41.5*	*48.4*	*43.8*	*40.4*	*46.8*	*39.7*	*35.2*
18:2 n-6	15.1	11.7	9.8	14.3	11.3	9.0	13.3	9.7	7.7
20:4 n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sum n-6 PUFA[c]	*15.4*	*12.1*	*10.4*	*14.6*	*11.8*	*9.5*	*13.7*	*10.1*	*8.2*
18:3 n-3	8.7	11.5	14.2	8.9	11.8	14.0	8.4	11.1	12.7
18:4 n-3	1.0	1.6	2.1	0.9	1.5	2.0	0.9	1.5	1.9
20:5 n-3 (EPA)	2.7	2.7	3.0	2.4	2.6	2.7	2.4	2.5	2.5
22:5 n-3	0.5	0.8	1.0	0.5	0.8	0.9	0.5	0.8	1
22:6 n-3 (DHA)	3.9	6.4	8.4	3.9	6.6	8.5	4.1	6.6	8.1
Sum n-3 PUFA[d]	*17.3*	*23.9*	*30.0*	*17.1*	*24.4*	*29.5*	*16.9*	*23.5*	*27.4*
n-6:n-3 ratio	0.90	0.51	0.35	0.85	0.49	0.32	0.81	0.44	0.30
Sum EPA + DHA	6.6	9.1	11.4	6.3	9.2	11.2	6.5	9.1	10.6

[a] Includes 20:0, 22:0,
[b] Includes 22:1 (n−11) + (n-9) + (n-7), 24:1 n-9,
[c] Includes 18:3 n-6, 20:2 n-6, 20:3 n-6, 22:4 n-6,
[d] Includes 20:3 n-3, 20:4 n-3, 21:5 n-3.

overfeeding) and to determine actual feed intake. The collector system consisted of a cone funnel (diameter 2 m) centred over the bottom of the cage 2 m under the surface and connected to a tube through which the feed spill was pumped up and collected on a sieve. The collected feed pellets were counted, and the mass of feed spill calculated based on average pellet weight for the feed used. The average pellet weight was obtained before commencement of feeding, by exact counting and weighing of 100 pellets of each feed batch.

Water temperature at 1, 3 and 5 m depth, salinity and oxygen concentration at 3 m were logged and registered daily. Water transparency was measured daily by means of a Secchi disc. The results are shown in Fig. 1. The water temperature (average of the three depths) varied between 3.1 and 14.8 °C during the experiment. The average temperatures for the three feeding periods were 8.9 °C on 600-feed (Oct-Nov 2019), 5.1 °C on 1200-feed (Nov 2019-Jun 2020) and 12.5 °C on 2500-feed (Jun-Sep 2020). The average temperature for the whole feeding trial was 7.7 °C.

All cages were inspected for mortalities every day, as far as possible. Dead fish were removed, weighed and registered.

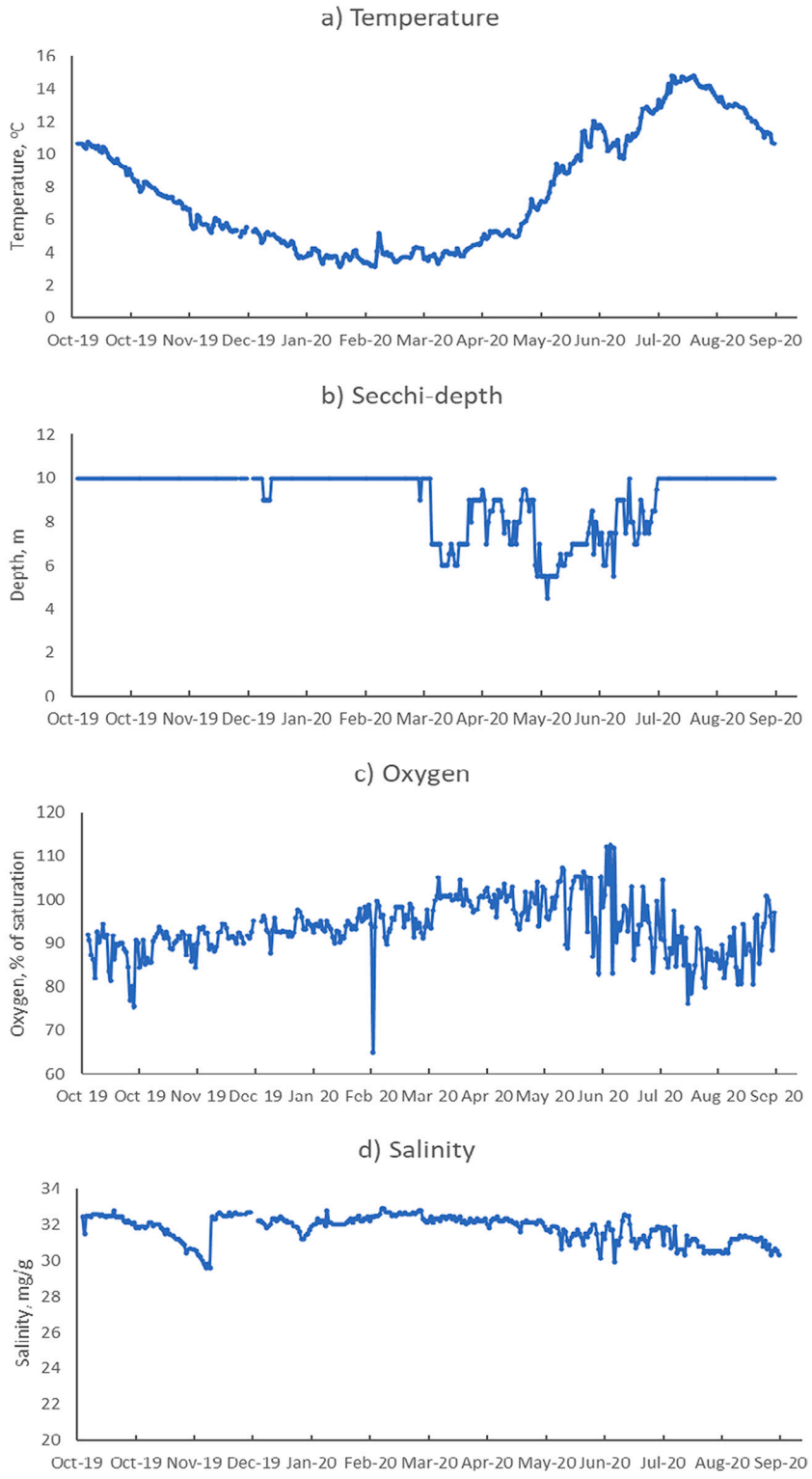

Fig. 1. Water quality parameters measured daily during the experiment: a) temperature in average of measurements at 1, 3 and 5 m depth; b) water transparency measured as Secchi depth; c) oxygen saturation logged and registered at 3 m depth; d) salinity logged and registered at 3 m depth.

2.3. Recordings and sampling

The fish were individually weighed, and samples taken for analyses at start and termination of the trial. Three pooled samples of 10 fish each were taken at the start, and 10 fish per cage at the final sampling.

The 30 fish taken for start samples were anaesthetised in 80 mg L^{-1} benzocaine (Bensoak vet., ACD Pharmaceuticals AS, Leknes, Norway), gill arches cut, and the fish left to bleed completely. The fish were then weighed, length measured and stored at −20 °C for later processing and analyses.

Prior to the final sampling the fish were fasted for 3 days. Ten fish were netted, anaesthetised, weighed, and length measured, and then tagged with a sheep tag (Os ID, Os, Norway) in the gill arch to gain full traceability of individual fish in the further transportation and

processing. The gill arches were cut, and the fish left to bleed in seawater for approximately 10 min. The fish were then gutted, the kidney removed by using a spoon, and the gutted fish were then weighed to calculate slaughter yield. The skin of a rectangular area between the dorsal fin and the midline of the left fillet was taken for chemical analyses, frozen on dry ice, and stored at −80 °C until analysis.

The livers and mid intestines from the ten fish in each cage were pooled in one sample per tissue and stored at −20 °C for later lipid analyses. The gutted fish were stored on ice in polystyrene transport boxes (5 fish in each) and shipped to Nofima (Ås, Norway) the following day, for further processing and quality assessments. Extra ice was added when the boxes arrived at Nofima, where they were stored in a refrigerated room until further processing. Nine days post-mortem, the ten fish per net pen were evaluated externally for visual score of cataracts (score 0–4 for each eye)(Wall and Bjerkas, 1999), scale loss (on a scale from 0 to 3; 0 = none, 1 = minimal, 2 = some, 3 = significant) and fin damage (active and healed damage combined, scale 0 to 3)(Noble et al., 2018), as well as for general appearance (e.g. deformities). The fish were then manually filleted by an experienced technician. The fillet yield was calculated relative to the whole-body weight and relative to the gutted weight (100 x fillet weight x weight^{-1}). Fillets were examined for degree of gaping (i.e. slits and holes in the fillet; score 0–5; Andersen et al., 1994) and presence of dark spots (score 0–8, and count of affected muscle segments)(Mørkøre, 2012)(Fig. 2). Fillet firmness and thickness were also measured instrumentally (TA-XT-2, Stable Micro Systems Ltd., England)(Mørkøre and Einen, 2003). Fillet firmness was analysed at two positions on the fillet; between the lateral line and the pin bones at the most anterior part of the dorsal fin, and in the dorsal part of the Norwegian Quality Cut (NQC, NS 9401:1994). Results are presented as the average of the two measurements. Colour (CIELab colour space) in muscle and skin was determined in duplicate by Minolta Chroma meter (CR-400 Minolta, Konica Minolta Sensing Inc., Japan) on the dorsal part of the NQC. The NQC was further processed as described in NS 9402:1994 "Atlantic salmon – Measurement of colour and fat", before chemical analyses in pooled samples per pen.

2.4. Registration and scoring of fillet melanin spots

In addition to the ten fish per pen examined at Nofima, an additional 20 fish were caught from each pen, killed, gutted and filleted, and fillets examined and scored for the presence of dark spots at the farm (Mørkøre, 2012). In total for each pen, 60 fillets from 30 fish were scored for melanin spots, 20 at Nofima, Ås, and 40 at the farm site. The scoring at Nofima and Gifas were carried out by different, experienced individuals. Within site, the same person did the scoring of all fillets.

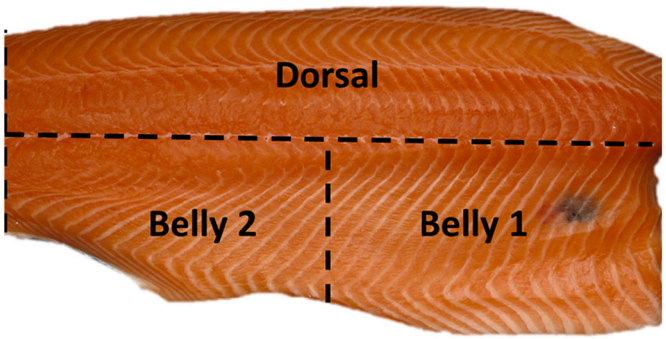

Fig. 2. Picture showing the areas of the fillet examined for melanin spots (the caudal part, not shown in the figure, was examined without registration of any spots). Example of a melanin spot in the anterior ventral part ("Belly 1").

2.5. Chemical analyses of fish samples

Astaxanthin (Ax) in muscle samples (NQC) was analysed according to (Bjerkeng et al., 1997), in pooled samples per cage. Total fat was extracted from pooled muscle, liver, mid-intestine and skin homogenates according to Folch et al. (1957). Fatty acid composition was then analysed by GC after trans-esterification to fatty acid methyl esters (FAME) as described by (Bou et al., 2017a).

2.6. Calculations

Specific growth rate (%BW d^{-1}); SGR = ln (W_2/W_1) x (t_2-t_1)$^{-1}$ × 100,

where W_1 and W_2 are live weights (g) at time (days) t_1 and t_2, respectively.

Thermal growth coefficient; TGC = ($W_2^{1/3}$ - $W_1^{1/3}$) x (T x d)$^{-1}$ × 1000

Where (T x d) is the day-degree sum for the period.

Daily feed intake (g /cage) was calculated from ration and collected feed spill, adjusting for dry matter and recovery:

Feed intake = ((Ration x DM_{feed}) – (Spill feed x DM_{spill}/Recovery %))/TS_{feed}

Feed conversion ratio; FCR = feed intake, g/biomass gain, g

Where biomass gain is (final biomass) + (biomass of mortalities) - (start biomass).

FA (or Ax) intake = Feed intake (g/fish) x dietary FA concentration

Fillet FA (or Ax) deposition = 0.61 x (W_2 x C_2 – W_1 x C_1),

where C_1 and C_2 are FA concentration in fillet (NQC) at time 1 and 2, respectively, and 0.61 is fillet yield (fillet weight/live weight), based on data from the end of the trial.

Ax retention, % = 100 x Fillet Ax deposition/Ax intake

Condition factor = 100 x BW x L^{-3},

where BW is body weight in g and L is the fork length (cm) of the fish

Hepatosomatic index; HSI (%) = liver weight/body weight x 100

Slaughter yield (%) = dressed weight/live weight

2.7. Statistics

Possible differences between diets were assessed by means of one-way ANOVA followed by Tukey HSD test. The statistical computations were done using SYSTAT® 13 (Systat Software Inc., Chicago, IL, USA).

Statistical analyses on data from quality assessment was based on individual data, to enable correction for e.g. body size. Cage was included in the model as a random variable. Non-parametric data (fillet gaping, dark spot prevalence) were tested by the Kruskal–Wallis test. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Mortality

In total, 52 fish died during the experiment, which corresponds to 4.4% of the initial number of fish. Of these, 19 (4.9%) were fed STD, 11 (2.8%) on MED and 22 (5.6%) on HIGH. Most of the mortality occurred in the mid-winter (Dec-Jan; $N = 27$).

3.2. Growth and feed utilisation

The fish grew from 0.7 to 4.7 kg during the experiment (Table 4). There were no significant dietary effects on fish weights, overall growth or FCR.

3.3. Tissue fat content and fatty acid composition

Crude fat content and fatty acid composition in muscle, mid intestine, liver and skin are shown in Tables 5–8. Crude fat content did not differ in any of the tissues between fish on the different diets.

Table 4
Fish weights, growth rates and feed conversion ratio in salmon fed the different experimental diets (mean ± s.d., N = 3 cages/diet).

	STD	MED	HIGH	P (ANOVA)
Start weight, g	706±4	703±9	702±3	0.67
Final weight, g	4735 ±140	4720±73	4542±81	0.11
Specific growth rate (SGR)	0.59 ±0.01	0.59 ±0.00	0.58 ±0.00	0.12
Thermal growth coefficient (TGC)	3.20 ±0.07	3.20 ±0.03	3.12 ±0.03	0.12
Feed conversion ratio (FCR)	1.04 ±0.02	1.03 ±0.01	1.04 ±0.02	0.51

Table 5
Fat content and fatty acid composition of muscle (NQC) of salmon fed the three experimental diets (mean ± s.d., $N = 3$). At final sampling, dietary groups sharing a common superscript^{abc} were not significantly different ($P < 0.05$; ANOVA followed by Tukey's HSD test).

	Start	Final sampling			*P*-value
		STD	MED	HIGH	(ANOVA)
Crude fat, %	10.9 ±0.21	16.7 ±1.10	15.2 ±0.30	15.2 ±0.23	0.052
% of total FA					
14:0	2.6±0.04	1.4±0.02	1.4±0.02	1.4±0.01	0.030
16:0	11.1 ±0.13	9.7±0.10	9.9±0.13	9.8±0.14	0.20
18:0	2.9±0.02	2.7±0.02^{b}	2.8±0.04^{a}	2.8±0.00^{a}	0.003
Total saturates[1]	*17.8 ±0.14*	*14.8 ±0.05*	*15.2 ±0.19*	*15.1 ±0.11*	*0.057*
16:1 n-7	2.8±0.02	1.6±0.10	1.5±0.00	1.6±0.06	0.25
18:1 n-9	32.8 ±0.18	41.4 ±0.11^{a}	36.8 ±0.13^{b}	33.8 ±0.04^{c}	<0.001
18:1 n-7	3.0±0.02	2.8±0.01	2.8±0.18	2.9±0.01	0.38
20:1 n-9	3.1±0.03	2.4±0.02^{a}	2.1±0.02^{b}	1.9±0.01^{c}	<0.001
Total monoenes[2]	*47.5 ±0.13*	*51.8 ±0.12^{a}*	*47.5 ±0.17^{b}*	*45.1 ±0.08^{c}*	*<0.001*
18:2 n-6	13.4 ±0.06	13.8 ±0.00^{a}	11.1 ±0.09^{b}	9.5±0.01^{c}	<0.001
20:2 n-6	1.0±0.03	1.1±0.01^{a}	0.8±0.01^{b}	0.7±0.01^{c}	<0.001
20:4 n-6 ARA	0.28 ±0.01	0.14 ±0.00	0.15 ±0.01	0.15 ±0.01	0.59
Total n-6 PUFA[3]	*15.1 ±0.02*	*15.4 ±0.02^{a}*	*12.5 ±0.07^{b}*	*10.7 ±0.00^{c}*	*<0.001*
18:3 n-3	5.7±0.02	7.7±0.01^{a}	10.5 ±0.05^{b}	12.3 ±0.06^{c}	<0.001
20:5 n-3 EPA	3.6±0.10	2.2±0.02^{a}	2.4±0.02^{b}	2.5±0.03^{c}	<0.001
22:5 n-3	1.3±0.05	1.0±0.01^{a}	1.3±0.00^{b}	1.4±0.01^{c}	<0.001
22:6 n-3 DHA	6.4±0.07	5.2±0.13^{a}	8.1±0.08^{b}	9.8±0.06^{c}	<0.001
Total n-3 PUFA[4]	*18.3 ±0.13*	*17.1 ±0.02^{a}*	*23.5 ±0.11^{b}*	*27.4 ±0.01^{c}*	*<0.001*
n-6:n-3 ratio	0,83 ±0,006	0,90 ±0,001	0,53 ±0,003	0,39 ±0,000	<0.001

[1] Includes 15:0, 17:0, 20:0, 22:0, 24:0;
[2] Includes 14:1 n-5, 16:1 n-9, 16:1 n-5, 17:1 n-7, 18:1 n-11, 20:1 n-7, 20:1 n-11, 22:1 n-7, 22:1 n-9, 22:1 n-11, 24:1 n-9,
[3] Includes 18:3 n-6, 20:3 n-6, 22:4 n-6;
[4] Includes 16:2 n-3, 20:4 n-3, 20:3 n-3.

The fatty acid profiles mirrored those of the diets: as the level of ALA (18:3n-3) and DHA in the diet increased, so did the corresponding levels in the muscle, intestine, liver and skin (Tables 5–8). In the final sampling, the relative content of ALA and DHA increased from 7.7% and 5.2% in STD, to 12.3% and 9.8%, respectively, in the muscle of fish fed

Table 6
Fat content and fatty acid composition of liver of salmon fed the three experimental diets (mean ± s.d., N = 3). Diets sharing a common superscript^{abc} are not significantly different ($P < 0.05$; ANOVA followed by Tukey's HSD test).

	STD	MED	HIGH	*P* (ANOVA)
Crude fat, % (Folch)	8.1±0.7	7.5±0.2	6.8±0.8	0.11
% of total FA				
14:0	0.8±0.03	0.7±0.04	0.7±0.04	0.84
16:0	7.8±0.45^{b}	8.1±0.10^{ab}	9.4±0.75^{a}	0.020
18:0	4.2±0.16	4.2±0.15	4.6±0.35	0.13
Total saturates[1]	13.1±0.61^{b}	13.7±0.21^{ab}	15.2±1.13^{a}	*0.033*
16:1 n-7	1.1±0.03	1.1±0.05	1.0±0.10	0.43
18:1 n-9	39.4±1.02^{a}	34.5±0.72^{b}	29.5±1.85^{c}	<0.001
18:1 n-7	3.1±0.03	3.1±0.03	3.0±0.13	0.44
20:1 n-9	4.6±0.17^{a}	4.1±0.40^{a}	3.3±0.24^{b}	0.005
Total monoenes[2]	50.9±1.23^{a}	46.4±0.45^{b}	41.2±2.34^{c}	*0.001*
18:2 n-6	10.2±0.14^{a}	8.0±0.25^{b}	6.4±0.30^{c}	<0.001
20:2 n-6	2.1±0.08^{a}	1.7±0.12^{b}	1.4±0.04^{c}	<0.001
20:4 n-6	0.70±0.04	0.68±0.09	0.84±0.14	0.20
Total n-6 PUFA[3]	13.6±0.19^{a}	10.9±0.02^{b}	9.1±0.11^{c}	*<0.001*
18:3 n-3	5.1±0.18^{b}	7.2±0.43^{ab}	7.8±1.49^{a}	0.025
20:3 n-3	1.3±0.10^{b}	2.1±0.11^{a}	2.3±0.21^{a}	<0.001
20:5 n-3 EPA	3.4±0.13^{b}	3.5±0.04^{ab}	4.1±0.47^{a}	0.031
22:5 n-3	1.2±0.07^{b}	1.5±0.02^{ab}	1.9±0.08^{a}	<0.001
22:6 n-3 DHA	10.6±0.81^{b}	13.0±0.49^{ab}	16.7±2.38^{a}	0.007
Total n-3 PUFA[4]	21.7±0.77^{c}	27.5±0.24^{b}	33.0±1.22^{a}	*<0.001*

[1] Includes 15:0, 17:0, 20:0, 22:0, 24:0;
[2] Includes 14:1 n-5, 16:1 n-5, 17:1 n-7, 18:1 n-11, 20:1 n-11, 20:1 n-7, 22:1 n-7, 22:1 n-11, 22:1 n-9, 24:1 n-9;
[3] Includes 16:2 n-6, 18:3 n-6, 20:3 n-6;
[4] Includes 20:4 n-3.

Table 7
Fat content and fatty acid composition of mid intestine of salmon fed the three experimental diets (mean ± s.d., N = 3). Diets sharing a common superscript^{abc} are not significantly different ($P < 0.05$; ANOVA followed by Tukey's HSD test).

	STD	MED	HIGH	*P* (ANOVA)
Crude fat, % (Folch)	3.3±0.6	3.1±0.3	3.0±0.2	0.60
% of total FA				
14:0	1.0±0.0	1.0±0.0	1.0±0.0	0.22
16:0	14.6±0.6	15.7±0.7	15.9±0.5	0.085
18:0	6.0±0.5	6.8±0.6	6.7±0.2	0.13
Total saturates[1]	*22.6±1.0*	*24.5±1.5*	*24.6±0.6*	*0.14*
18:1 n-9	26.4±1.6^{a}	21.5±2.2^{b}	20.2±0.4^{b}	0.007
18:1 n-7	2.5±0.1	2.4±0.2	2.5±0.1	0.60
20:1 n-9	1.7±0.1^{a}	1.4±0.1^{b}	1.2±0.0^{b}	<0.001
Total monoenes[2]	*34.5±1.8^{a}*	*29.0±3.1^{b}*	*28.4±0.6^{b}*	*0.023*
18:2 n-6	8.7±0.6^{a}	6.3±0.6^{b}	5.5±0.1^{b}	0.001
20:2 n-6	1.2±0.0^{a}	0.9±0.1^{b}	0.8±0.0^{c}	0.001
20:4 n-6	1.3±0.2	1.4±0.1	1.3±0.0	0.97
Total n-6 PUFA[3]	*11.9±0.4^{a}*	*9.3±0.3^{b}*	*8.2±0.1^{c}*	*<0.001*
18:3 n-3	4.2±0.4^{b}	5.0±0.7^{ab}	5.6±0.4^{a}	0.043
20:5 n-3 EPA	4.5±0.1	4.6±0.2	4.7±0.2	0.57
22:5 n-3	1.7±0.1	1.8±0.0	1.7±0.0	0.62
22:6 n-3 DHA	18.8±1.4^{b}	23.0±1.1^{a}	24.6±1.2^{a}	0.003
Total n-3 PUFA[4]	*30.0±1.2^{c}*	*35.1±0.6^{b}*	*37.6±0.9^{a}*	*<0.001*

[1] Includes 15:0, 17:0, 20:0, 22:0, 24:0;
[2] Includes 14:1 n-5, 16:1 n-7, 16:1 n-5, 17:1 n-7, 18:1 n-11, 20:1 n-11, 20:1 n-7, 22:1 n-7, 22:1 n-11, 22:1 n-9, 24:1 n-9;
[3] Includes 16:2 n-6, 18:3 n-6, 20:3 n-6, 22:4 n-6;
[4] Includes 20:4 n-3, 20:3 n-3.

Table 8
Final fat content and fatty acid composition in skin of salmon fed the three experimental diets (mean ± s.d., N = 3). Diets sharing a common superscript[abc] are not significantly different (P < 0.05; ANOVA followed by Tukey's HSD test).

	STD	MED	HIGH	*P* (ANOVA)
Crude fat, % (Folch)	7.1±1.1	9.5±1.7	9.9±2.1	0.17
% of FA				
14:0	1.5±0.04	1.5±0.02	1.6±0.01	0.10
16:0	11.5±0.42	11.1±0.16	11.2±0.15	0.31
18:0	3.3±0.14	3.2±0.05	3.2±0.03	0.43
Total saturates[1]	*17.2±0.46*	*16.9±0.20*	*17.1±0.11*	*0.46*
16:1 n-7	1.5±0.09	1.6±0.02	1.6±0.07	0.55
18:1 n-9	41.0±0.24^{a}	37.2±0.10^{b}	34.6±0.25^{b}	<0.001
18:1 n-7	2.8±0.01^{b}	2.9±0.01^{a}	3.0±0.03^{a}	0.001
20:1 n-9	2.4±0.01^{a}	2.1±0.02^{b}	1.9±0.03^{c}	<0.001
22:1 n-7	0.9±0.10^{c}	1.4±0.03^{b}	1.6±0.02^{a}	<0.001
Total monoenes[2]	*50.9±0.26^{a}*	*47.8±0.25^{b}*	*45.5±0.47^{c}*	*<0.001*
18:2 n-6	12.9±0.16^{a}	10.8±0.10^{b}	9.3±0.10^{c}	<0.001
20:4 n-6 ARA	0.4±0.03	0.3±0.03	0.3±0.01	0.019
Total n-6 PUFA[3]	*14.7±0.14^{a}*	*12.3±0.09^{b}*	*10.7±0.07^{c}*	*<0.001*
18:3 n-3	6.9±0.08^{c}	9.6±0.10^{b}	11.3±0.20^{a}	<0.001
20:5 n-3 EPA	2.5±0.09	2.5±0.06	2.6±0.09	0.21
22:5 n-3	0.9±0.01^{c}	1.2±0.01^{b}	1.3±0.02^{a}	<0.001
22:6 n-3 DHA	5.3±0.28^{c}	7.2±0.14^{b}	8.6±0.25^{a}	<0.001
Total n-3 PUFA[4]	*16.5±0.44^{c}*	*21.8±0.12^{b}*	*25.3±0.12^{a}*	*<0.001*

[1] Includes 15:0, 17:0, 20:0, 22:0, 24:0;
[2] Includes 17:1 n-7, 18:1 n-11, 20:1 n-11, 20:1 n-7, 22:1 n-11, 22:1 n-9, 24:1 n-9;
[3] Includes 18:3 n-6, 20:2 n-6, 20:3 n-6;
[4] Includes 16:2 n-3, 20:3 n-3, 20:4 n-3.

HIGH (Table 5). Total n-6 fatty acid content of the muscle decreased with increasing dietary high n-3 canola oil inclusion level. This resulted in a major change in the n-6:n-3 ratio in the fillets, from 0.9 in diet STD to 0.4 in diet HIGH (Table 5). Saturated fatty acid content was not different in muscle between the diet groups, while monounsaturated fatty acid content decreased, reflecting the higher monounsaturated fatty acid content in the STD diet. There was a strong, linear correlation between dietary intake and quantitative (g DHA/kg) muscle deposition of DHA (Fig. 3).

The fatty acid composition of the liver (Table 6) was strongly influenced by the fatty acid composition of the feeds. With increasing ALA and DHA levels in the diet, the relative levels of these fatty acids increased in the liver from 5.1% and 10.6% to 7.8% and 16.7% of total fatty acids, respectively. The content of 18:2 n-6 was reduced from 10.2% in STD to 6.4% in HIGH. The fatty acid composition of the mid intestine was also similarly affected by the diet composition, although to a lesser extent than in the liver (Table 7). Table 8 shows that the fatty acid composition of the skin was very similar to that of the muscle in the different diet groups.

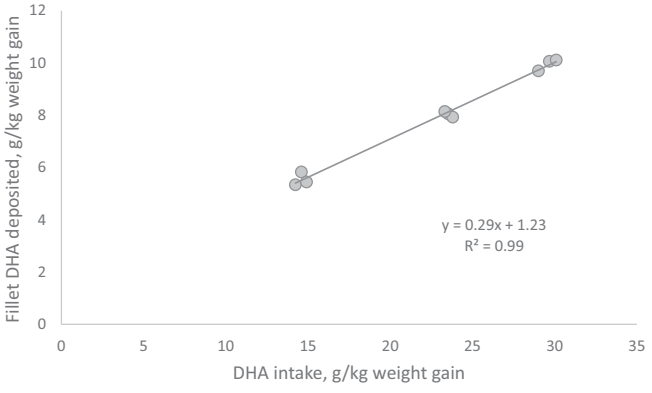

Fig. 3. Relationship between DHA intake and muscle DHA deposition. Each symbol represents one cage.

3.4. Body indices, product yield, and fillet texture

There were no differences in weight of sampled fish between diets when adjusted for cage effect (Table 9), but the average weight of fish sampled from STD were 0.3 kg higher than in HIGH and these differences were accounted for in the statistical analyses. The condition factor of the fish fed STD was significantly higher than the others, but numerically the difference in K values was very small, 1.17 for fish fed STD and 1.12 for MED and HIGH. Slaughter yield and fillet yield was normal and similar for all groups.

There were no significant effects of diet on fillet thickness, although the fillets of fish fed STD tended to be higher than for MED and HIGH for the posterior measurement (NQC) ($P = 0.06$, Table 9). This seemed to be associated with numerically higher body weight in this group, since when weight was included in the model, the trend disappeared ($P = 0.90$). Fillet gaping was low and similar for all groups, with no fish having gaping considered problematic. Fillet firmness, measured as the force required to break the surface of the fillet, did not differ among diet groups, all showing values considered adequate/firm, with only one fish below what is considered the threshold for fillets prone to being soft (7 N, Mørkøre, 2008). The total work (N x *sec*) required to reach 90% of

Table 9
Body indices, product yield, fillet thickness, texture and exterior colour and welfare markers of salmon sampled for quality evaluation at the end of the experiment (mean ± sd, N = 3).

	STD	MED	HIGH	*P* (ANOVA)
Round body weight, kg	4.82 ± 0.31	4.66 ± 0.31	4.52 ± 0.11	0,27
Gutted body weight, kg	4.30 ± 0.28	4.07 ± 0.24	3.99 ± 0.10	0,28
Condition factor (round weight)	1.35 ± 0.01^{a}	1.28 ± 0.01^{b}	1.29 ± 0.01^{b}	0,034
Condition factor (gutted weight)	1.17 ± 0.01^{a}	1.12 ± 0.01^{b}	1.12 ± 0.02^{b}	0,035
Slaughter yield, %	86.9 ± 0.3	87.0 ± 0.6	87.2 ± 0.3	0,57
Fillet yield, % of round weight	60.6 ± 0.1	61.2 ± 1.0	61.4 ± 0.6	0,41
Fillet yield, % of gutted weight	69.5 ± 0.1	70.1 ± 0.1	70.2 ± 0.2	0,39
Fillet quality:				
Gaping	0.3 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	0,78
Thickness anterior, mm	35.9 ± 0.7	34.6 ± 0.6	34.6 ± 0.9	0,11
Thickness posterior, mm	27.1 ± 0.5	25.7 ± 0.8	25.7 ± 0.2	0,058
Firmness - breaking force, N	9.5 ± 0.3	9.0 ± 0.2	9.1 ± 0.3	0,59
Firmness - total work, N x sec	281 ± 17^{a}	240 ± 10^{b}	247 ± 9^{b}	0,015
Exterior registrations:				
Cataract, sum of both eyes (score 0–4)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.89
Fin damage (score 0–3)	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.68
Scale loss (score 0–3)	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.36
Skin lightness (L*-value)	64.9 ± 1.3	61.9 ± 1	64.1 ± 1.2	0.20
Skin redness (a*-value)	−1.2 ± 0.1	−1.1 ± 0.2	−0.9 ± 0.2	0.53
Skin yellowness (b*-value)	−0.2 ± 0.3	0.3 ± 0.4	0.8 ± 0.2	0.072

fillet thickness was, however, significantly higher for fish fed STD compared to MED and HIGH, although the numerical differences were small. This effect could not be explained by weight differences.

Fish from all groups had similar and low scores for cataract, scale loss and dorsal fin damage, and skin colour measurements by Minolta Chroma Meter revealed no significant differences between the dietary groups for L*-, a*- or b*-value (Table 9).

3.5. Muscle pigmentation

Muscle astaxanthin and total carotenoid (astaxanthin + idoxanthin) concentrations did not differ significantly between diet groups (Table 10). Redness a* and yellowness b* measured instrumentally was significantly higher for the diets containing higher levels of DHA (MED and HIGH) than for STD. The groups MED and HIGH had also significantly higher levels of chroma (C*, colour saturation), than STD. There were no significant differences between the groups for colour hue (hue angle, H°, data not shown). No differences were seen between diets in muscle lightness, L*.

3.6. Dark spots in fillet

In total, 20% of the investigated fish had melanized dark spots in the fillets, with fish fed the STD diet (31%) having significantly higher prevalence than those fed MED (9%) (Table 11). The STD group had a significantly higher score (larger and more marked spots), more affected muscle segments and a higher prevalence of distinct spots (score > 1) than groups MED and HIGH (Table 11). Prevalence of spots followed a similar pattern in all parts of the fillet (Fig. 4), although was most pronounced in the dorsal muscle.

4. Discussion

The major overall aim of this work was to investigate the use of oil from genetically modified canola crop, high n-3 canola oil (Aquaterra®) as an n-3 fatty acid-rich fat source in feed for Atlantic salmon under exposed conditions in sea cages. There were no effects on fish growth of feeding diets with the high n-3 canola oil from approximately 0.7 kg up to 4.5–4.7 kg in seawater. This is in accordance with previous studies showing no effects on growth of salmon fed different inclusion levels of high n-3 canola oil in the freshwater stage and early sea water stage (Ruyter et al., 2022; Ruyter et al., 2019). It is also in accordance with previous literature showing generally little or no effect of dietary oil source on growth performance and feed efficiency, provided the quality of the oil used is good (Turchini et al., 2009).

The present study confirms the previous findings of (Ruyter et al., 2022; Ruyter et al., 2019), that the high n-3 canola oil is an efficient source of n-3 FA for deposition of these fatty acids in tissues of salmon. The increasing percentages of n-3 PUFA and reduced n-6 PUFA in the fillet with increasing dietary inclusion of high n-3 canola oil is particularly important, since this is considered beneficial to human nutrition (Simopoulos, 2006). These results are in accordance with a vast number of studies showing that fatty acid composition of both fillet and skin of Atlantic salmon to a large extent reflect that of the dietary oil (Bou et al., 2017a; Grisdale-Helland et al., 2002; Sargent et al., 2003; Thomassen and Røsjø, 1989; Torstensen et al., 2005). The tissue FA composition is a result of both dietary composition and metabolism in the fish. Although a clear influence of diet was seen in all studied tissues, liver and mid intestine differed more from the feed, than did skin and muscle, e.g. by containing higher proportions of n-3 FA and lower of n-6 FA. This agrees with previous studies (Bou et al., 2017a), and may be explained partly by differences in the proportions of structural and storage lipids, and partly by differences in endogenous metabolism between tissues.

The higher condition factor of fish fed the STD diet than in the other groups may be related to the larger fish (not significant) sampled in this group. However, the differences seen were small both when calculated on round weight (1.35–1.28) and gutted weight basis (1.17–1.12). Condition factor may be expected to affect slaughter yield, but no such trend was seen in this experiment, supporting that the small differences seen in condition factor were of little practical significance. The fish fed STD were also observed to have firmer fillets, measured as total work (Ns), than the two other groups. The differences were, however, small, all firmness values were within normal ranges and none of the fillets were considered as "soft" (breaking force <7 N) (Mørkøre, 2008). A similar trend was observed when comparing diets with fish oil (EPA + DHA) and algae meal (mainly DHA) by (Kousoulaki et al., 2020), but in that case the difference was even smaller than in the present trial.

The effects of diet on black melanin spots in fillets observed in the present study are interesting and highly relevant for the salmon farming industry, since this has been pointed out as the largest fillet quality problem in farmed Atlantic salmon (Mørkøre et al., 2015; Nordberg, 2018). The observed reduction in melanin spots with increasing dietary DHA level is in accord with several recent reports showing similar effects of feeds with increase in either sum of EPA and DHA (Lutfi et al., 2022; Sissener et al., 2016) or DHA alone (Kousoulaki et al., 2020). Melanin spots, usually referred to in the scientific literature as melanized focal changes, are melanomacrophage accumulation involved in a chronic focal inflammation, with an unknown initial cause (Bjørgen et al., 2019; Bjørgen et al., 2020; Larsen et al., 2012; Malik et al., 2021). The anti-inflammatory effects of dietary EPA and DHA due to their role as precursors in the immune system, have therefore been suggested as a possible explanation for their reducing effects on dark melanin spots (Sissener et al., 2016). Also, an association between infection with PRV (piscine rheovirus), the virus causing heart and skeletal muscle inflammation (HSMI), and development of melanin spots has been shown,

Table 10
Muscle pigment deposition and colour in salmon fed the experimental diets (mean ± s.d., N = 3 cages/diet; *N* = 30 fish/diet for colour data). All analyses were carried out in the "Norwegian Quality Cut" (NQC; NS 9401).

	STD	MED	HIGH	*P* (ANOVA)
Muscle carotenoid concentrations				
Astaxanthin, mg/kg [1]	5.3±0.4	5.9±0.5	5.4±0.3	0.20
Idoxanthin, mg/kg [1]	0.15 ±0.02	0.18 ±0.03	0.21 ±0.02	0.12
Total carotenoids, mg/kg	5.4±0.4	6.1±0.5	5.6±0.3	0.19
% idoxanthin of total	2.9±0.6	3.0±0.4	3.7±0.5	0.16
Muscle retention, % of eaten				
Astaxanthin	6,6±0,7	7,6±0,7	6,8±0,5	0.21
Total carotenoid	6,8±0,7	7,8±0,8	7,0±0,5	0.20
Muscle colour (Minolta)				
Lightness, L*	41.5±0.1	42.3±1.0	43.0±0.6	0.12
Redness, a*	11.0 ±0.3^{b}	12.7 ±0.4^{a}	12.2 ±0.4^{a}	0.004
Yellowness, b*	12.5 ±0.1^{b}	14.2 ±0.3^{a}	13.9 ±0.5^{a}	<0.001
Muscle chroma C*	16.6 ±1.5^{b}	19.0 ±1.7^{a}	18.5 ±1.7^{a}	<0.001

[1] Initial concentrations: 4.3 mg/kg astaxanthin and 0.17 mg/kg idoxanthin.

Table 11
Dark spot prevalence, intensity (score) and number of affected muscle segments of all salmon evaluated for dark spots (average ± SE, *N* = 90 per diet).

	STD	MED	HIGH	*P* (ANOVA)
Prevalence, %	31 ± 5^{a}	9 ± 3^{b}	20 ± 4^{ab}	<0.001
Prevalence of score ≥ 2, %	19 ± 4^{a}	3 ± 2^{b}	7 ± 3^{b}	<0.001
Average score	0.7 ± 0.2^{a}	0.1 ± 0.1^{b}	0.3 ± 0.1^{b}	<0.001
N of affected segments	4.8 ± 1.3^{a}	0.4 ± 0.2^{b}	0.6 ± 0.1^{b}	<0.001

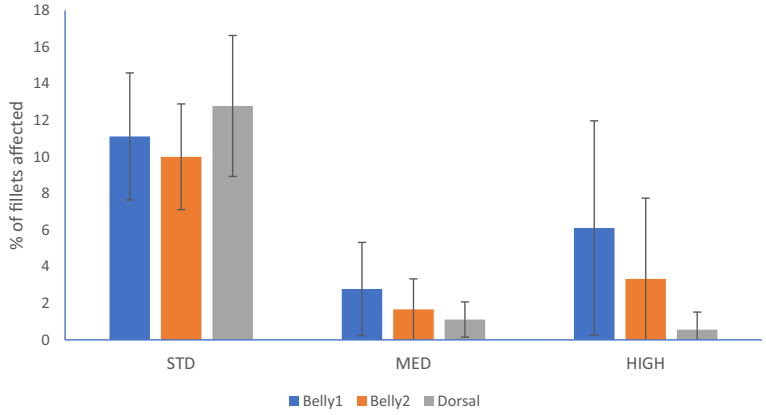

Fig. 4. Percentage of fillets with melanin deposits in the different fillet segments ($N = 60$ fillets examined/cage).

although probably not as the initial cause (Malik et al., 2021). A reduction in the inflammatory reactions to PRV infection by feeding functional feeds with elevated EPA content has been shown by (Martinez-Rubio et al., 2012). (Kousoulaki et al., 2020) however, reported reduced number of muscle segments with melanin discoloration in fish fed a diet added a DHA-rich algal meal (*Schizochytrium limacinum*), compared to a diet with fish oil, although the total dietary EPA and DHA content in their diets was similar, only differing in their ratio. Since EPA also has strong anti-inflammatory effects (Calder, 2014; Simopoulos, 2002) this may suggest another, specific role of DHA in preventing melanin deposits, besides reducing inflammation.

The improved muscle colour in the present trial is in accordance with (Ruyter et al., 2022) who found increased Salmofan™ colour score in salmon fed 25% high n-3 canola oil compared to a 0% control diet. As in the present study, no difference was seen in astaxanthin deposition by (Ruyter et al., 2022), but a slight reduction in idoxanthin level was observed with increasing high n-3 canola oil inclusion. (Kousoulaki et al., 2020) found improved muscle astaxanthin content in fish fed DHA-rich algal biomass compared to a FO-diet suggesting a specific effect of DHA on astaxanthin deposition. The differences seen in individually measured colour scores in the present trial were not confirmed by astaxanthin analysis of pooled muscle samples. Both redness a* and yellowness b* are usually highly correlated to astaxanthin concentration (Christiansen et al., 1995), however, it is possible that feed composition may have affected muscle structure and composition in ways contributing to the differences seen in fillet colour. Improved colour, scored by means of Salmofan™ (DSM), was also seen in salmon with increasing levels of EPA and DHA in the study of (Lutfi et al., 2022), despite no significant differences in astaxanthin concentration.

5. Conclusion

This work is consistent with previous findings of high n-3 canola oil as a safe and effective source of n-3 FA (Ruyter et al., 2022; Ruyter et al., 2019). Whereas previous results were obtained in controlled tank conditions and with relatively small fish, this work confirms the safety and efficacy of this oil in a full annual cycle in seawater cages, where the fish were exposed variable environmental conditions similar to those experienced in normal commercial cage culture. Further, the study clearly demonstrated the benefits of increased dietary supply of DHA and other n-3 FA through addition of high n-3 canola oil, on fillet quality in harvest size Atlantic salmon. The results show that high n-3 canola oil could be a good alternative to fish oil as a source of n-3 FA, thus supporting continued growth in production of healthy salmon, without increasing the pressure on wild fish stocks.

CRediT authorship contribution statement

Bjarne Hatlen: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding acquisition, Project administration, Visualization, Writing – original draft. **Thomas Larsson:** Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – review & editing. **Tone-Kari Østbye:** Investigation, Writing – review & editing. **Odd Helge Romarheim:** Methodology, Resources, Writing – review & editing. **Laura Martinez Rubio:** Methodology, Writing – review & editing. **Bente Ruyter:** Conceptualization, Methodology, Investigation, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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