



# Growth performance, swimming capacity, and fillet quality in rainbow trout fed a transgene-derived omega-3 and carotenoid-enriched oil

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## ABSTRACT

A new oil from transgenic *Camelina sativa* was evaluated for its potential to serve as a primary source for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and astaxanthin in feed for rainbow trout (*Oncorhynchus mykiss*). Experimental diets were formulated containing either the transgene-derived oil (CAM) or a blend of fish and vegetable oils (CTL). The two diets were given to quadruplicate tanks of 30 fish (body weight  $294 \pm 32$  g) for 10 weeks. Some of the fish were exercised in a swim tunnel prior to sampling. At sampling, muscle tissue was collected for fillet quality assessment and plasma to measure oxidative stress markers. Dietary treatment had no significant impact on final body weight. The fatty acid profile of rainbow trout fillets reflected that of the experimental diets with higher levels of linoleic acid, arachidonic acid, EPA and DHA in fish fed CAM. Levels of corresponding lipid inflammatory mediators, except for those derived from DHA, were pre-exercise elevated in plasma of fish fed CAM. Similarly, dietary ketocarotenoid levels were reflected in the fillet of fish with lower astaxanthin in fish fed CAM compared to CTL, which agreed with differences in fillet color. Dietary treatment had no significant impact on swimming performance. Plasma cortisol and 8-isoprostane were elevated post-exercise, irrespective of dietary treatment. Feeding the transgene-derived oil supported normal growth and increased fillet EPA and DHA levels above standard. A comparable swimming capacity and stress response suggests that the inclusion of the transgenic camelina oil did not impair fish welfare.

## 1. Introduction

Farmed fish is an important source for omega-3 polyunsaturated fatty acids (n-3 PUFA) in the human diet, especially for the long chain n-3 PUFA (n-3 LC-PUFA) eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA). Meanwhile, the aquaculture industry faces a supply gap of raw materials to produce aquafeeds (Tocher et al., 2019). For this reason, in modern aquafeed formulations, fish oil is partly substituted with land-based oils derived from vegetables or terrestrial animals. However, these oils differ substantially from fish oil in their fatty acid profile, being devoid of EPA and DHA. In addition,

marine fish have a limited ability to produce n-3 LC-PUFA themselves (Gregory et al., 2016). To boost n-3 LC-PUFA levels in the fillet of farmed fish, non-conventional sources of n-3 LC-PUFA are therefore needed. An option that promises high production yields is the use of oils derived from genetically modified terrestrial crops (Sprague et al., 2017). Agri-biotechnology and bioengineering research successfully identified enzymes and regulatory elements involved in the synthesis of EPA and DHA from short-chain n-3 PUFA in terrestrial oilseed crops, especially in the two host species camelina and canola (Napier et al., 2015). Previous studies on the replacement of fish oil with oil derived from transgenic camelina gave promising results observing similar growth performance

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(Betancor et al., 2016a, 2016b, 2017a, 2021). For instance, Atlantic salmon (*Salmo salar*) fed transgenic camelina oil showed n-3 LC PUFA fillet levels comparable to those of fish fed a diet with full inclusion of fish oil (Betancor et al., 2021). Recently, Aquaterra®, an omega-3 DHA-rich oil derived from genetically engineered canola, was the first transgene-derived oil to receive approval for its inclusion in salmon feeds in Norway by the competent Norwegian food safety authority (Thorstensen et al., 2023). In the future, targeted development of these new oils offers the possibility to further optimize the nutritional profile of transgenic oils towards the needs of the aquafeed industry (Napier and Betancor, 2023).

Besides their fatty acid profile, another important quality criterion of fish fillet is its color, which is especially true for salmonids like Atlantic salmon or rainbow trout (*Oncorhynchus mykiss*). The distinct pink color of their fillet is the result of deposition of xanthophyll carotenoid pigments (Storebakken et al., 1987). Wild fish obtain these pigments through their prey, such as krill and shrimp. In farmed salmonids, astaxanthin is supplemented, in synthetic or natural form, to obtain the desired fillet color profile (Lim et al., 2018). A comparison of various commercially available synthetic and natural astaxanthin products in rainbow trout showed that the coloring ability and physiological function differs between astaxanthin sources (Zhao et al., 2022). Also, the role of other nutrients, including lipids, on the retention and fillet deposition of dietary carotenoids remains unclear. A correlation between polyunsaturated fatty acid (PUFA) and pigment levels in salmon fillets indicates a nutrient interaction in the process of carotenoid fillet deposition, possibly related to their lipid soluble nature (Bjerkeng et al., 1999; Ytrestøyl et al., 2023). On the other hand, no significant effect by the replacement of fish oil with terrestrial oils on muscle astaxanthin content was reported in rainbow trout (Choubert et al., 2006).

Astaxanthin not only provides color, but also brings other benefits to the fish by functioning as a strong antioxidant (Kalinowski et al., 2019). Antioxidants are scavengers of free radicals and are required to maintain a cellular redox balance preventing oxidative stress. This is especially important to protect cellular compounds, including PUFA, from oxidation (Halliwell and Gutteridge, 2007). During any activity, free radicals are generated as a result of the consumption of oxygen for energy. Therefore, under exercised-induced stress the redox balance in the cell must be maintained through adaptations in the antioxidant system (Nikolaidis et al., 2012). Astaxanthin as an antioxidant aligns with the cell membrane helping to maintain its structure either through donation of electrons or bonding with free radicals forming non-reactive products.

The aim of this study was to assess a novel oil, derived from a genetically modified *Camelina sativa*, containing both n-3 LC-PUFA and astaxanthin, as a new raw ingredient for feeds given to juvenile rainbow trout over a period of 10 weeks. The deposition and dynamics of both astaxanthin and n-3 LC-PUFA were assessed in the flesh of rainbow trout. Additionally, athleticism and stress tolerance were tested in a swim tunnel experiment in response to the dietary treatment. Markers of the antioxidant metabolism were measured to identify possible interactions between the exhaustive exercise stress and the dietary treatment.

## 2. Material and methods

### 2.1. Experimental diets

Two diets were formulated to meet the macro- and micronutrient requirements of rainbow trout as defined by (NRC, 2011). The control diet (CTL) was formulated to represent a commercial standard containing a mix of fish and terrestrial oils. In the camelina oil diet (CAM), all oils and astaxanthin source (Carophyll Red, DSM) were replaced with the transgene-derived camelina oil containing EPA, DHA, and carotenoids (Table 1). The plant line containing these novel ingredients was generated by crossing the EPA and DHA line DHA2015.1 (Han et al., 2020) with a line accumulating ketocarotenoids (Xie et al., 2019). Seeds

**Table 1**

Formulation (% inclusion) and analyzed proximate composition (%) of the experimental diets.

	CTL	CAM
Fishmeal	17	17
Plant protein <sup>1</sup>	56.4	56.4
Fish oil	8.0	–
Rapeseed oil	6.0	–
Linseed oil	3.6	–
Palm oil	2.4	–
Camelina oil	–	20
Rapeseed lecithin	2.0	2.0
Vitamin premix <sup>2</sup>	1.0	1.0
Mineral premix <sup>3</sup>	3.6	3.6
Cellulose	–	0.05
Carophyll pink <sup>4</sup> (10 % astaxanthin)	0.05	–
Analyzed composition <sup>5</sup>		
Dry matter	95.5 ± 0.4	93.0 ± 0.2
Crude protein	45.5 ± 0.8	43.4 ± 1.1
Total lipid	24.8 ± 0.9	26.0 ± 1.5
Ash	6.8 ± 0.1	6.7 ± 0.0

<sup>1</sup> Protein mix: Wheat gluten (31.9 %), potato protein concentrate (30.1 %), soy protein concentrate (14.2 %) and whole wheat (23.8 %).

<sup>2</sup> Vitamin premix (per kg diet): retinyl acetate, 5000 IU; cholecalciferol, 2500 IU; DL- $\alpha$ -tocopheryl acetate, 100 IU; sodium menadione bisulfate, 10 mg; thiamin-HCl, 1 mg; riboflavin, 4 mg; niacin, 10 mg; D-calcium pantothenate, 20 mg; pyridoxine-HCl, 3 mg; myo-inositol, 0.3 g; D-biotin, 0.2 mg; folic acid, 1 mg; cyanocobalamin, 0.01 mg; L-ascorbyl-2-polyphosphate, 50 mg; choline-HCl, 1 g. BHA, 0.80 mg; BHT, 0.75 mg; sepiolite, 200 mg. All ingredients were diluted with calcium carbonate.

<sup>3</sup> Mineral premix (per kg diet): CaHPO<sub>4</sub>·2H<sub>2</sub>O (18 % P; 22 % Ca), 30.15 g; CaCO<sub>3</sub> (40 % Ca), 2.15 g; MgOH<sub>2</sub> (42 % Mg), 1.24 g; KCl (52 % K), 0.9 g; NaCl (39 % Na), 0.4 g; ZnSO<sub>4</sub>·H<sub>2</sub>O (36 % Zn), 140 mg; FeSO<sub>4</sub>·H<sub>2</sub>O (33 % Fe), 20 mg; MnO (77 %), 10 mg; NaF (45 % F), 10 mg; CuSO<sub>4</sub>·5H<sub>2</sub>O (25 % Cu), 5 mg; CaI<sub>2</sub> (86 % I), 3 mg; Na<sub>2</sub>SeO<sub>3</sub> (46 % Se), 0.25 mg; CoCO<sub>3</sub> (50 % Co), 0.05 mg; BHA, 0.75 mg; BHT, 0.75 mg; propyl gallate, 0.15 mg; sepiolite, 200 mg.

<sup>4</sup> DSM Firmenich, Netherlands.

<sup>5</sup> Average values of 6 feed samples collected over the course of the experiment.

from this genetic cross (designated CASX) were obtained from plants grown under permit (18/R8/01) on the Experimental Farm at Rothamsted Research (Harpenden, UK), and the oil was extracted and analyzed by New Holland Extractions Ltd. (Hull, UK) using their pilot-plant facility. Technical information provided by the manufacturer is included in the Supplementary Data (ST1 + ST2). Diets were produced in the INRAE experimental facilities (St.-Pee-sur-Nivelle, France) using a twin-screw extruder (45 BCE, Cleextral, Firminy, France) and vacuum-coated with the oils. Diet samples were collected at six time points over the course of the experiment to assess feed stability and analyzed as described below. Proximate composition, fatty acid profile and carotenoid levels were monitored in the feeds over the course of the experiment with average values displayed in Table 1 and Table 2, respectively.

Fatty acid profiles of the experimental diets revealed that total PUFA levels were higher in CAM compared to CTL (60 vs 39 % of total fatty acids). CAM also showed higher proportions of n-6 PUFAs but lower proportions of saturated and mono-unsaturated fatty acids (SFA and MUFA, respectively). The diets were formulated to contain similar levels of carotenoids (50 mg/kg) coming from either supplementation with synthetic astaxanthin in CTL or the transgene-derived camelina oil in CAM. Carotenoid analysis revealed that CAM had slightly lower total carotenoid levels compared to CTL (42 vs 46 mg/kg). In addition, the carotenoid profile of CAM was more complex with astaxanthin representing the major carotenoid in the diet, about 38 %, while in total 48 % of the peaks remained unidentified. Example chromatograms of both feeds are provided in supplement (SF1). The unidentified peaks possibly represent esterified astaxanthin compounds or other ketocarotenoids. In comparison, 84 % of total carotenoids in CTL were identified as

**Table 2**

Fatty acid and pigment profile of the experimental diets.

	CTL	CAM
Fatty acids [% of total]		
16:0	16.7 ± 0.2	9.0 ± 0.3
<b>Total SFA<sup>1</sup></b>	<b>25.1 ± 0.4</b>	<b>19.5 ± 0.3</b>
18:1n-9	27.1 ± 0.2	8.8 ± 0.1
<b>Total MUFA<sup>2</sup></b>	<b>35.5 ± 0.2</b>	<b>20.7 ± 0.1</b>
18:2n-6	15.8 ± 0.3	24.4 ± 0.3
20:4n-6	0.4 ± 0.0	1.8 ± 0.0
22:5n-6	0.1 ± 0.0	0.0 ± 0.0
<b>Total n-6 PUFA<sup>3</sup></b>	<b>16.4 ± 0.2</b>	<b>29.6 ± 0.2</b>
18:3n-3	10.2 ± 0.1	10.6 ± 0.1
20:5n-3	6.0 ± 0.2	6.9 ± 0.1
22:5n-3	0.8 ± 0.0	3.6 ± 0.1
22:6n-3	3.1 ± 0.1	5.3 ± 0.1
<b>Total n-3 PUFA<sup>4</sup></b>	<b>21.3 ± 0.4</b>	<b>30.1 ± 0.5</b>
<b>Total PUFA</b>	<b>39.3 ± 0.5</b>	<b>59.7 ± 0.3</b>
<b>22:6n-3/20:5n-3</b>	<b>0.5 ± 0.0</b>	<b>0.8 ± 0.0</b>
Carotenoids [mg/kg]		
Astaxanthin	39.1 ± 1.2	16.2 ± 1.9
Xanthophylls	0.9 ± 0.0	4.0 ± 0.6
Adonirubin/astacene	1.5 ± 0.2	1.9 ± 0.3
Other	5.0 ± 0.6	20.3 ± 1.9
<b>Total</b>	<b>46.5 ± 1.4</b>	<b>42.5 ± 4.1</b>

Average values of 6 feed samples collected over the course of the experiment presented as standard deviation.

<sup>1</sup> Saturated fatty acids (SFA): 14:0, 15:0, 16:0, 18:0, 20:0, 22:0, 24:0.

<sup>2</sup> Mono-unsaturated fatty acids (MUFA): 16:1n-9, 16:1n-7, 17:1, 18:1n-9, 18:1n-7, 20:1n-11, 20:1n-9, 20:1n-7, 22:1n-11, 22:1n-9, 24:1n-9.

<sup>3</sup> Total omega-6 polyunsaturated fatty acids (n-6 PUFA): 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.

<sup>4</sup> Total omega-3 polyunsaturated fatty acids (n-3 PUFA): 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3 22:6n-3.

astaxanthin (Table 2).

## 2.2. Fish husbandry, feeding and sampling

Farmed juvenile rainbow trout (*Oncorhynchus mykiss*), were purchased from a local farm (Leroy, Flatråker, Norway) and transported to the Institute of Marine Research in Matre (Masfjorden, Norway). Eight freshwater tanks (1 m<sup>2</sup>, 300 l each, 10 °C, 12 L:12D) in a flow-through system were allocated to the two experimental treatments in quadruplicate. Each holding tank was stocked with 30 fish at an initial body weight of 294 ± 32 g. The fish were fed with the experimental diets continuously (see 2.1) in excess by automatic feeders for 12 h a day from 08:00 am to 08:00 pm during the light period. The feeding trial lasted for 10 weeks. After 6 weeks, data on intermediate growth were collected through bulk weighing of fish in each tank. At the end of the feeding trial after 10 weeks, sampling was done tank-wise. Feeding in a tank was stopped 24 h before the respective tank was sampled. During the sampling, all fish per tank were measured for body weight and length. Ten fish per tank were immediately killed by an overdose of metacaine sulphate (Finquel, 500 mg/l). Blood was collected through the caudal vein before organ samples were dissected in non-exercised and exercised fish. Blood samples were centrifuged immediately during the sampling (3000 g, 4 °C, 3 min) and the plasma supernatant stored at −80 °C. Liver and viscera weight were recorded. Muscle fillet was collected using the Norwegian quality cut. The organ samples were immediately placed on dry ice and stored at −20 °C until further analysis. A sub-section of the liver was preserved in RNAlater™ (Thermo Fisher Scientific, UK) and placed in a fridge overnight before frozen. Meanwhile, another six fish per tank were slightly sedated (Finquel, 100 mg/l) and transferred into a swim tunnel setup for a critical swim speed trial the following day as described below.

## 2.3. Swim tunnel setup and test protocol

To assess swimming capacities and stress response to exhaustive exercise in rainbow trout fed the two experimental diets, a large Brett-type swim tunnel system was used previously described in detail (Remen et al., 2016). Briefly summarized, the 252 cm<sup>3</sup> cylindrical swim section had a camera mounted downstream to allow remote observation of the fish. A steady water supply was ensured through a 2000 l holding tank. Current speeds were created using a motor driven propeller (Flygt 4630, 11° propeller blade, Xylem Water Solutions, Norway) calibrated by a flow meter (Höntzsch Flow Measuring Technology).

To prepare for a swim trial, the velocity in the swim tunnel was set to a low current speed of 20 cm s<sup>−1</sup> to acclimatize the fish grouped by tank origin overnight. The next morning, at onset of light, fish in the swim tunnel were exercised by increasing the current speed every 20 min by 10 cm s<sup>−1</sup>. At each time interval, tail beat frequencies were measured by timing tail beat counts six independent times and in different fish, if possible. The swim trial continued until all fish had reached fatigue. Fatigue was defined when a fish was no longer able to maintain continuous swimming effort. Once deemed fatigue, fish were removed from the tunnel and time was recorded. The fish were then euthanized and sampled as described above (see 2.2).

## 2.4. Proximate, fatty analysis and TBARS

Determination of dry matter in the experimental diets was done through drying in an oven at 110 °C overnight (AOAC, 2000). Crude protein was determined through the Kjeldahl method in a nitrogen analyzer (LiquidLine Kjeldahl analyzer and autosampler, Opsis, Sweden) (AOAC, 2006). Extraction of total lipids was done on pooled flesh samples ( $n = 10$  fish in pre-exercise and  $n = 6$  fish in exercised treatment) (Folch et al., 1957). Therefore, one gram of sample was homogenized with an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK) in 20 ml of 2:1 (by vol.) chloroform/methanol. Afterwards, the lipid layer was separated by addition of 5 ml 0.88 % (w/v) KCl and resting for 1 h on ice. The lower lipid layer was evaporated under a stream of oxygen-free nitrogen and the lipid content determined gravimetrically after drying in a desiccator overnight. Determination of the fatty acid profile was done following (Christie, 1996) as previously described (Betancor et al., 2017b). Briefly, gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) was used to separate fatty acid methyl esters on a 30 m × 0.32 mm capillary column (CP-Wax 52CB, Chrompak, London, UK) using hydrogen as carrier gas. The column injection was done at 50 °C following 50 to 150 °C at 40 °C min<sup>−1</sup> and then 230 °C at 2.0 °C min<sup>−1</sup>. Thiobarbituric acid reactive substances (TBARS) were analyzed in 1 g of pooled flesh samples ( $n = 10$  fish in pre-exercise and  $n = 6$  fish in exercised treatment) as previously described (Wischhusen et al., 2024). Briefly, the samples were homogenized in 7.5 % trichloroacetic acid solution before filtered (110 nm, Whatman No1 filter paper) and 1 ml 0.02 M thiobarbituric acid solution was added to the filtrate. After, the samples were heated in a water bath to 100 °C for 35 min, precipitates were removed through centrifugation (6 min, 13,226 g) and the extracts read at 532 nm in a spectrophotometer (UV-1900i spectrophotometer, Shimadzu, Japan).

## 2.5. Plasma analysis

The plasma osmolality was measured in thawed samples by freeze point determination (Fiske 210 Micro-Sample Osmometer, Advanced Instruments). The concentration of plasma lactate, glucose, Na<sup>+</sup>, and Cl<sup>−</sup> were measured using an ABL90 FLEX blood gas analyzer (Radiometer), while plasma cortisol was measured with an ELISA assay kit (standard range 20 to 800 ng ml<sup>−1</sup>, IBL International GmbH).

To extract isoprostanes, a modified method based on Ostermann et al. (2015) and Dupuy et al. (2016) was used: 10 µl of 1.4 pmol/µl d4 8-iso prostaglandin F2a and d4 8-iso prostaglandin E2 standard were

added to 400 µl of plasma, followed by 50 µl of 0.2 mg/ml BHT in methanol and 800 µl of 1 M methanolic potassium hydroxide solution. The samples were then vortexed and heated at 40 °C for 30 min followed by centrifugation (10 min, 17,000 g, 10 °C). After, the supernatant was mixed with 800 µl of 1 M HCl, 1200 µl acetic acid solution (320 mM, pH 4) and 1000 µl distilled water. Then, the samples were centrifuged (2 min, 3400 g) to sediment solids and the supernatant passed through a 0.5 g SepPak tC18 SPE cartridge from where isoprostanes were eluted with 1 ml of methyl formate. The samples were dried under nitrogen before resuspended in 40 µl of 50:50 methanol/water solution, centrifuged (2 min, 17,000 g) and the supernatant used for LC-MS/MS analysis. Lipid mediators were analyzed using an internal standard mixture containing 11 deuterated standards at 1.5 pmol/µl, respectively. First, 10 µl of standard, 0.2 mg/ml BHT in methanol and 1200 µl ice-cold methanol was added to 400 µl plasma. The samples were then vortexed and incubated on ice for 15 min before being centrifuged (10 min, 17,000 x g, 4 °C). The supernatant was dried under vacuum centrifugal evaporation (2 h, 41 °C) and re-dissolved in 800 µl of 90:10 water/methanol solution, acidified with 32 µl of pure acetic acid before passed through a 0.5 g SepPak tC18 SPE cartridge and further processed as detailed above for isoprostane extraction. Isoprostane and lipid mediators were both analyzed on a Waters Xevo TQ-S, with a Waters I class UPLC system (Acquity BEH C18 column, 2.1 × 100 mm; injection: 15 µl; solvent A: water/acetonitrile (80:20 v/v) and formic acid (0.02 % v/v); solvent B: acetonitrile/isopropyl alcohol (50:50 v/v)). The following gradient was used: 100 % A, 0.5 min; ramped linearly to 55 % solvent B, 7 min; 100 % solvent B, 3 min; 4 min hold; return to 100 % solvent A over 3 min and conditioned for a further 2 min. The TQ-S was run in negative mode, with a capillary voltage of -3 kV, desolvation temperature of 300 °C, a desolvation gas flow rate of 800 l/h and a cone gas flow rate of 150 l/h. The system was run in MRM mode, with transitions detailed in supplementary table ST3.

## 2.6. Carotenoids and flesh color

Pigments were extracted as described below using a method based on Rodriguez-Amaya (2001), Rodriguez-Amaya and Kimura (2004), Schierle et al. (2008), Weber (1990). To extract pigments from feed, 100 µl of the protease Protex 6 L (Genecor International, NY, USA), 25 mg BHT and 1.5 ml of distilled water was mixed with 0.75 g of grounded feed before the samples were placed in an ultrasonic water bath (50 °C; 30 min). Then, 10 ml of ethanol and 12.5 ml of dichloromethane were added while shaking in-between. The samples were left in the dark overnight to cool down before diluted to volume with dichloromethane. Following, 5 ml from the supernatant was taken and evaporated under nitrogen to dryness before re-suspended in a known volume of isohexane:acetone (82:18 v/v) and analyzed by HPLC (Nexera HPLC (LC-40D), Shimadzu, Japan, column: Roc Silica, 5 µm, 150 × 4.6 mm; mobile phase: isohexane:acetone (82:18 v/v); injection volume: 50 µl; absorbance: 470 nm). Muscle samples (1 g, pooled per tank) were homogenized in 10 ml ethyl acetate:ethanol (1:1 v/v) before centrifuged (3000 g; 5 min), washed with 5 ml of ethyl acetate and 5 ml of isohexane, respectively, and supernatant collected. The supernatant was then dried under nitrogen and left in the desiccator in darkness overnight before the samples were re-suspended in 5 ml isohexane and measured by HPLC (same settings as for feed).

To measure fillet color, homogenized pooled flesh samples ( $n = 10$  fish in pre-exercise and  $n = 6$  fish in exercised treatment) were spread into a petri dish to achieve samples of similar shape. Then, the petri dish was placed in a photo box having a LED light ring at the top to create standardized light conditions. A colorimeter (CSM4, PCE Instruements, UK) and a digital SalmoFan™ (DSM-Firmenich, Netherlands) were directly placed on the surface of the homogenized fillet samples to obtain color values. In addition, a picture of the homogenized fillet samples was taken from the top of the photo box using a 12-megapixel camera (iPhone 13 mini, Apple). Image processing was done in

Phyton to convert the RGB color space of the picture to CIELAB using the `skimage.color.rgb2lab` function of the `scikit-image` library.

## 2.7. Gene expression

Total RNA was extracted from 100 mg liver tissue using 1 ml of TRIzol™ (Invitrogen, Thermo Fisher Scientific, UK). Quality and quantity of RNA was checked by running 200 ng of total RNA on a 1 % agarose gel complemented by Nanodrop ND-1000 (Labtech Int., East Sussex, UK) and Qubit RNA IQ Assay via Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Prior to reverse transcription (High-Capacity cDNA Reverse Transcription kit, Applied Biosystems, REF4368813, Warrington, UK with random primers) 6 µg total RNA of two samples per tank were pooled to obtain a total of 6 samples per treatment. Quantitative real-time PCR (qPCR, Biomtra TOptical Gradient 96 Real-Time PCR Thermal Cycler, Göttingen, Germany) was done in a total 10 µl reaction volume comprising of 5 µl Luminaris Color HiGreen qPCR Master Mix (Thermofisher Scientific Waltham, MA, USA), 1.5 µl of nuclease-free water, 2.5 µl of the cDNA template and 0.5 µl of forward and reverse primer, respectively. The utilized primers are listed in Table 3.

The amplification condition was 120 s at 50 °C, 600 s at 95 °C followed by 40 cycles of 15 s at 95 °C and 30 s at the primer's specific annealing temperatures. All melting curves were systematically screened for quality control. The relative expression of target genes was calculated using geometric means of the housekeeping genes *ef1α* and *β-actin* by  $\Delta\Delta CT$  method on the average of the control treatment CTL sampled prior to the stress challenge.

## 2.8. Formulas

The daily weight gain (DWG) of the fish over the course of the experiment was determined using the following equation:

$$DWG [\%] = \left( \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \right) * \frac{100}{\text{number of days}}$$

While the specific growth rate (SGR) was determined as follows:

$$SGR = \frac{(\ln(\text{final weight}) - \ln(\text{initial weight})) * 100}{\text{number of days}}$$

The weight (W) – length (L) relationship of a fish is expressed through the condition factor (CF) using the following formula:

$$CF = \frac{W \times 100}{L^3}$$

The viscera and hepatosomatic index (VSI and HSI) were calculated as a ratio of total body weight:

$$SI = \frac{\text{Organ weight}}{\text{Total body weight}} \times 100$$

The critical swimming speed ( $U_{crit}$ ) is a standardized test of the prolonged swimming capacity in fish and was calculated according to (Brett, 1964). In which  $U_f$  represents the water velocity of the last completed velocity increment,  $t_f$  is the time spent at the last velocity increment,  $t_i$  is the time spent at each velocity and  $U_i$  is the magnitude of the velocity increment:

$$U_{crit} = U_f + \frac{t_f \times U_i}{t_i}$$

The difference (dE) between two colors ( $L^*, a^*, b^*$ )<sub>p</sub> and ( $L^*, a^*, b^*$ )<sub>v</sub> in the CIELAB color space was calculated using the following equation:

$$dE = \sqrt{(L_p^* - L_v^*)^2 + (a_p^* - a_v^*)^2 + (b_p^* - b_v^*)^2}$$

The color difference was calculated between a baseline fillet sample collected prior to the beginning of the feeding period and the fillets



**Table 3**  
Oligonucleotide primers used in RT-qPCR to assay mRNA abundance.

Gene	Forward	Reverse	Tm	Efficiency	Reference
<i>fads2a</i> (Δ6)	TAGTTTCCCGACGCTTTGTGTC	CCAAAAATGATTAAACAATTACG	57	1.82	NM_001124287
<i>elovl5</i>	CTATGGGCTCTCTGCTGTCC	TATCGTCTGGGACATGGTCA	57	1.90	AY605100
<i>elovl2</i>	GATGCCTGCTCTTCCAGTTC	CATTGGTGAGACAGTGTGG	57	2.00	KM244737
<i>gpx1a</i>	AATGTGGCGTCACTCTGAGG	CAATTCTCCTGATGGCCAAA	57	1.99	HE687021
<i>gpx4a1</i>	GAAAGGCTTCTCTGGGAAATG	CTCCACCACACTGGGATCAT	57	1.99	HE687024
<i>gstp</i>	TCGCTGACTGGACGAAAGGA	CGAAGGTCCTCAACGCCATC	60	1.97	BX302932.3
<i>gr</i>	CTAAGCGCAGCGTCATAGTG	ACACCCCTGCTGACGACAT	56	1.85	HF969248.1
<i>gclc1</i>	AGGCCAGAGTATGGCAGCTA	CAGCCTAACCTTGGGAATGA	56	1.92	GS0NMT00071788001
<i>ef1a</i>	TCCTCTTGGTCTGTTTCGCTG	ACCCGAGGGACATCCTGTG	56	2.02	AF498320.1
<i>β-actin</i>	GATGGGCCGAAAGACAGCTA	TCGTCCCGTGGTGACGAT	56	1.97	AJ438158.1

*fads2*, fatty acid desaturase 2; *elovl*, fatty acid elongase; *gpx*, glutathione peroxidases; *gstp*, glutathione-s-transferases pi; *gr*, glutathione reductase; *gclc*, glutamate-cysteine ligase catalytic subunit; *ef1a*, alpha subunit of the elongation factor-1; *β-actin*, beta-actin.

collected from fish at final sampling.

## 2.9. Statistical analysis

The results are presented as average  $\pm$  standard deviation. All statistical analyses were performed using R (R Core Team, 2024). A statistical significance was assumed at  $p < 0.05$  in all analyses. A Kaplan-Maier equation followed by a log-rank test was applied to compare the fatigue probability of fish of the two dietary treatments over time in the swim tunnel (R, survminer, version 0.4.9 and survival, version 3.3–1). Tail beat count data followed a Poisson distribution and a generalized linear model with Poisson family was applied. Following, a non-linear regression was used to model tail beat count over current speed. Linear regression was used to model Ucrit and muscle EPA/DHA levels. A heatmap of color distance was created using the pipeline provided by the R package colordistance, version 1.0.1. Otherwise, statistical differences were tested by *t*-test and two-way ANOVA or by Kruskal-Wallis as non-parametric alternative when data were not normally distributed and had unequal variance. Significant differences between groups were further elaborated using Tukey's HSD postdoc test.

## 3. Results

### 3.1. Growth performance during the feeding trial

Fish fed CAM had a significantly lower body weight compared to fish fed CTL at the first intermediate weighing (Table 4). However, none of the biometric measures including final body weight, body length and condition factor were significantly different between the two groups at the end of the 10-week period (Table 4).

**Table 4**

Biometric measures of body weight [g], body length [cm], condition factor, daily weight gain [%], specific growth rate and hepato- and viscera somatic index in rainbow trout fed a diet either with a mix of traditional vegetable oils and fish oil (CTL) or a transgenic camelina oil (CAM) over a 10-week period.

	CTL	CAM	p-value
Body weight, start	298 $\pm$ 31	291 $\pm$ 33	0.12
Body length, start	27 $\pm$ 1	26 $\pm$ 1	0.15
Condition factor, start	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1	0.66
Body weight, intermediate	484 $\pm$ 4 <sup>a</sup>	452 $\pm$ 8 <sup>b</sup>	<0.01
Body weight, final	537 $\pm$ 106	513 $\pm$ 91	0.06
Body length, final	33 $\pm$ 2	33 $\pm$ 2	0.22
Condition factor, final	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	0.06
Daily weight gain	1.4 $\pm$ 0.1	1.3 $\pm$ 0.1	0.13
Specific growth rate	1.0 $\pm$ 0.0	1.0 $\pm$ 0.1	0.09
Hepatosomatic index	1.0 $\pm$ 0.2	1.1 $\pm$ 0.2	0.15
Viscera somatic index	10.8 $\pm$ 1.2	11.0 $\pm$ 1.0	0.52

Mean  $\pm$  standard deviation. Statistical analysis as *t*-test (start  $n = 100$ ; intermediate, daily weight gain and specific growth rate  $n = 4$ ; others  $n = 76$ ). Superscript letters indicate significant differences between groups.

### 3.2. Swimming performance

In the swim tunnel, the number of tail beats increased with increasing current speed following a non-linear relationship by 2nd order polynomial. The maximum number of tail beats per second was recorded at current speeds above 110 cm s<sup>-1</sup> in both dietary treatments (Fig. 1A). The fatigue probability of exercised fish was not significantly affected by the dietary treatment over time (Fig. 1B). Similarly, the absolute Ucrit with 103  $\pm$  3 in CAM and 101  $\pm$  3 in CTL and the relative Ucrit with 14.4  $\pm$  0.4 in CAM and 10.9  $\pm$  0.3 in CTL was not significantly different between dietary groups (Fig. 1C). However, Ucrit correlated with the EPA and DHA levels measured in the muscle tissue of fish. The data showed a linear relationship between Ucrit and EPA/DHA across both dietary groups having a correlation coefficient of 0.64 (Fig. 1D). On the other hand, no correlation was found between Ucrit and muscle AST (correlation coefficient < 0.20).

### 3.3. Fatty acid profile of rainbow trout fillet

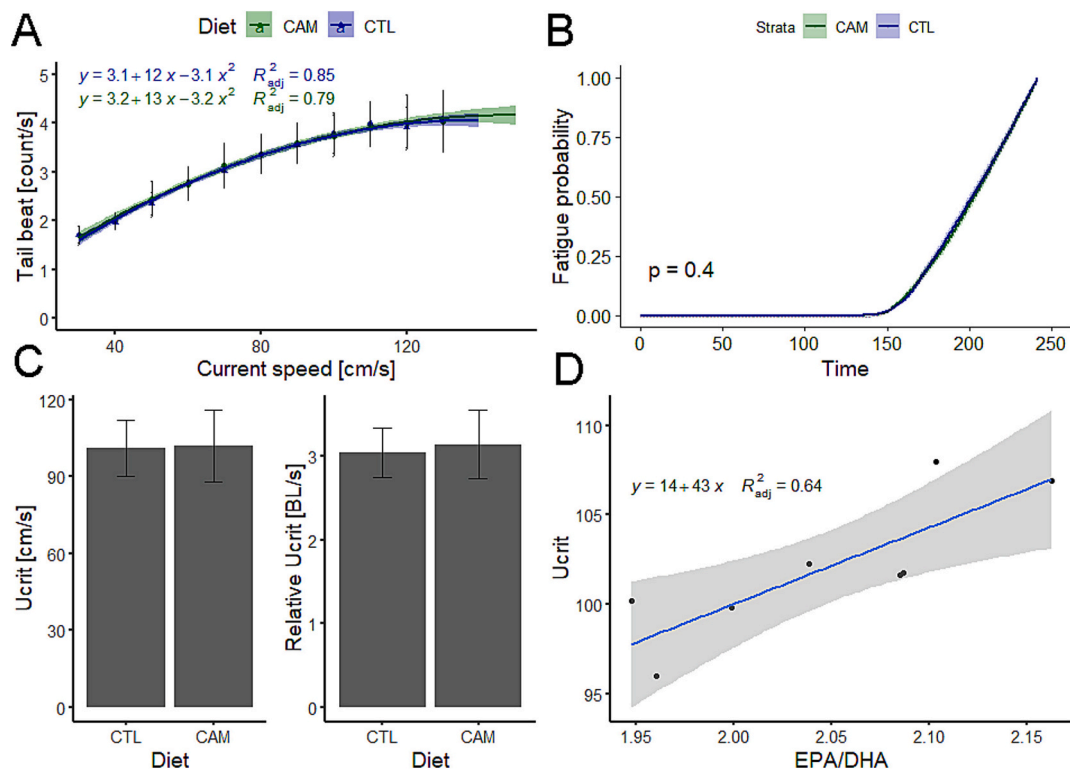
The total lipid content in the rainbow trout fillet was not significantly different between the two dietary groups (average = 5.7 %; Table 5). In general, the fatty acid profile of the rainbow trout fillet reflected the fatty acid profile of the two experimental diets. Fish fed CTL had higher proportions of SFAs and MUFAs compared to fish fed CAM. On the other hand, fish fed CAM had significantly higher fillet PUFA levels (Table 5). A standard portion of 140 g rainbow trout fillet would provide 260 mg of EPA and 550 mg of DHA when the fish were fed CTL, but 343 mg of EPA and 682 mg of DHA when the fish were fed CAM (supplementary data; ST4).

The swim tunnel exercise had no significant impact on the fatty acid composition of rainbow trout fillet (Table 5). TBARS levels were not significantly different in the fillets of fish fed CTL or CAM and sampled before or after the swim tunnel exercise (Table 5).

### 3.4. Plasma levels of lipid oxidative products

Fish fed CAM displayed higher plasma levels of the eicosanoids 13-HOTrE and 15-HETrE derived from linoleic acid as well as 5-HETE, 12-HETE, 15-HETE and 5,15-di-HETE derived from arachidonic acid and 5-HEPE, 12-HEPE and 15-HEPE derived from EPA compared to fish fed CTL (Table 6). PGE2 was only detected in fish plasma after the exercise stress and was also higher in fish fed CAM compared to CTL. The levels of 8-isoprostanes were significantly higher in fish fed CAM compared to CTL. Dietary treatment had no significant effect on eicosanoid levels derived from DHA including 17-HpDHA, 14-HDHA, 17-HDHA and protectin D1.

Exercised fish showed higher plasma levels of 13-HOTrE, 13-HODE, 5,15-diHETE, PGE2 and protectin D1 compared to fish before exercise (Table 6). Also, 8-isoprostane levels were significantly higher in plasma of stressed fish compared to fish sampled prior to the exercise challenge.



**Fig. 1.** Performance of rainbow trout exercised in a swim tunnel after fed two experimental diets formulated on a mix of traditional vegetable oils and fish oil (CTL) or a transgenic camelina oil (CAM) over a 10-week period: (A) Tail beat frequency at increasing current speed; (B) Model of fatigue probability of exercised fish over time; (C) Absolute and relative Ucrit of fish in the swim tunnel; (D) Linear regression model of Ucrit and EPA/DHA ratio in the muscle tissue of fish.  $N = 24$  per treatment in A, B and C and  $n = 8$  in D.

**Table 5**

Lipid content (%), fatty acid profile (% fatty acids) and TBARS (mmol/g) level in rainbow trout fillet sampled from fish fed the experimental diets either containing a mix of traditional vegetable oils and fish oil (CTL) or a transgenic camelina oil (CAM) over a 10-week period before and after subjected to exercise stress.

	Main effects				Treatment effects				P-value		
	Diet		Exercise		Before exercise		After exercise		D		
	CTL	CAM	Before	After	CTL	CAM	CTL	CAM	D	E	DxE
Total lipid	5.4 ± 0.3	5.6 ± 0.5	5.7 ± 0.4	5.4 ± 0.4	5.5 ± 0.3	5.9 ± 0.4	5.4 ± 0.4	5.4 ± 0.4	0.34	0.13	0.29
16:0	15.1 <sup>a</sup> ± 0.2	12.4 <sup>b</sup> ± 0.3	13.7 ± 1.4	13.8 ± 1.5	15.0 <sup>a</sup> ± 0.1	12.4 <sup>b</sup> ± 0.3	15.2 <sup>a</sup> ± 0.1	12.4 <sup>b</sup> ± 0.3	<0.01	0.21	0.57
Total SFA	22.9 <sup>a</sup> ± 0.2	20.5 <sup>b</sup> ± 0.5	21.6 ± 1.3	21.8 ± 1.4	22.8 <sup>a</sup> ± 0.1	20.4 <sup>b</sup> ± 0.5	23.1 <sup>a</sup> ± 0.2	20.6 <sup>b</sup> ± 0.4	<0.01	0.21	0.65
18:1n-9	29.1 <sup>a</sup> ± 0.5	18.8 <sup>b</sup> ± 0.7	24.0 ± 5.6	23.9 ± 5.5	29.2 <sup>a</sup> ± 0.5	18.8 <sup>b</sup> ± 1.0	29.1 <sup>a</sup> ± 0.5	18.7 <sup>b</sup> ± 0.4	<0.01	0.79	0.94
Total MUFA	39.7 <sup>a</sup> ± 0.7	30.4 <sup>b</sup> ± 0.9	35.1 ± 5.2	35.0 ± 5.0	39.9 <sup>a</sup> ± 0.8	30.4 <sup>b</sup> ± 1.28	39.6 <sup>a</sup> ± 0.8	30.4 <sup>b</sup> ± 0.6	<0.01	0.75	0.78
18:2n-6	12.3 <sup>b</sup> ± 0.1	17.0 <sup>a</sup> ± 0.3	14.7 ± 2.6	14.5 ± 2.5	12.3 <sup>b</sup> ± 0.0	17.1 <sup>a</sup> ± 0.2	12.2 <sup>b</sup> ± 0.1	16.8 <sup>a</sup> ± 0.4	<0.01	0.11	0.44
20:4n-6	0.5 <sup>b</sup> ± 0.1	1.3 <sup>a</sup> ± 0.1	0.9 ± 0.5	0.9 ± 0.4	0.5 <sup>b</sup> ± 0.0	1.3 <sup>a</sup> ± 0.1	0.5 <sup>b</sup> ± 0.1	1.3 <sup>a</sup> ± 0.0	<0.01	0.41	0.66
22:5n-6	0.2 <sup>a</sup> ± 0.0	0.1 <sup>b</sup> ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 <sup>a</sup> ± 0.0	0.1 <sup>b</sup> ± 0.0	0.2 <sup>a</sup> ± 0.0	0.1 <sup>b</sup> ± 0.0	<0.01	0.06	0.42
Total n-6 PUFA	14.1 <sup>b</sup> ± 0.1	21.5 <sup>a</sup> ± 0.5	17.9 ± 4.1	17.7 ± 3.9	14.1 <sup>b</sup> ± 0.1	21.6 <sup>a</sup> ± 0.4	14.0 <sup>b</sup> ± 0.1	21.3 <sup>a</sup> ± 0.5	<0.01	0.26	0.40
18:3n-3	6.8 <sup>b</sup> ± 0.1	7.1 <sup>a</sup> ± 0.2	6.9 ± 0.2	6.9 ± 0.2	6.8 <sup>b</sup> ± 0.1	7.1 <sup>a</sup> ± 0.24	6.7 <sup>b</sup> ± 0.2	7.0 <sup>a</sup> ± 0.1	<0.01	0.38	0.82
20:3n-3	0.3 <sup>b</sup> ± 0.0	0.8 <sup>a</sup> ± 0.0	0.5 ± 0.3	0.5 ± 0.3	0.3 <sup>b</sup> ± 0.0	0.8 ± 0.04 <sup>a</sup>	0.3 <sup>b</sup> ± 0.0	0.8 <sup>a</sup> ± 0.0	<0.01	0.27	0.51
20:5n-3	4.0 <sup>b</sup> ± 0.2	4.9 <sup>a</sup> ± 0.2	4.4 ± 0.5	4.5 ± 0.5	4.0 <sup>b</sup> ± 0.2	4.9 ± 0.24 <sup>a</sup>	4.1 <sup>b</sup> ± 0.2	5.0 <sup>a</sup> ± 0.2	<0.01	0.39	0.84
22:5n-3	1.4 <sup>b</sup> ± 0.1	2.6 <sup>a</sup> ± 0.2	2.0 ± 0.7	2.0 ± 0.6	1.4 <sup>b</sup> ± 0.1	2.6 <sup>a</sup> ± 0.22	1.4 <sup>b</sup> ± 0.1	2.5 <sup>a</sup> ± 0.1	<0.01	0.69	0.51
22:6n-3	8.4 <sup>b</sup> ± 0.3	9.8 <sup>a</sup> ± 0.4	9.0 ± 0.8	9.1 ± 0.9	8.4 <sup>b</sup> ± 0.3	9.6 <sup>a</sup> ± 0.5	8.4 <sup>b</sup> ± 0.3	9.9 ± 0.3 <sup>a</sup>	<0.01	0.56	0.48
Total n-3 PUFA	22.6 <sup>b</sup> ± 0.7	27.5 <sup>a</sup> ± 1.0	25.0 ± 2.7	25.1 ± 2.7	22.6 <sup>b</sup> ± 0.7	27.4 <sup>a</sup> ± 1.3	22.6 <sup>b</sup> ± 0.7	27.6 ± 0.7 <sup>a</sup>	<0.01	0.81	0.84
Total PUFA	37.4 <sup>b</sup> ± 0.8	49.1 <sup>a</sup> ± 1.3	43.3 ± 6.5	43.2 ± 6.3	37.4 <sup>b</sup> ± 0.8	49.2 <sup>a</sup> ± 1.8	37.3 <sup>b</sup> ± 0.9	49.0 <sup>a</sup> ± 0.9	<0.01	0.88	0.94
22:6n-3/20:5n-3	2.1 <sup>a</sup> ± 0.0	2.0 <sup>b</sup> ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.1 <sup>a</sup> ± 0.0	2.0 <sup>b</sup> ± 0.0	2.1 <sup>a</sup> ± 0.0	2.0 <sup>b</sup> ± 0.1	<0.01	0.43	0.29
TBARS	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.04	0.2 ± 0.0	0.2 ± 0.0	0.35	0.11	0.75

Mean ± standard deviation. Superscript letters indicate significant differences of main effects as obtained by two-way ANOVA ( $n = 4$  pooled samples per treatment; D = Diet, E = Exercise). Following, a Tukey's HSD post hoc test was used to find differences between groups (treatment effect).

Lipid mediators in plasma derived from arachidonic acid and EPA showed high correlation with the levels of their respective precursor fatty acid measured in the muscle tissue of fish (Fig. 2B and C). However, there was no correlation between muscle arachidonic acid and plasma 8-isoprostane levels (Fig. 2B). Lipid mediators in plasma derived from DHA showed only a weak correlation with muscle DHA levels (Fig. 2D). Similarly, 9-HODE and 13-HODE showed no correlation to linoleic acid,

while 13-HOTrE and 15-HETrE were moderately correlated with linoleic acid (Fig. 2A).

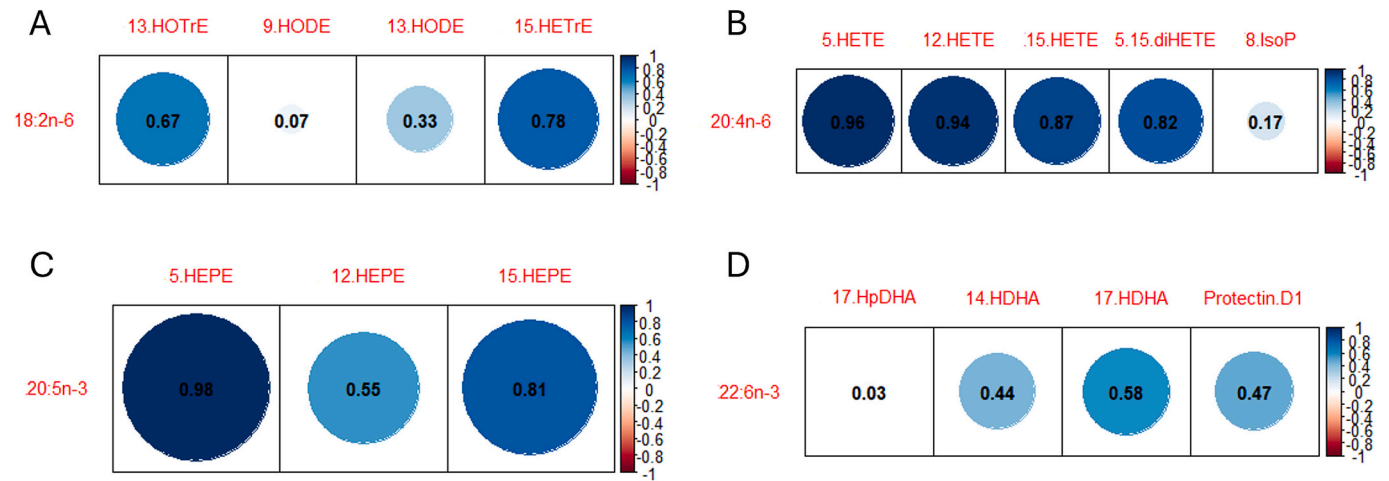
### 3.5. Other plasma parameters

Plasma ion levels including  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Cl^-$  remained unaffected by the dietary treatment (Fig. 3). Similarly, plasma glucose, lactate and

**Table 6**  
Eicosanoid and 8-isoprostane levels [pmol/ml] detected in plasma of rainbow trout fed the experimental diets either containing a mix of traditional vegetable oils and fish oil (CTL) or a transgenic camelina oil (CAM) over a 10-week period before and after subjected to exercise stress.

	Main effect				Treatment effect				P-value		
	Diet		Exercise		Before exercise		After exercise		D	E	DxE
	CTL	CAM	Before	After	CTL	CAM	CTL	CAM			
LA, 18:2 n-6											
13-HOTrE	30 <sup>b</sup> ± 17	58 <sup>a</sup> ± 24	37 <sup>b</sup> ± 25	52 <sup>a</sup> ± 23	25 <sup>c</sup> ± 19	48 <sup>ab</sup> ± 25	36 <sup>bc</sup> ± 13	68 <sup>a</sup> ± 20	<0.01	0.01	0.48
9-HODE	29 ± 10	31 ± 10	29 ± 10	31 ± 13	28 ± 11	29 ± 14	30 ± 9	33 ± 12	0.57	0.32	0.17
13-HODE	208 ± 102	252 ± 105	184 <sup>b</sup> ± 98	276 <sup>a</sup> ± 85	165 <sup>b</sup> ± 99	203 <sup>ab</sup> ± 97	252 <sup>ab</sup> ± 88	301 <sup>a</sup> ± 79	0.11	<0.01	0.83
15-HETrE	0.9 <sup>b</sup> ± 1.4	4.9 <sup>a</sup> ± 3.8	3.1 ± 4.2	2.7 ± 2.7	1.1 <sup>b</sup> ± 1.9	3.8 <sup>a</sup> ± 2.5	0.6 <sup>b</sup> ± 0.6	4.7 <sup>a</sup> ± 2.3	<0.01	0.77	0.24
ARA, 20:4 n-6											
5-HETE	6.5 <sup>b</sup> ± 2.4 <sup>b</sup>	30 <sup>a</sup> ± 10 <sup>a</sup>	20 ± 16	17 ± 12	6.9 <sup>b</sup> ± 2.7	33 <sup>a</sup> ± 12	6.2 <sup>b</sup> ± 2.0	27 <sup>a</sup> ± 7.0	<0.01	0.11	0.20
12-HETE	31 ± 13 <sup>b</sup>	99 ± 36 <sup>a</sup>	63 ± 46	66 ± 42	28 <sup>b</sup> ± 15	99 <sup>a</sup> ± 37	34 <sup>b</sup> ± 10	98 <sup>a</sup> ± 37	<0.01	0.76	0.65
15-HETE	5.7 <sup>b</sup> ± 3.0	27 <sup>a</sup> ± 14	17 ± 17	16 ± 12	5.5 <sup>b</sup> ± 3.6	25 <sup>a</sup> ± 12	6.0 <sup>b</sup> ± 2.4	26 <sup>a</sup> ± 7.9	<0.01	0.76	0.95
5,15-diHETE	2.9 <sup>b</sup> ± 1.3	5.8 <sup>a</sup> ± 3.6	3.5 <sup>b</sup> ± 2.0	5.3 <sup>a</sup> ± 3.6	2.4 <sup>b</sup> ± 0.8	4.6 <sup>ab</sup> ± 2.3	3.5 <sup>b</sup> ± 1.5	7.1 <sup>a</sup> ± 4.2	<0.01	0.02	0.36
PGE2	0.5 <sup>b</sup> ± 0.6	1.3 <sup>a</sup> ± 2.2	n.d.	1.7 ± 2.0	n.d.	n.d.	1.0 <sup>b</sup> ± 0.5	2.5 <sup>a</sup> ± 2.5	0.04	<0.01	0.04
8-isoprostane	1.9 <sup>b</sup> ± 1.1	2.5 <sup>a</sup> ± 1.3	1.8 <sup>b</sup> ± 1.3	2.6 <sup>a</sup> ± 1.1	1.7 <sup>b</sup> ± 1.4	1.9 <sup>ab</sup> ± 1.4	2.1 <sup>ab</sup> ± 0.9	3.1 <sup>a</sup> ± 1.1	0.03	0.01	0.78
EPA, 20:5 n-3											
5-HEPE	20 <sup>b</sup> ± 8	57 <sup>a</sup> ± 21	39 ± 23	38 ± 26	22 <sup>b</sup> ± 9	56 <sup>a</sup> ± 18	19 <sup>b</sup> ± 7	57 <sup>a</sup> ± 24	<0.01	0.87	0.74
12-HEPE	6.2 <sup>b</sup> ± 7.6	11.2 <sup>a</sup> ± 9.4	10.1 ± 11.3	7.3 ± 5.3	4.7 <sup>b</sup> ± 5.3	9.9 <sup>a</sup> ± 6.1	5.0 <sup>b</sup> ± 2.7	9.6 <sup>a</sup> ± 6.3	<0.01	0.98	0.86
15-HEPE	16 <sup>b</sup> ± 13	35 <sup>a</sup> ± 19	26 ± 22	25 ± 15	16 <sup>b</sup> ± 17	35 <sup>a</sup> ± 22	15 <sup>b</sup> ± 8	35 <sup>a</sup> ± 15	<0.01	0.88	0.97
DHA, 22:6 n-3											
17-HpDHA	13 ± 7	15 ± 9	13 ± 7	15 ± 9	13 ± 8	13 ± 6	13 ± 7	18 ± 10	0.25	0.28	0.24
14-HDHA	22 ± 24	26 ± 23	27 ± 32	21 ± 10	15 ± 13	23 ± 14	21 ± 12	20 ± 8	0.33	0.62	0.20
17-HDHA	27 ± 26	35 ± 26	36 ± 35	27 ± 11	20 ± 13	33 ± 18	25 ± 14	28 ± 8	0.06	0.98	0.18
Protectin D1	1.8 ± 1.4	1.8 ± 1.6	1.1 <sup>b</sup> ± 0.9	2.4 <sup>a</sup> ± 1.7	1.0 <sup>b</sup> ± 0.9	1.3 <sup>ab</sup> ± 0.9	2.6 <sup>a</sup> ± 1.4	2.3 <sup>ab</sup> ± 1.9	0.96	<0.01	0.45

Mean ± standard deviation. Superscript letters indicate significant differences of main effects as obtained by two-way ANOVA ( $n = 16$  with 4 fish per tank and 4 tanks per treatment; D = Diet, E = Exercise). Following, a Tukey's HSD post hoc test was used to find differences between groups (treatment effect). Abbreviation n.d. for non-detected.



**Fig. 2.** Correlation plot of plasma lipid mediators and 8-isoprostane with their precursor fatty acids linoleic acid, 18:2n-6 (A), arachidonic acid, 20:4n-6 (B), eicosapentaenoic acid, 20:5n-3 (C) and docosahexaenoic acid, 22:6n-3 (D) measured in muscle tissue of fish ( $n = 8$  tanks).

cortisol levels were not significantly different between the two dietary treatments.

The exercise challenge had a significant effect on most analyzed blood parameters with an increase in blood ions  $K^+$ ,  $Na^+$  and  $Ca^{2+}$ , which was associated to higher blood osmolality (Fig. 3). In addition, exercised fish showed higher glucose, lactate and cortisol levels in blood plasma compared to fish sampled pre-exercise.

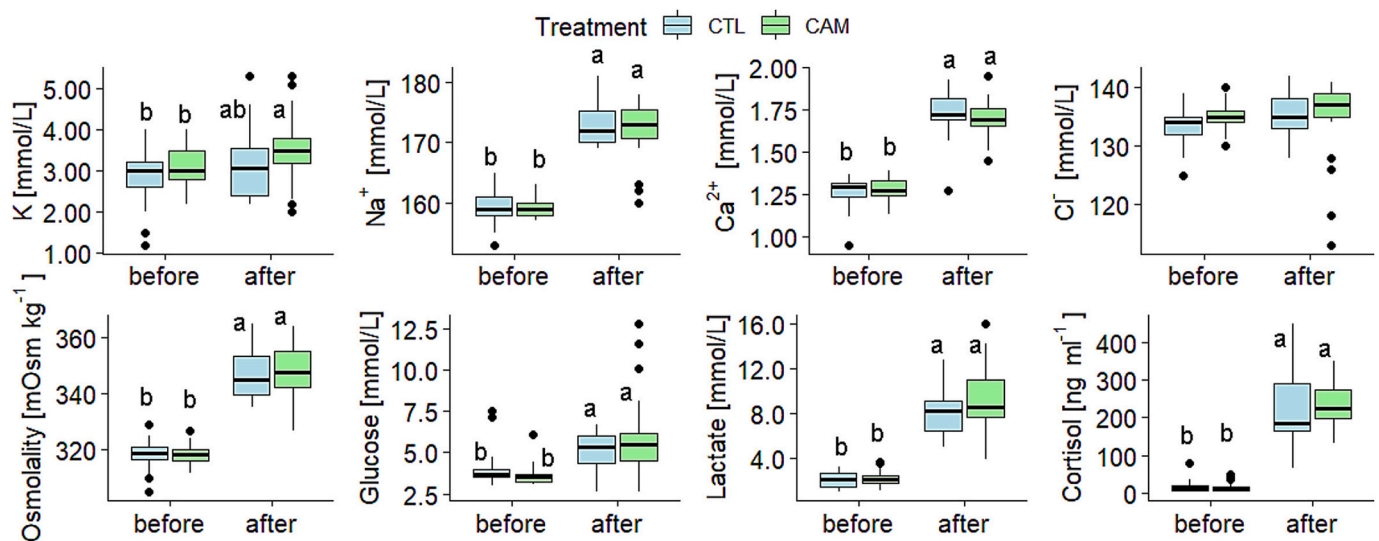
3.6. Effect on fillet pigments and color

Total carotenoid levels were significantly lower in fish fed CAM compared to CTL (Table 7). Astaxanthin levels were significantly lower in CAM compared to CTL, while the levels of other carotenoids including xanthophylls, adonirubin or astacene were significantly higher in CAM compared to CTL. However, astaxanthin was the major carotenoid detected in flesh irrespective of dietary treatment. In fish fed CTL, fillet

astaxanthin levels were significantly higher in fish after the exercise challenge compared to fish sampled prior to the exercise (Table 7).

The color difference of rainbow trout fillet by dietary treatment was also perceived visually. Measurements with a colorimeter and a digital SalmoFan showed that the A\* value, representing the green-red axis, shifted towards red in CTL compared to CAM (Table 7). A color distance map based on photographs taken of the samples shows clear clustering according to the dietary treatments except for samples collected from tank 6 fed CAM and collected after stress that showed more similarity to the baseline sample compared to other samples (Fig. 4).

Further processing of the color values obtained from these images showed a significant shift towards red in CTL compared to CAM in agreement with the colorimeter and SalmoFan data (Table 7). On the other hand, image analysis also found significant differences in L\* with higher lightness values in CAM compared to CTL, although not significantly different between groups according to the posthoc test. Also, the



**Fig. 3.** Plasma K, Na<sup>+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>, glucose, lactate, osmolality and cortisol levels measured in fish fed the experimental diets and before and after exercise in a swim tunnel ( $n = 40$  before stress and  $n = 24$  after stress).

**Table 7**

Fillet pigment levels [mg/kg] and visual measurements of fillet color by colorimeter, digital SalmoFan and picture image analysis in rainbow trout fed the experimental diets either containing a mix of traditional vegetable oils and fish oil (CTL) or a transgenic camelina oil (CAM) over a 10-week period before and after subjected to exercise stress.

	Main effect				Treatment effect				P-value		
	Diet		Exercise		Before exercise		After exercise		D		
	CTL	CAM	Before	After	CTL	CAM	CTL	CAM	D	E	DxE
<b>Carotenoid</b>											
Astaxanthin	6.5 <sup>a</sup> ± 0.7	3.2 <sup>b</sup> ± 0.3	4.6 <sup>b</sup> ± 1.5	5.1 <sup>a</sup> ± 2.0	6.0 <sup>b</sup> ± 0.5	3.2 <sup>c</sup> ± 0.2	7.0 <sup>a</sup> ± 0.6	3.2 <sup>c</sup> ± 0.4	<0.01	0.04	0.05
Xanthophylls	0.2 <sup>b</sup> ± 0.0	0.3 <sup>a</sup> ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.2 <sup>b</sup> ± 0.0	0.3 <sup>a</sup> ± 0.1	0.2 <sup>b</sup> ± 0.0	0.3 <sup>a</sup> ± 0.0	<0.01	0.08	0.62
Adonirubin/ Astacene	0.0 <sup>b</sup> ± 0.0	0.3 <sup>a</sup> ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 <sup>b</sup> ± 0.0	0.3 <sup>a</sup> ± 0.0	0.0 <sup>b</sup> ± 0.0	0.3 <sup>a</sup> ± 0.0	<0.01	0.96	0.22
Other	0.0 <sup>b</sup> ± 0.0	0.2 <sup>a</sup> ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 <sup>b</sup> ± 0.0	0.2 <sup>a</sup> ± 0.0	0.0 <sup>b</sup> ± 0.0	0.2 <sup>a</sup> ± 0.0	<0.01	0.88	0.30
Total	6.7 <sup>a</sup> ± 0.7	4.0 <sup>b</sup> ± 0.3	5.2 ± 1.3	5.6 ± 1.8	6.3 <sup>a</sup> ± 0.5	4.0 <sup>b</sup> ± 0.2	7.2 <sup>a</sup> ± 0.3	4.0 <sup>b</sup> ± 0.2	<0.01	0.07	0.06
<b>Colorimeter</b>											
L	66.9 ± 1.8	67.1 ± 2.6	66.5 ± 2.3	67.5 ± 2.0	67.4 ± 2.2	65.6 ± 2.4	66.4 ± 1.4	68.6 ± 2.0	0.84	0.35	0.07
A	16.9 <sup>a</sup> ± 0.9	12.5 <sup>b</sup> ± 1.0	14.0 <sup>b</sup> ± 2.4	15.4 <sup>a</sup> ± 2.4	16.2 <sup>b</sup> ± 0.3	11.9 <sup>c</sup> ± 0.9	17.6 <sup>a</sup> ± 0.8	13.2 <sup>c</sup> ± 0.5	<0.01	<0.01	0.90
B	24.8 ± 1.3	24.9 ± 0.8	24.1 <sup>b</sup> ± 0.6	25.5 <sup>a</sup> ± 1.0	23.9 <sup>b</sup> ± 0.6	24.4 <sup>ab</sup> ± 0.4	25.7 <sup>a</sup> ± 1.1	25.3 <sup>ab</sup> ± 1.1	0.86	0.01	0.21
dE	35.4 <sup>b</sup> ± 1.3	39.2 <sup>a</sup> ± 0.7	38.1 <sup>a</sup> ± 1.6	36.6 <sup>b</sup> ± 2.5	36.6 <sup>b</sup> ± 0.3	39.6 <sup>a</sup> ± 0.4	34.3 <sup>c</sup> ± 0.8	38.9 <sup>a</sup> ± 0.8	<0.01	<0.01	0.02
<b>SalmoFan</b>											
L	43.3 ± 1.6	44.5 ± 1.5	43.8 ± 1.9	43.9 ± 1.4	43.2 ± 2.1	44.5 ± 1.7	43.4 ± 1.1	44.4 ± 1.6	0.17	0.92	0.90
A	14.1 <sup>a</sup> ± 1.4	10.6 <sup>b</sup> ± 0.8	12.0 ± 2.2	12.7 ± 2.1	13.8 <sup>a</sup> ± 1.3	10.1 <sup>b</sup> ± 0.6	14.3 <sup>a</sup> ± 1.6	11.1 <sup>b</sup> ± 0.7	<0.01	0.24	0.73
B	15.4 ± 1.2	15.6 ± 0.7	15.4 ± 0.7	15.6 ± 1.2	15.2 ± 0.7	15.5 ± 0.7	15.6 ± 1.6	15.6 ± 0.9	0.77	0.66	0.86
dE	10.9 <sup>a</sup> ± 2.0	8.2 <sup>b</sup> ± 1.1	9.3 ± 2.1	9.8 ± 2.2	10.7 <sup>a</sup> ± 1.9	7.8 <sup>a</sup> ± 1.2	11.1 <sup>a</sup> ± 2.3	8.5 <sup>a</sup> ± 1.1	0.01	0.53	0.88
Score	29.3 <sup>a</sup> ± 1.0	27.0 <sup>b</sup> ± 0.6	27.9 ± 1.5	28.4 ± 1.5	29.1 <sup>a</sup> ± 0.9	26.7 <sup>b</sup> ± 0.5	29.5 <sup>a</sup> ± 1.2	27.3 <sup>b</sup> ± 0.5	<0.01	0.24	0.75
<b>Image</b>											
L	40.3 <sup>b</sup> ± 0.8	42.4 <sup>a</sup> ± 1.7	41.4 ± 1.3	41.4 ± 2.0	40.5 <sup>a</sup> ± 0.9	42.2 <sup>a</sup> ± 1.2	40.1 <sup>a</sup> ± 0.7	42.6 <sup>a</sup> ± 2.2	0.01	0.99	0.57
A	20.6 <sup>a</sup> ± 1.1	15.4 <sup>b</sup> ± 0.9	17.6 ± 2.6	18.4 ± 3.2	19.9 <sup>a</sup> ± 1.0	15.4 <sup>b</sup> ± 0.8	21.2 <sup>a</sup> ± 0.6	15.5 <sup>b</sup> ± 1.2	<0.01	0.13	0.21
B	39.5 <sup>a</sup> ± 1.1	37.8 <sup>b</sup> ± 1.0	38.8 ± 1.0	38.5 ± 1.9	39.1 <sup>ab</sup> ± 1.2	38.5 <sup>ab</sup> ± 0.6	39.9 <sup>a</sup> ± 0.9	37.1 <sup>b</sup> ± 1.5	0.01	0.61	0.07
dE	19.1 <sup>a</sup> ± 1.5	14.5 <sup>b</sup> ± 1.6	16.7 ± 2.1	16.9 ± 3.5	18.4 <sup>a</sup> ± 1.6	15.1 <sup>b</sup> ± 1.1	19.9 <sup>a</sup> ± 1.1	13.9 <sup>b</sup> ± 2.0	<0.01	0.80	0.10

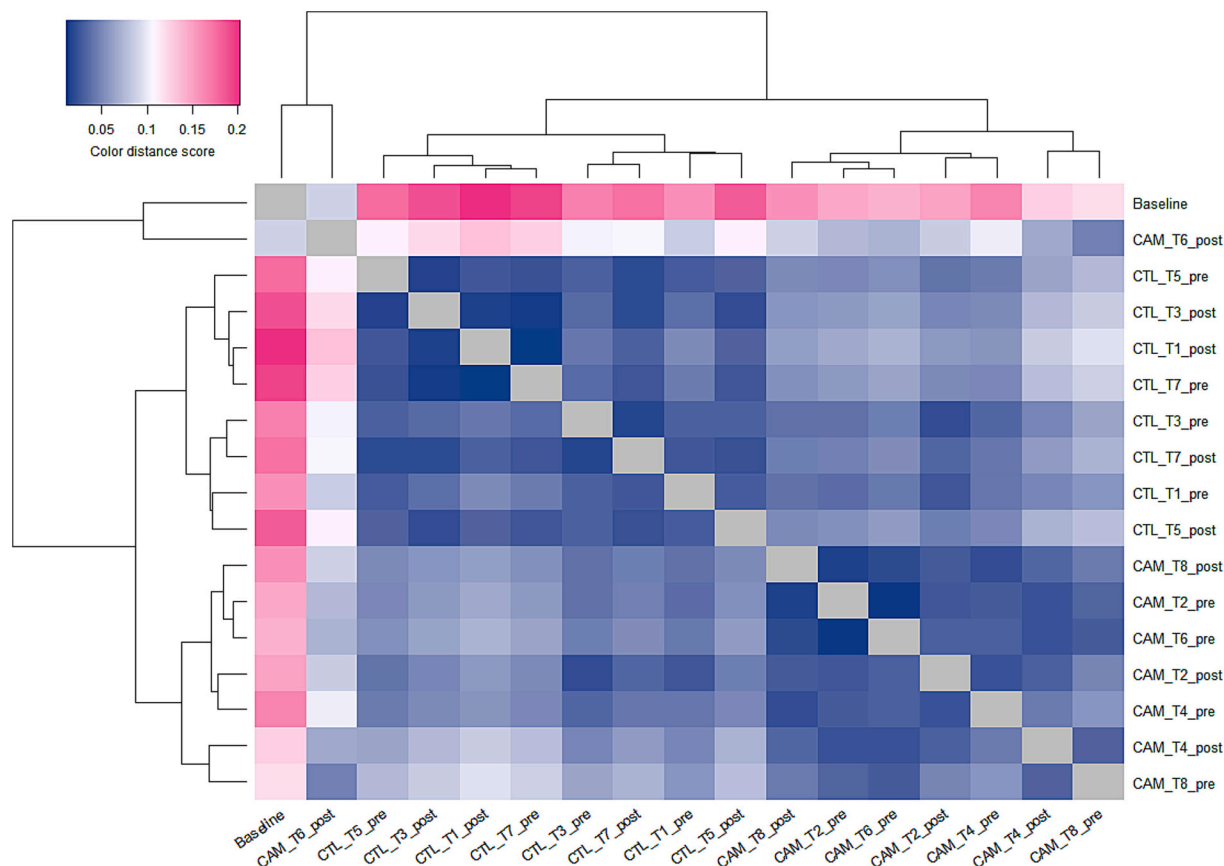
Mean ± standard deviation. Superscript letters indicate significant differences of main effects as obtained by two-way ANOVA ( $n = 4$  pooled samples per treatment; D = Diet, E = Exercise). Following, a Tukey's HSD post hoc test was used to find differences between groups (treatment effect).

shift towards yellow in the blue-yellow axis in CTL compared to CAM detected by image analysis was not detected by the colorimeter or SalmoFan. Overall, the color image analysis showed that all samples showed a significant increase in color compared to the baseline fish, sampled at the beginning of the feeding trial, however, the difference in fillet color between a baseline fish, and the fillet color of the fish at the end of the feeding trial was higher in CTL compared to CAM (Table 7).

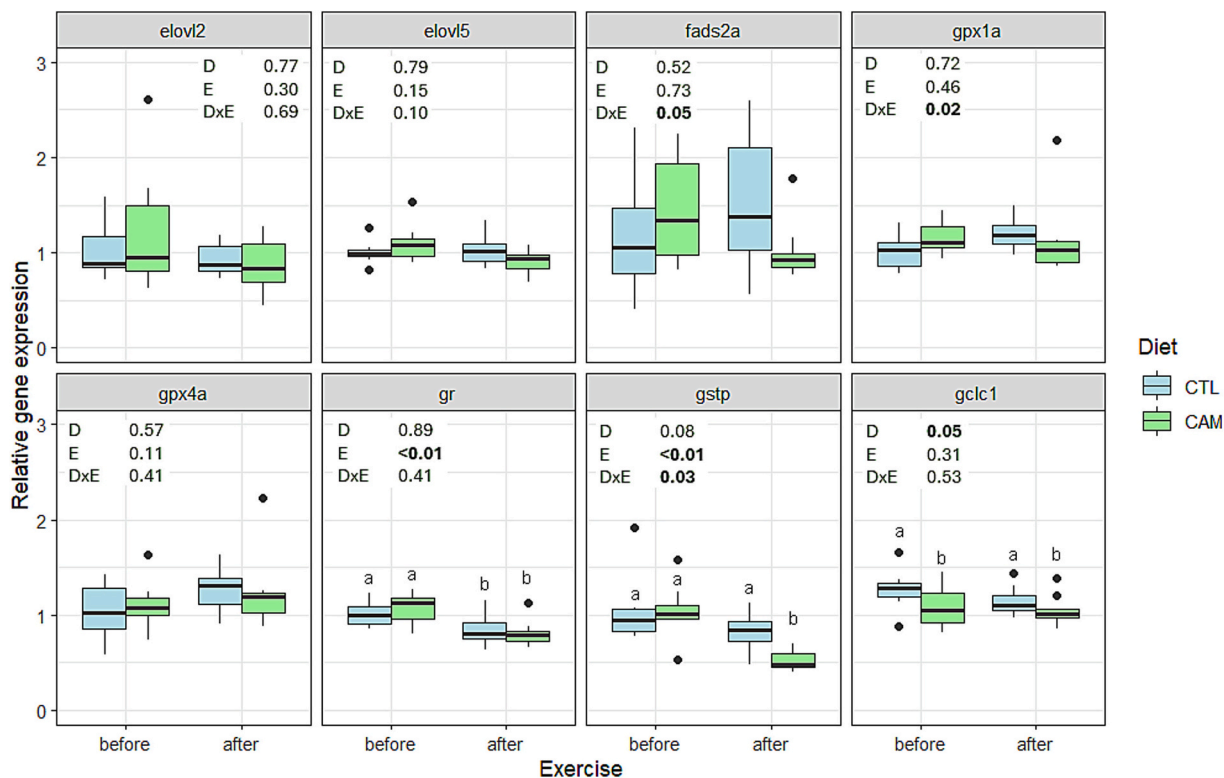
The heatmap shows no clustering of fillet samples according to the

stress challenge (Fig. 4). Similarly, no significant effect of exercise on the color values obtained from the images or measured by the digital SalmoFan could be detected. However, the colorimeter results indicated a significant shift towards red and yellow colors in the fillet of exercised fish (Table 7).





**Fig. 4.** Heatmap of color distance. Clustering is based on average color values retrieved from images of homogenized rainbow trout fillets ( $n = 4$  per treatment). The rainbow trout were fed either of two experimental diets (CTL: fish/vegetable oil mix and synthetic astaxanthin and CAM: transgenic camelina oil containing EPA, DHA and ketocarotenoids) for 10 weeks prior to sampling. In addition, part of the fish was exercised in a swim tunnel before sampling.



**Fig. 5.** Relative gene expression in liver tissue of fish fed one of the experimental diets (CTL, CAM) before and after subjected to an exercise challenge.

### 3.7. Gene expression

The dietary treatment had no significant effect on the expression of the LC-PUFA biosynthetic genes *fatty acid elongase 2 (elovl2)*, *fatty acid elongase 5 (elovl5)* or *fatty acid desaturase 2 (fads2a)* (Fig. 5). Although *fads2a* and the antioxidant enzyme *glutathione peroxidase 1a (gpx1a)* showed a significant interaction between the factors diet and stress, no significant differences between the groups were detected in the post hoc test. The expression of *glutamate-cysteine ligase catalytic subunit 1 (gclcl1)*, in the glutathione synthesis pathway, was significantly down-regulated in fish fed CAM compared to fish fed CTL. Also, *glutathione-s-transferase pi (gstp)* was less expressed in CAM compared to CTL, however, this effect was only detected in exercised fish. Exercised fish showed lower expression of *glutathione reductase (gr)* compared to fish that were not exercised (Fig. 5).

## 4. Discussion

### 4.1. Effect of diet on fish growth performance

Following the introduction of plant-based ingredients in aquafeeds for Atlantic salmon, n-3 LC-PUFA levels in aquafeeds generally decreased (Sprague et al., 2016). However, this trend has been reversed over the past years recognizing the essentiality of n-3 PUFA for fish health and welfare. Salmonids require about 1 % of EPA and DHA on dry matter basis in their feed for normal growth, but possibly more to achieve optimal welfare or under stress (Løvmo et al., 2022; Løvmo et al., 2021; Lutfi et al., 2023; NRC, 2011). In the current study both the CTL as well as the CAM diet, formulated with the transgene-derived oil, covered this minimum requirement. The growth performance of rainbow trout fed the novel oil was comparable to that of fish fed the control diet over the whole duration of this experiment, which agrees with earlier studies testing n-3 LC-PUFA rich transgenic camelina oil in this salmonid species (Osmond et al., 2021) as well as other species such as Atlantic salmon, gilthead seabream or European sea bass (Betancor et al., 2021; Betancor et al., 2017a; Betancor et al., 2016a; Tocher et al., 2024). However, in the present study the body weight of fish fed the camelina oil-based diet at first intermediate sampling was lower compared to fish fed the control diet. A similar effect has been observed in European sea bass where the short-term use of a transgene-derived camelina oil diet gave reduced growth (Betancor et al., 2021). In European sea bass it was related to a low initial palatability however, feed intake was not measured in the present study. It seems that sensitivity to gene-derived camelina oil inclusion varies by species, as Atlantic salmon or gilthead seabream did not show differences in feed intake (Betancor et al., 2017a; Betancor et al., 2016a). Nevertheless, in the present as well as the previous experiment (Betancor et al., 2021) fish eventually showed a compensatory growth in the second half of the trial yielding in similar body weight after 10 or 16 weeks of feeding. In a different study using juvenile rainbow trout, fish fed transgenic camelina oil in blend with non-transgenic camelina oil at a dietary inclusion level of 12.5 g/kg, which is below the levels used in the current trial, showed higher body weight as compared to fish fed a fish oil-based control diet after 12 weeks of feeding (Osmond et al., 2021). The authors speculated that this might be related to a better bioavailability of n-3 LC-PUFA from the transgenic camelina compared to the fish oil. Indeed, lipid apparent digestibility showed to be higher in feeds containing genetically modified camelina derived oil than in feed containing fish oil as the only lipid source in Atlantic salmon (Betancor et al., 2016b). Additional long-term studies on the timely growth dynamics of rainbow trout fed transgenic n-3 LC-PUFA oils might be warranted in the future.

### 4.2. Dietary effect on fillet fatty acid profile and plasma lipid inflammatory mediator levels

It has been well established that the fatty acid profile in fish fillets

reflects that of the feed (Turchini et al., 2018), which was also observed in this study. In agreement with previous trials the present data show that transgenic camelina oil is a rich source of EPA and DHA with the potential to increase n-3 LC-PUFA in feed and fillet levels above those obtained by current commercial formulations (Betancor et al., 2017a). Overall, in the current trial n-3 PUFA fillet concentrations from both dietary treatments were in the upper range of those observed in commercially farmed rainbow trout and Atlantic salmon (Kolanowski, 2021; Sprague et al., 2020). However, in this experiment, eating two portions of 140 g fillets per week, as recommended by NHS, UK to achieve an n-3 PUFA intake of 3.5 g (NHS, 2022), would only be fulfilled if the fish were fed CAM, while two portions of fillet from fish fed the CTL diet would fall short by 16 %. Camelina oil also contains naturally high levels of n-3 short-chain PUFA, which are known to induce the expression of genes involved in the metabolism of n-3 LC-PUFA in salmonids, particularly when the dietary levels of the end-products (EPA and DHA) are low (Mock et al., 2019). However, in the present study there were no differences in the expression levels of LC-PUFA biosynthetic genes, probably because both diets contained sufficient LC-PUFA to fulfill requirements (>1 % of the diet) (Lutfi et al., 2023). This is opposite to results obtained in earlier studies with Atlantic salmon (Betancor et al., 2016b). However, in European sea bass, like the present results, the expression of LC-PUFA synthesis limiting gene *fads2* was not affected by supplementation of transgenic EPA and DHA-rich transgenic oils, which might be related to the availability of both EPA and DHA well above requirement (Betancor et al., 2021).

Studies in Atlantic salmon have shown that high levels of n-6, especially arachidonic acid (20:4n-6), can give rise to eicosanoids, prostaglandins and isoprostanes (Araujo et al., 2014; Cardona et al., 2024). However, the responses are complex, and eicosanoid levels were not always correspondent to the levels of their respective precursor fatty acids (Bell et al., 1996; Hundal et al., 2021; Sissener et al., 2020). In the present study, lipid mediators derived from arachidonic acid and EPA showed strong correlation with the levels of their respective precursor fatty acids in the muscle tissue. However, it was less apparent for linoleic acid and not found for DHA. In general, fish fed transgenic camelina oil had higher levels of both arachidonic acid and EPA. These fatty acids compete for the COX and LOX enzymes in eicosanoid production. In the present study, both arachidonic acid- and EPA-derived eicosanoids were elevated in fish fed the transgenic oil, although it was suggested that arachidonic acid might be the preferred substrate for eicosanoid production (Ghioni et al., 2002). Nevertheless, a strong response on cytokine and pro-inflammatory transcription factors has been observed in vitro under co-supplementation of arachidonic acid and EPA (Holen et al., 2015). DHA-derived lipid mediators have received little attention in fish and were not elevated with dietary DHA levels in the present study.

### 4.3. Effect of transgene-derived oil on carotenoid levels and fillet color

Natural astaxanthin sources derived from yeast, krill or algae exist on the market and have generally been shown to effectively increase pigment levels and fillet color in salmonids at comparable levels to synthetic astaxanthin (Zhao et al., 2022). However, the limited availability of these natural resources and the high price of natural and synthetic astaxanthin products fosters the search for alternative solutions. This study proved that astaxanthin derived from a transgenic camelina oil can increase the levels of astaxanthin in rainbow trout fillet. However, the transgenic oil yielded lower fillet carotenoid levels as well as in fillet color likely due to the lower total astaxanthin level in the diet compared to the pure synthetic astaxanthin. Although camelina oil is naturally yellow, the additional accumulation of the pinkish astaxanthin pigment in the plant can be achieved through transgenic modification. The resulting oil contained a mix of carotenoid compounds including their stereoisomers. Astaxanthin stereoisomers generally display a lower bioavailability and compete in the uptake of astaxanthin (Bjerkeng

et al., 1997). In comparison to a previous study using astaxanthin derived from transgenic maize and re-diluted in yellow pigmented canola oil (Breitenbach et al., 2016), the present study found no shift towards yellow in the fillet color of fish fed the transgenic oil. This lack in color change may be related to differences in the levels of yellow carotenoids and total astaxanthin levels of the experimental diets. Nevertheless, the results indicate that the removal of competing carotenoids in favor of astaxanthin in transgenic oils might be warranted to yield high astaxanthin level and availability as well as the desired color profile in salmonid fillets.

The bioavailability of dietary astaxanthin might also depend on the dietary fatty acid composition although findings in the literature are not consistent. Atlantic salmon fed herring, capelin, sand eel or Peruvian high DHA oil showed no differences in fillet astaxanthin levels or fillet color (Bjerkeng et al., 1999). This was similar to observations in salmon fed either fish oil or olive oil (Choubert et al., 2006). On the other hand, Osmond et al. (2021) described higher orange fillet color in rainbow trout fed an EPA and DHA-rich transgenic camelina oil compared to fish oil, however without measurement of fillet pigment concentration. In addition, the deposition of algae-based astaxanthin was higher compared to synthetic astaxanthin in Atlantic salmon but lowered when fish were fed a diet based on tallow compared to a diet based on canola oil (Courtot et al., 2022). Thereby, high levels of SFA seemed to inhibit the fillet deposition of carotenoids, while high levels of MUFA, especially oleic acid (18:1n-9), were reported to positively correlate with the fillet deposition of algae-based carotenoids. This observation might be due to the lower digestibility of SFA compared to MUFA (Menoyo et al., 2003), which in turn, reduces the digestibility of lipid soluble carotenoids. In the present study, lipid and total carotenoid levels were comparable in the experimental diets, but the percentage of MUFA and SFA were higher in the control diet compared to the diet formulated on the transgene-derived oil, while astaxanthin levels higher in the control. Future feeding trials might therefore use feed formulations based on equal astaxanthin rather than on total carotenoid concentrations to establish the effect of dietary fatty acid composition on the deposition of astaxanthin which might also differ between astaxanthin sources.

#### 4.4. Swimming performance and muscle fatty acids under exercise stress

In fish, gentle swimming is powered by aerobic generation of adenosine triphosphate in red muscle, using substrates such as lipids, protein or carbohydrates to burn, and oxygen as an electron acceptor (McKenzie, 2011). Swimming that leads to exhaustion such as done in this experiment, is partly fueled by anaerobic metabolism through glycolysis in white muscle tissue, where glucose is converted to pyruvate and subsequently to lactate. Indeed, in the present experiment significantly higher plasma glucose levels in both dietary groups suggest a mobilization of energy to accommodate the cost of swimming as previously described (Hvas et al., 2018; Madaro et al., 2023). In addition, blood lactate levels increased in fish after the exercise indicating that the anaerobic metabolism was active, and fatigue reached (Wendelaar Bonga, 1997; Wood, 1991). The high lactate can then cause a severe acidosis, which in turn is regulated through changes in plasma ion concentration (Hvas et al., 2018) as similarly observed in this study.

The muscular fatty acid composition has been suggested to influence the swimming physiology of fish. In Atlantic salmon a negative relationship was observed between Ucrit and dietary n-3 LC-PUFA levels, while a highly positive relationship was found between Ucrit and 18-carbon unsaturated fatty acids in muscle tissue, particularly with linolenic acid (18:2n-3) and oleic acid (18:1n-9) (McKenzie et al., 1998; Wagner et al., 2004). In the present study, oleic acid levels were significantly lower in both diet and tissues of fish fed the transgenic camelina oil compared to the control diet, however, no significant differences in Ucrit were observed between the dietary treatments. The positive relationship between EPA, DHA and the cardio-vascular health has been demonstrated in various studies on fish (Bell et al., 1993; Bell

et al., 1991). The deviations between the present results and observations of McKenzie et al. (1998) may be due to species differences (rainbow trout vs Atlantic salmon) or variations in muscle sampling. The present measurements were done in fillet tissue in the region of the Norwegian quality cut representing mainly white muscle tissue mixed with smaller amounts of red muscle tissue, while McKenzie et al. (1998) analyzed locomotory muscle tissue, possibly red muscle.

Although there was a tendency in the present study for a lower condition factor in fish fed the transgenic camelina oil, no differences in absolute or relative Ucrit were observed between the dietary groups. Similar to Atlantic salmon (Warren-Myers et al., 2023), rainbow trout in the present study increased tail beat frequency with increasing velocity in a linear relationship up to 90 cm s<sup>-1</sup>, but reached maximum at 110 cm s<sup>-1</sup> without differences according to dietary treatment. Together these results indicate that fish of both dietary groups had similar health and athleticism. In line with the present results, in chinook salmon (*Oncorhynchus tshawytscha*) no significant effect of replacing anchovy or poultry fat with canola oil was found on swimming performance (Regan et al., 2010). Additionally, the cardio-vascular physiology of Atlantic salmon was not impaired by replacing anchovy with sunflower or flaxseed oil under repeat swimming performance (Wagner et al., 2004). Therefore, moderate differences in fish feed and muscle fatty acid composition might not be the dominating factor in determining the athleticism and respiratory capacity in salmonids.

#### 4.5. Stress markers and astaxanthin in exercised fish

In the present study, exhaustive swimming led to a rise in cortisol across all diet groups. The cortisol response in stress coping is multifaceted (Madaro et al., 2023; Madaro et al., 2015; Wendelaar Bonga, 1997). In this context, cortisol promotes coping capacity not only releasing energy but also improving oxygen uptake through increased permeability of the gill surface epithelium, enhancing gas transfer (Hvas et al., 2018). However, the increased gill permeability may cause a loss of ions in freshwater acclimated fish. Cortisol is known for its osmoregulatory function in the gill at both high and low salinities particularly mitigating osmotic disturbances from exercise by reducing passive ion loss and promoting increased active uptake at the gills (Evans et al., 2005). In this study, plasma analyses showed that Na<sup>+</sup> and Ca<sup>2+</sup> levels were higher in both diet groups after the swim challenge, while Cl<sup>-</sup> levels remained unchanged. Generally, ion levels and osmolality increase post-stress, partly due to fluid shifts within tissues. However, as observed in a previous study (Hvas et al., 2018), plasma Cl<sup>-</sup> levels may remain unchanged due to pH regulation through modulation of branchial HCO<sub>3</sub><sup>3-</sup>/Cl<sup>-</sup> transport (Evans et al., 2005; Marshall, 2002).

Antioxidants such as astaxanthin can mitigate the effects of certain stressors that cause oxidative stress (Nakano and Wiegertjes, 2020). However, in the present study, fatigue after exercise was associated with an increase in muscle astaxanthin levels. A similar tendency of higher muscle astaxanthin levels has also been observed in rainbow trout after the exposure to oxygen stress in a previous trial (Kalinowski et al., 2019). The metabolic pathway leading to elevated muscle astaxanthin in stressed fish remains unknown but might be related to a mobilization in response to redox changes. In the present study, a simultaneous decrease in the hepatic mRNA levels of certain antioxidant enzymes indicate an activation of the antioxidant system in post-exercise fish. These enzymes include glutathione reductase and glutathione-s-transferase, which utilize glutathione, the major cellular redox protein, as a substrate. However, the decrease in the gene expression of glutathione-s-transferase was only observed in fish fed the diet with transgenic camelina oil. Indeed, the higher astaxanthin levels in the control diet might have improved glutathione recycling as previously suggested in rainbow trout (Kalinowski et al., 2019) and indicated in the present study by a lower expression of the *gclc* gene in exercised fish fed the transgenic camelina oil. Together these results indicate that exercise might have induced oxidative stress, and the dietary treatment affected the response in the

cellular glutathione system. Polyunsaturated fatty acids are especially sensitive to oxidative stress but were not significantly affected by the exercise challenge in the present study. Similarly, TBARS as a marker of lipid peroxidation were similar in non-exercised and exercised fish. However, 8-isoprostane levels, a non-enzymatic product of arachidonic acid oxidation, were elevated in plasma of exercise-stressed fish. This might be related to the termination of fish directly at the end of the exercise, while it might take a longer time for TBARS to accumulate in the muscle tissue (Richards et al., 2002). In plasma, on the other hand, the levels of some lipid mediators were elevated. These PUFA oxidation products function as signaling molecules in physiological systems. Although dietary n-6/n-3 ratio has been reported to increase hepatic eicosanoid levels in Atlantic salmon under repeated acute hypoxia stress (Hundal et al., 2021), no interaction between diet and exercise stress could be observed in the present study. However, higher levels of 8-isoprostanes in exercised fish fed CAM compared to CTL might directly relate to the high levels of arachidonic acid in the feed rather than oxidative status as suggested by van 't Erve et al. (2015).

## 5. Conclusion

The oil derived from transgenic camelina engineered to accumulate n-3 LC-PUFA and ketocarotenoids supported normal growth and increased fillet EPA and DHA levels above that of current commercial standard suggesting that it could be used as a novel n-3 LC-PUFA source in aquafeeds. The swimming performance and response to exhaustive exercise stress were similar between groups indicating that inclusion of the transgenic camelina oil did not impair the health and welfare of the fish. However, fillet pigmentation and tissue carotenoid levels were lower in fish fed the transgenic oil compared to the supplementation of synthetic astaxanthin which suggests that it might be advantageous to formulate feed based on equal astaxanthin rather than total carotenoid levels in future studies.

## CRediT authorship contribution statement

**Pauline Wischhusen:** Writing – original draft, Investigation, Formal analysis, Data curation. **Angelico Madaro:** Project administration, Investigation, Funding acquisition, Conceptualization. **Malthe Hvas:** Writing – review & editing, Methodology. **Richard Broughton:** Methodology, Data curation. **Lihua Han:** Resources. **Karla Fernandez Quiroz:** Writing – review & editing, Investigation. **Kasidis Chaiyasut:** Investigation, Formal analysis. **Akhil Gupta:** Formal analysis. **Rolf Erik Olsen:** Writing – review & editing, Conceptualization. **Stéphanie Fontagné-Dicharry:** Writing – review & editing, Resources. **Johnathan A. Napier:** Resources. **Mónica B. Betancor:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

## Ethical approval

All animal experiments and sampling procedures followed national guidelines of the UK and Norway implementing the EU Directive 2010/63/EU as minimum standards. In addition, the study has been approved by the Animal Welfare and Ethical Review Board (AWERB) of the University of Stirling (AWERB/2022/8188/6558). This trial has also been approved by the Norwegian food safety authority under FOTS id 29931. All researchers and personnel involved in the experiment obtained appropriate training.

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## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2025.742453>.

## Data availability

Data will be made available on request.

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