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# Transgenic camelina oil is an effective source of eicosapentaenoic acid and docosahexaenoic acid in diets for farmed rainbow trout, in terms of growth, tissue fatty acid content, and fillet sensory properties

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## Abstract

The oilseed *Camelina sativa* was genetically engineered to produce eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at levels similar to fish oil (FO), which we tested as a lipid source in diets for rainbow trout. Three experimental diets were tested, a FO control, a low-level transgenic camelina oil (LCO) diet (no FO with 12.5 g/kg camelina), and a high-level transgenic camelina oil (HCO) diet (no FO with 130 g/kg transgenic camelina). Trout (initial weight 49.8 ± 11 g/fish) were fed for 12 weeks and were evaluated for growth performance, fatty acid content (muscle, liver, brain, and eye), and sensory properties of fillets. The final lengths and weights of fish fed LCO and HCO diets were higher compared to fish fed FO. There were no differences in weight gain, condition factor, specific growth rate, feed intake, and feed conversion ratio. EPA in muscle tissue was higher in fish fed HCO and FO diets compared to the LCO diet. Compound-specific stable isotope analysis revealed that rainbow trout fed the FO diet stored isotopically enriched DHA compared to the lighter DHA in fish fed

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HCO. Trout fillets from the HCO treatment were firmer in texture and had a higher orange intensity compared to the FO group.

**KEYWORDS**

camelina, DHA, EPA, rainbow trout, transgenic

## 1 | INTRODUCTION

Omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA) play a key role in physiological functions in all vertebrate organisms, where they support neurological development and function, cardiovascular health, visual acuity, growth, reproduction, and the immune system (Brenna, Salem, Sinclair, & Cunnane, 2009; Calder, 2015; Swanson, Block, & Mousa, 2012). As many vertebrates are generally unable to efficiently synthesize the n-3 LC-PUFA, eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) in sufficient quantities to meet nutritional needs, these are often obtained via dietary consumption (Arts, Ackman, & Holub, 2001; Calder, 2015). EPA and DHA are primarily produced by microalgae and wild fish obtain these fatty acids (FA) as they are trophically transferred through food webs. The most common dietary source of EPA and DHA is directly consumed from seafood, particularly from fish, as the natural production of these FA, on a global scale, is primarily aquatic-based (Colombo, Rodgers, Diamond, et al., 2020).

Culturing salmonid fishes requires a dietary source of EPA and DHA at levels up to 1% of the dry diet for normal growth and development, though specific inclusion rates vary depending on species and life stage (NRC, 2011). Wild-sourced fish oil (FO) is, currently, the main commercial source of dietary EPA and DHA. However, increases in production demands and continued reliance on wild stocks have further highlighted the need to identify and validate new dietary sources of EPA and DHA for use in aquaculture. For the most part, commercial aquaculture feeds have reduced FO levels and included other terrestrial-based lipid sources (Turchini, Hermon, & Francis, 2018). For example, nearly 70% of the oil fraction in feeds for Atlantic salmon in Norway consists of canola oil (Ruyter et al., 2019). However, some FO (and fish meal) must remain to supply EPA and DHA, as terrestrial oilseed plants do not naturally produce n-3 LC-PUFA. This not only reduces the EPA and DHA in the feed but also directly impacts the lipid profile of the final fish fillet for human consumption (Sprague, Dick, & Tocher, 2016). It is therefore evident that the reliance on FO as a source of dietary EPA and DHA has become a major constraint for further growth of the aquaculture industry.

There has been recent interest in the use of genetically engineered products in aquafeeds (Osmond & Colombo, 2019). The crucifer oilseed, *Camelina sativa*, in its wild form, is naturally high in the n-3 LC-PUFA precursor, alpha-linolenic acid (ALA, 18:3n-3), but does not produce EPA or DHA. However, in one of its transgenic forms, camelina produces EPA and DHA at levels comparable to that of FO (Napier, Usher, Haslam, Ruiz-Lopez, & Sayanova, 2015). A specific set of seven nonhost gene segments, mainly from marine microalgae, were used, by Ruiz-Lopez, Haslam, Napier, and Sayanova (2014), to produce the n-3 LC-PUFA synthesis pathway, with the expressed phenotype of EPA and DHA accumulation in the seeds of camelina. The oil, extracted from this transgenic camelina, has been tested as a lipid source in diets for Atlantic salmon (*Salmo salar*) and gilthead seabream (*Sparus aurata*; Betancor et al., 2015; Betancor et al., 2016; Betancor et al., 2016; Betancor et al., 2017). However, it has not yet been tested in another salmonid species of commercial interest.

In post-smolt Atlantic salmon, transgenic camelina oil was highly digestible overall, and, in particular, had high apparent digestibility values for EPA and DHA (Betancor, Sprague, Sayanova, et al., 2016). In that study, salmon fed transgenic camelina oil (20% of the diet) with EPA and DHA showed no signs of negative impacts to fish health or growth performance compared to salmon fed diets with FO or non-transgenic camelina seed oil over the 11-week

study (Betancor, Sprague, Sayanova, et al., 2016). Total levels of n-3 LC-PUFA in Atlantic salmon fed transgenic camelina oil diets were comparable to fish fed diets containing FO as a lipid source, and both were significantly higher compared to fish fed non-transgenic camelina oil (Betancor, Sprague, Sayanova, et al., 2016). Similar results were observed in gilthead seabream (Betancor, Sprague, Montero, et al., 2016). In the study with gilthead seabream, another variety of transgenic camelina was tested that contained EPA only, in addition to the transgenic camelina oil with EPA and DHA. After 11 weeks of feeding, the transgenic varieties of camelina oil had no apparent negative impacts on fish growth performance; however, final weight of fish fed diets containing transgenic camelina with EPA only was slightly lower in comparison to fish fed transgenic camelina with both EPA and DHA and to fish fed the control diet (Betancor, Sprague, Sayanova, et al., 2016). It should be noted that as limited biosynthesis of these fatty acids was observed, the n-3 LC-PUFA was said to be accumulated through dietary sources (Betancor et al., 2016, b). Another transgenic oilseed has also been tested as a dietary lipid source for Atlantic salmon. Fingerling salmon fed diets with transgenic canola oil (with high DHA) for up to 83 days had approximately the same EPA and DHA content in whole body as salmon fed FO diets (Ruyter et al., 2019). Gene expression, lipid composition, and oxidative stress-related enzyme activities showed only minor differences between the dietary groups. Collectively, these studies suggest that transgenic oilseeds represent promising lipid sources that could completely replace FO in aquafeeds. However, transgenic camelina has yet to be evaluated in another commercially-relevant salmonid species, such as rainbow trout (*Oncorhynchus mykiss*).

Rainbow trout is of particular interest in North America, as it is one of the most widely produced commercial species. In Canada, production was over 9,000 t and revenue from fish sales totaled \$56.8 million CAD in 2018 (DFO, 2018). In the United States, production was over 21,000 t and revenue from fish sales totaled \$95.6 million USD in 2019 (FAO, 2020; USDA, 2020). Although rainbow trout do have the ability (albeit limited) to synthesize n-3 LC-PUFA when provided with essential dietary ALA, they still require EPA and DHA supplementation in intensive aquaculture production for optimal growth and development (Hixson, Parrish, & Anderson, 2014) and final product quality (Thanuthong, Francis, Senadheera, Jones, & Turchini, 2011; Yildiz, Eroldoğan, Ofori-Mensah, Engin, & Baltacı, 2018).

In this study, we evaluated the efficacy of transgenic camelina as a dietary lipid source for juvenile rainbow trout, in terms of growth performance, as well as n-3 LC-PUFA storage and synthesis. We also used compound-specific stable isotope analysis (CSIA) as a tool to discriminate the origin of DHA in the muscle tissue of the rainbow trout. With the introduction of transgenic plant-based oils, it is essential to determine how EPA and DHA, obtained from a plant-based source, are metabolized and used in comparison to EPA and DHA from a marine-based oil. As such, the overall aim of this study was to determine the impact of transgenic camelina oil on fish growth performance, tissue-specific fatty acid composition, and sensory properties of juvenile rainbow trout. We hypothesize that transgenic camelina oil can fully replace FO in rainbow trout diets at commercially relevant inclusion levels (i.e., 100 g/kg).

## 2 | MATERIALS AND METHODS

### 2.1 | Test ingredient

The transgenic camelina variety used in this study was grown, harvested, and single cold-pressed (to extract the seed oil) by Rothamsted Research (West Commons, UK). The variety used in this study was the DHA2015.1 line, as described by Han et al. (2020). The fatty acid composition of the oil (presented as % and µg/mg in Table 1) contained 9.5% EPA and 7.8% DHA, for a total n-3 LC-PUFA proportion of 17.3%. The transgenic camelina seed oil also included ALA (12.2%), linoleic acid (LNA; 18:2n-6; 21.0%), and oleic acid (18:1n-9; 5.8%). The sum of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and PUFA was found in the following proportions, 14.9, 15.8, and 69.4%, respectively. Total n-3 and total n-6 were 40.1 and 29.3%, respectively. The n-3/n-6 ratio was 1.4 for this transgenic camelina oil.

## 2.2 | Experimental diet formulation and composition

All diets were formulated as isonitrogenous and isolipidic to meet the requirements of rainbow trout (NRC, 2011). Diets were produced at the Chute Animal Nutrition lab, Faculty of Agriculture, Dalhousie University (Truro, NS, Canada). Three experimental treatments were produced as follows: a control diet containing a fish (herring) oil (FO; 100 g/kg) and canola oil (100 g/kg) blend, a low transgenic camelina oil inclusion (LCO; no FO with 12.5 g/kg transgenic camelina) canola oil (70 g/kg) blend, and a high transgenic camelina oil inclusion (HCO; no FO with 130 g/kg transgenic camelina) canola oil (70 g/kg) blend (Table 2). The LCO diet represents a practical, commercial diet without FO to determine if a minimum inclusion of transgenic camelina would be sufficient in providing n-3 LC-PUFA for acceptable growth performance. The HCO diet utilizes transgenic camelina as a complete FO replacement, while also provides the majority of dietary lipid. In addition to the transgenic camelina oil, FO, and canola oil lipid sources, fish meal (10% lipid), ground wheat (1.8% lipid), poultry by product meal (14% lipid), corn protein concentrate (2% lipid), and soybean meal (1% lipid) all contribute dietary lipid; however, remain consistent across all treatments. Diet mash was steam pelleted through a California Steam Pellet Mill (San Francisco, USA) with a 3 or 5 mm die plate. Pellets were dried in a JWP ST series industrial cabinet oven at 60°C for 4 hr. Excessive fines were sifted using a 3 mm sieve. Diets were stored at -20°C in airtight and darkened containers to reduce oxidization of fats until needed. Diets were only exposed to room temperature during periods of feeding. Fish were fed 3.0 mm pellets at the beginning of the experiment; however, pellet size was increased to 5.0 mm as fish increased in size. The FA profiles of experimental diets can be viewed in Table 3.

## 2.3 | Experimental fish

Juvenile rainbow trout ( $n = 225$ ;  $49.8 \pm 10.9$  g/fish initial weight  $\pm$  SD) were obtained from Fraser's Mills Hatchery (Antigonish, NS, Canada) and transported to the Aquaculture Centre, Faculty of Agriculture, Dalhousie University (Truro, NS, Canada). Guidelines for ethical treatment of fish were followed by the Canadian Council of Animal Care (Dalhousie, approved protocol #2017-101). The rainbow trout were randomly distributed into nine 232 L circular fiberglass experimental tanks, with each tank containing 25 fish. Each experimental diet was fed to three replicate tanks, with tank as the experimental unit ( $n = 3$  per treatment). Experimental tanks were supplied with a flow-through system of freshwater at a rate of 2-3 L/min, with water supplied from the local aquifer on campus. The dissolved oxygen concentration (10 mg/L, 100%) and target water temperature (12°C) were monitored and recorded daily. Fish were reared on a natural photoperiod over the 16-week trial (average 14 hr light: 10 hr dark). Fish were fed twice daily to apparent satiation and feed intake was recorded weekly. There were no observed mortalities throughout the trial.

## 2.4 | Tissue sampling

Fish sampling occurred initially at Week 0, the day before feeding the experimental diets and final sampling occurred again after 12 weeks of feeding. Five fish per tank were randomly netted and euthanized with an overdose of anesthetic (tricaine methane sulfonate; MS-222) and clinical signs of death were confirmed prior to sampling. Fish were weighed and fork length was measured. Viscera was removed and weighed. Liver tissue samples were taken for lipid analysis. Skin was removed from the left side of the fish under the dorsal fin, above the lateral line and skin-less dorsal muscle tissue was sampled from this location for lipid and protein analysis. At Week 12, the same sampling regime was conducted; however, brain and eye tissue were also sampled at this time. All tissue samples were placed in 2 mL cryogenic vials, flash frozen in liquid nitrogen, and stored at -80°C until analysis.

**TABLE 1** Fatty acid composition of transgenic camelina oil

Fatty acid	Transgenic camelina oil (%)	Transgenic camelina oil ( $\mu\text{g}/\text{mg}$ )
14:0	0.1	1.9
15:0	<0.1	0.8
16:0	6.5	138.8
16:1n-7c	0.1	2.6
16:1n-7t	<0.1	0.7
17:0	0.1	1.3
17:1n-7	<0.1	0.2
18:0	4.8	102.4
18:1n-9c	5.8	123.8
18:1n-9t	0.2	3.1
18:1n-12c	0.3	6.1
18:1n-7c	1.3	28.8
18:1n-7t	<0.1	0.3
19:0	<0.1	0.6
18:2n-6 (LNA)	21.0	446.8
20:0	2.5	52.3
18:3n-6	2.2	46.0
20:1n-15	0.1	2.1
20:1	0.1	1.9
20:1n-9	6.7	142.4
18:3n-3 (ALA)	12.2	260.3
18:2n-6t	<0.1	0.3
18:4n-3	1.4	30.5
20:2n-6	1.2	24.7
22:3n-3	0.1	2.4
22:0	0.8	16.6
20:3n-6	0.8	17.5
22:1n-9	0.7	14.9
20:3n-3	1.2	24.9
20:4n-6 (ARA)	3.1	65.4
20:4n-3	2.4	50.1
22:2n-6	0.2	3.4
24:0	0.1	1.6
20:5n-3 (EPA)	9.5	202.9
24:1n-9	0.3	5.8
22:4n-6	0.8	16.9
22:5n-6	0.1	1.6
22:5n-3	5.6	118.4
22:6n-3 (DHA)	7.8	165.8
$\Sigma\text{SFA}$	14.9	316.5
$\Sigma\text{MUFA}$	15.8	335.7
$\Sigma\text{PUFA}$	69.4	1,475.5
$\Sigma\text{MUFA}_{\geq 18\text{C}}$	15.6	332.1
$\Sigma\text{MUFA}_{>18\text{C}}$	7.9	169.9
$\Sigma\text{C18 PUFA}$	36.8	783.9
$\Sigma\text{C20 PUFA}$	18.1	385.6

(Continues)

TABLE 1 (Continued)

Fatty acid	Transgenic camelina oil (%)	Transgenic camelina oil (µg/mg)
ΣC22 PUFA	14.4	306.0
ΣEPA and DHA	17.3	368.7
Σn-6	29.3	622.5
Σn-3	40.1	853.0
ΣOdd chain	0.2	3.2
n-3/n-6	1.4	1.4

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LNA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

## 2.5 | Analytical methods

### 2.5.1 | Growth performance

Growth performance was assessed at Week 0 (initial) and Week 12 (final) sampling. Final weight, final length, weight gain, specific growth rate, condition factor, apparent feed intake, feed conversion ratio, and visceral somatic index were determined based on the following calculations:

$$\text{Weight gain (g/fish)} = \text{Final weight} - \text{initial weight (calculated by tank)}$$

$$\text{Condition factor} = \frac{\text{Body mass (g)}}{\text{Length (cm)}^3} * 100 \text{ (calculated by individual fish)}$$

$$\text{Visceral somatic index (\%)} = 100 * \frac{\text{Viscera mass (g)}}{\text{Body mass (g)}} \text{ (calculated by individual fish)}$$

$$\text{Specific growth rate (\%/day)} = \frac{(\ln(\text{final body weight}) - \ln(\text{initial body weight}))}{\text{Number of days in period}} * 100 \text{ (calculated by tank)}$$

$$\text{Apparent feed intake (g/fish)} = \frac{\text{Feed consumed (g)}}{\text{Number of fish per tank}} \text{ (calculated by tank)}$$

$$\text{Feed conversion ratio} = \frac{\text{Feed intake (per fish)}}{\text{Weight gain (g/fish)}} \text{ (calculated by tank)}$$

### 2.5.2 | Diet and tissue nutrient composition

Dry matter and nutritional composition of the diets was analyzed at Department of Agriculture Laboratory Services (Truro, NS, Canada). Crude protein of rainbow trout muscle tissue was analyzed using a LECO FP-528 Nitrogen analyzer (Model FP-528, Leco Cooperation, St. Joseph, MI, USA) using the conversion (% nitrogen × 6.25).

### 2.5.3 | Total lipid and fatty acids

Total lipid and fatty acid content were analyzed from liver and muscle tissue sampled from fish at both Weeks 0 and 12, as well as brain and eye tissue at Week 12. Tissue samples were placed into 2-mL microcentrifuge tubes, flash

**TABLE 2** Diet formulation and composition (g/kg as fed basis) of experimental diets fed to rainbow trout

Ingredient <sup>a</sup> (g/kg)	FO	LCO	HCO
Fish meal	150	150	150
Fish (herring) oil	100	0	0
Transgenic camelina oil <sup>b</sup>	0	12.5	130
Camelina oil <sup>c</sup>	0	117.5	0
Ground wheat	117.5	117.5	117.5
Empyreal (corn protein concentrate)	250	250	250
Canola oil	100	70	70
Poultry by-product meal	170	170	170
Soybean meal	80	80	80
Vitamin and mineral mix <sup>d</sup>	2	2	2
Dicalcium phosphate	20	20	20
Pigment mix <sup>e</sup>	2.5	2.5	2.5
Lysine HCl	5	5	5
Choline chloride	3	3	3
<b>Chemical composition (as fed, g/kg)</b>			
Dry matter	943	937	925
Crude protein	472	489	477
Total lipid	314	260	299

<sup>a</sup>All ingredients were supplied and donated by Northeast Nutrition (Truro, Nova Scotia, Canada).

<sup>b</sup>Produced by Rothamsted Research (West Commons, UK).

<sup>c</sup>Commercial grade Camelina oil, produced by Smart Earth Seeds (Saskatoon, SK, Canada).

<sup>d</sup>Vitamin and mineral premix contains (per kg): zinc, 77.5 mg; manganese, 125 mg; iron, 84 mg; copper, 2.5 mg; iodine, 7.5 mg; vitamin A, 5000 IU; vitamin D, 4000 IU; vitamin K, 2 mg; vitamin B12, 4 µg; thiamine, 8 mg; riboflavin, 18 mg; pantothenic acid, 40 mg; niacin, 100 mg; folic acid, 4 mg; biotin, 0.6 mg; pyridoxine, 15 mg; inositol, 100 mg; ethoxyquin, 42 mg; wheat shorts, 1,372 mg.

<sup>e</sup>Pigment mix contains (per kg): selenium, 0.220 mg; vitamin E, 250 IU; vitamin C, 200 mg; astaxanthin, 60 mg; wheat shorts, 1988 mg.

frozen in liquid nitrogen, and were stored in a  $-80^{\circ}\text{C}$  freezer until they were shipped to Ryerson University (Toronto, ON, Canada) for fatty acid analysis. Muscle and liver tissues were freeze-dried and individually ground to a fine powder in liquid nitrogen using a ceramic mortar and pestle (which were washed with soap and water and lipid-cleaned three times with 2 mL chloroform: methanol [2:1; v/v] between each sample), and the resulting powder was subsampled and weighed to the nearest microgram. Total lipid was extracted using a modified Folch method (Folch, Lees, & Sloane Stanley, 1957), as in Hixson et al. (2016). Briefly, each sample was extracted three times, using 2 mL of chloroform/methanol (2:1; v/v) and then pooled (total 6 mL). Polar impurities were removed by adding 1.6 mL of KCl solution (0.9% w/v). The organic layer was removed using a lipid-cleaned glass pipette and pooled. The resulting lipid-containing solvent was concentrated to 2 mL by evaporating with nitrogen gas. The lipid extract was then prepared for gas chromatography (GC) by derivatizing into fatty acid methyl esters (FAME) using the Hildich reagent (1.5 H<sub>2</sub>SO<sub>4</sub>: 100 anhydrous MeOH) as the catalyst (Christie et al., 2003). Reagents were added in the proportion of 1.5 mL reagent per 4–16 mg of lipid. Samples were heated at 90°C for 90 min and vortexed halfway through the derivatization reaction. The FAME were extracted twice using hexane: diethyl ether (1:1; v/v), then dried under a gentle stream of nitrogen. The dry FAME extract was re-dissolved in hexane and individual FAME were separated using a GC (Shimadzu-2010 Plus, Nakagyo-ku, Kyoto, Japan) equipped with an SP-2560 column (Sigma-Aldrich, St. Louis, Missouri). All solvents used in the extraction and FAME derivatization procedures were of high

**TABLE 3** Fatty acid content ( $\mu\text{g}/\text{mg}$  dry weight) of experimental diets fed to rainbow trout

FA	FO	LCO	HCO
14:0	7.8	0.8	1.1
16:0	33.6	21.7	23.0
16:1n-7	10.0	2.8	3.1
18:0	8.1	7.5	9.9
18:1n-9	78.9	73.0	63.8
18:2n-6 (LNA)	37.0	55.1	56.2
18:3n-3 (ALA)	9.7	48.3	20.8
20:1n-9	4.8	19.7	10.9
20:4n-6 (ARA)	1.5	1.0	4.1
20:5n-3 (EPA)	16.6	2.3	12.9
22:1n-9	0.6	3.3	1.2
22:5n-3	2.0	0.8	6.7
22:6n-3 (DHA)	11.1	2.6	10.8
24:1n-9	0.7	0.9	0.6
$\sum$ SFA	52.7	33.7	39.0
$\sum$ MUFA	111.1	108.9	89.2
$\sum$ PUFA	83.1	116.1	124.2
$\sum$ n-3	42.6	56.5	57.3
$\sum$ n-6	40.5	59.6	66.9
n-3/n-6	1.0	0.9	0.9
Total	246.9	258.7	252.37

Note: Data expressed as  $\mu\text{g}/\text{mg}$  dry weight, values are means ( $n = 3$ )  $\pm$  standard deviation.

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; HCO, high camelina oil; LCO, low camelina oil; LNA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

purity HPLC grade (>99%). FAME in samples were identified by comparison of their retention times with a known standard (GLC-463 reference standard; Nu-chek Prep, Inc., Waterville, Minnesota) and quantified with a five-point calibration curve using this same standard. A known concentration of 5 $\alpha$ -cholestane (C8003, Sigma-Aldrich, St. Louis, MI, USA) was added to each sample prior to extraction to act as the internal standard to estimate extraction and instrument recovery efficiency. FAME were sent from Ryerson University to the University of Toronto (Toronto, ON, Canada) for CSIA.

### 2.5.4 | Compound-specific stable carbon isotope analysis

The  $\delta^{13}\text{C}$  values of DHA were analyzed by GC-combustion-isotope mass spectrometry (GC-C-IRMS) following methods by Lacombe, Giuliano, Colombo, Arts, and Bazinet (2017). FAME from transgenic camelina oil, FO, and rainbow trout muscle tissue from FO and HCO groups were analyzed. All  $\delta^{13}\text{C}$  values were reported relative to the Vienna Pee Dee Belemnite standard. An aliquot of the methanol used during methylation of FA was analyzed for the  $\delta^{13}\text{C}$  composition. The average  $\delta^{13}\text{C}$  of methanol was used to correct for the additional methyl group added to FA during transesterification, by subtracting the proportional contribution of methanol to the  $\delta^{13}\text{C}$  of FAME.

### 2.5.5 | Sensory properties

Texture and color analyses were completed at the Faculty of Agriculture, Dalhousie University (Truro, NS, Canada). Texture and color analyses were performed on fillet samples ( $n = 9$  per treatment) that were previously frozen and thawed prior to testing. Fillets were transferred to a refrigerator ( $4^{\circ}\text{C}$ ) 24 hr prior to sampling to thaw before analysis was undertaken (FAO, 2018). Each fillet was analyzed mid-loin, below the dorsal fin and above the lateral line. Each fillet sample was analyzed twice, and the average was used for data analysis.

Texture of the fillets was evaluated utilizing a TA.XT texture analyzer (Texture Technologies Corp., Scarsdale, New York, USA) equipped with a 9.5 mm cylindrical probe and data was recorded using Exponent stable micro systems software. The probe was pressed into the fillet at a pre-test speed of 5.0 mm/s. The probe penetration depth was 50% of the fillet thickness. The fillet thickness (mm) and the necessary force required to penetrate the surface (the breakpoint force) along with the maximum force (max force) reached during compression were recorded generating a fillet texture average.

The instrumental color analysis of the fillets was completed using a Hunter Lab MiniScan EZ 45/0 LAV (Hunter Associates Lab, Inc., Reston, VA, USA) colorimeter with EZMQC-OPT EasyMatch Quality Control Software. Color analysis was completed on the same fillet samples used for texture analysis. The Hunter color scale was used to determine lightness ( $\Delta L$ ), redness ( $\Delta a$ ), and yellowness ( $\Delta b$ ). Values were generated using daylight setting (D65, CIE L, a, b).

Fish were sampled for the sensory evaluation panel 1 day after the Week 12 final sampling period. The HCO and FO treatments only were presented to the sensory evaluation panel as the most distinct treatments that potentially could have detectable differences to untrained panelists. Prior to sampling, three fish from each dietary group were euthanized via submersion in tricaine methanesulfonate (MS-222) until clinical death was observed. Fish were filleted and portioned into  $\sim 3 \times 3 \text{ cm}^2$  squares from the mid-loin region, below the dorsal fin and above the lateral line, and placed into clear plastic sampling cups. The sensory evaluation was completed in a lab with standard lighting at the Aquaculture Centre. An untrained panel ( $n = 24$ ) of volunteers was recruited that consisted of faculty, staff, and students at the Faculty of Agriculture, Dalhousie University. Panelists were briefed in the testing procedure and fillet evaluation techniques. Untrained panelists were used in this study to evaluate consumer preference, to complement the instrumental analyses for color and texture. Panelists were introduced to different evaluation techniques (e.g., smelling and evaluating texture and color). Evaluation of the different testing procedures was discussed. The panelists completed a triangle test, a hedonic test, and a quantitative descriptive analysis (QDA) as in Hixson, Parrish, and D.M. (2014); Hixson et al. (2017) to evaluate fillet samples in terms of appearance, texture, odor, and color. Participants were given specific instructions on how to evaluate the given samples for appearance, texture, odor, and color, prior to beginning the test. Three fillet samples were provided to each volunteer: two fillet samples from the FO control group and one was from the HCO diet group. The triangle test required panelists to determine which one of the three provided samples appeared to be different in terms of texture, odor, color, and appearance. For 24 panelists, 13 assessors in a triangle test are required to give correct judgments at the 5% level. In the hedonic test, panelists were asked to rate fillets on a two anchored linear scale for odor, texture, and appearance of individual samples as per (Hixson et al., 2017; Hixson, Parrish, & D.M., 2014). Panelists were also required to complete an objective QDA test in which they were asked to rate fillet samples on a scale from 1 to 7 (1 = no intensity; 7 = distinct intensity) for a number of traits (such as brightness and orange intensity) as per (Hixson et al., 2017; Hixson, Parrish, & D.M., 2014).

### 2.6 | Statistical analysis

Growth performance results were analyzed by ANOVA using the general linear model in Minitab 18 Statistical Software. For individual measurements (e.g., weight, length, viscera somatic index, condition factor), a two-level

nested ANOVA was used to analyze growth data. This model was designed to test the effect of diet treatment (fixed factor) on the growth performance (response variable) and nested fish individuals (random factor) within tanks, to remove variability among fish within tanks, while also testing for effects of individual tanks (Ruohonen, 1998). For measurements that were based on tank means, fish individuals were not independent (e.g., weight gain, specific growth rate, feed intake, feed conversion ratio), a one-way ANOVA was conducted to test the effect of diet. Tukey' HSD post-hoc tests ( $p < .05$  significance level) were applied to assess differences among treatments.

For lipid and fatty acid content of the muscle, liver, brain and eye tissue, as well as instrumental analyses of color and texture, a one-way ANOVA was used to detect treatment differences, followed by a Tukey' post-hoc test for multiple comparisons ( $p < .05$  significance level). Multivariate analyses, including permutational multivariate analysis of variance (PERMANOVA) and Principal Coordinates Analysis (PCoA) were used to determine whole fatty acid profile changes in individual fish tissues among treatments. The non-metric Bray–Curtis dissimilarity statistic was used to quantify the compositional dissimilarity between samples in the PCO plot (Bray & Curtis, 1957). Multivariate statistics were conducted using PRIMER (PRIMER-E, version 7.0.13, Plymouth, UK).

### 3 | RESULTS

#### 3.1 | Growth performance

There was a significant difference in final weight, with trout fed the HCO and LCO diets were larger than trout fed the FO diet ( $p = .001$ ; Table 4). Trout fed the HCO diet also had a longer fork length than fish fed the FO diets ( $p = .008$ ). Final VSI of fish fed the LCO diet was found to be higher in comparison to fish fed the FO diet ( $p = .014$ ). There were no significant differences between the final VSI of fish fed LCO and HCO diets and FO and HCO diets. There were no significant differences in the initial weight ( $p = .328$ ), initial length ( $p = .062$ ), initial condition factor ( $p = .422$ ), final condition factor ( $p = .099$ ), initial VSI ( $p = .640$ ), SGR ( $p = .092$ ), weight gain ( $p = .155$ ), FCR ( $p = .145$ ), and apparent feed intake ( $p = .144$ ) among treatments.

#### 3.2 | Protein and dry matter composition of muscle and liver

There was no difference in protein content of muscle tissue among rainbow trout depending on treatment in either Week 0 or Week 12 ( $p > .05$ ) (Table 5). Muscle tissue dry weight showed no differences among treatments (Table 5). Liver tissue at Week 0 from trout fed FO diets had higher dry matter content than trout that were fed the LCO diet ( $p = .021$ ) (Table 6). At Week 12, liver tissue showed no differences in dry matter content in fish fed any of the experimental diets ( $p = .099$ ).

#### 3.3 | Fatty acid content of muscle tissue

Overall, the fatty acid content ( $\mu\text{g}/\text{mg}$ , dry weight) of rainbow trout muscle tissue showed notable differences in fish fed diets containing transgenic camelina oil in comparison to the FO diet (Table 5). ALA was significantly higher in fish fed LCO diets when compared to HCO and FO diets ( $p < .0001$ ). EPA accumulated in higher amounts in trout fed the FO diet compared to trout fed the LCO diet; however, no differences were found between fish fed FO and HCO diets ( $p = .014$ ). DHA was also stored in higher amounts in trout fed both FO and HCO diets in comparison to trout fed LCO diets but did not differ between trout fed FO and HCO ( $p < .0001$ ). Total MUFA in muscle tissue was

**TABLE 4** Growth performance of rainbow trout fed experimental diets for 12 weeks

Parameters	FO	LCO	HCO	p-value
Initial weight <sup>a</sup>	50.8 ± 7.9	46.4 ± 12.9	52.1 ± 11.2	.328
Final weight <sup>b</sup>	178.5 ± 30.7b	193.3 ± 25.0a	197.6 ± 25.0a	.001
Weight gain <sup>c</sup>	127.5 ± 8.8	146.9 ± 12.1	145.5 ± 13.4	.115
Initial length	15.7 ± 0.8	14.8 ± 0.9	15.6 ± 0.9	.062
Final length	23.5 ± 1.3b	23.9 ± 1.1ab	24.2 ± 1.4a	.008
Initial CF <sup>d</sup>	1.33 ± 0.15	1.39 ± 0.12	1.35 ± 0.12	.422
Final CF	1.37 ± 0.12	1.41 ± 0.10	1.39 ± 0.1	.099
Initial VSI <sup>e</sup>	12.47 ± 2.9	12.52 ± 2.9	11.77 ± 1.1	.640
Final VSI	10.6 ± 1.0 b	11.7 ± 1.2 a	10.9 ± 1.0 ab	.014
SGR <sup>f</sup>	1.44 ± 0.3	1.75 ± 0.5	1.59 ± 0.4	.092
AFI <sup>g</sup>	143.3 ± 2.2	139.3 ± 1.2	145.4 ± 4.2	.095
FCR <sup>h</sup>	1.13 ± 0.09	0.95 ± 0.08	1.01 ± 0.11	.145

Note: Means with different lowercase letters indicate significant differences among treatments ( $p > .05$ ).

Abbreviations: FO, fish oil; HCO, high camelina oil; LCO, low camelina oil.

<sup>a</sup>Initial measurements are mean ± standard deviation, body weight (g/fish), fork length (cm/fish);  $n = 45$  per treatment.

<sup>b</sup>Final measurements are mean ± standard deviation, body weight (g/fish), fork length (cm/fish). Weight, length, and condition factor are calculated from individual fish;  $n = 58$  (FO);  $n = 60$  (LCO);  $n = 58$  (HCO) per treatment.

<sup>c</sup>Weight gain (g/fish) = Final weight – initial weight (calculated by tank means;  $n = 3$ ).

<sup>d</sup>Condition factor = Body mass/length (cm)<sup>3</sup> (calculated by individual fish).

<sup>e</sup>Visceral somatic index (%) =  $100 * (\text{viscera mass}/\text{body mass})$ .

<sup>f</sup>Specific growth rate =  $(\ln(\text{final body weight}) - \ln(\text{initial body weight})) / \text{number of days in period} * 100$ .

<sup>g</sup>Apparent feed intake (g/fish) = Feed consumed (g)/number of fish per tank (calculated by tank means).

<sup>h</sup>(g/fish) = Feed intake (g/fish)/weight gain (g/fish) (calculated by tank means).

higher in fish fed FO diets than in fish fed HCO diets. The n-3/n-6 ratio was higher in trout fed the FO diet than trout fed the LCO and HCO diets ( $p < .0001$ ). No differences were found in LNA ( $p = .089$ ), total PUFA ( $p = .312$ ), total n-3, or total n-6 in the muscle tissue. Total lipid (wet weight and dry weight) in trout muscle was not different among treatments; however, total lipid in muscle tissue increased from Week 0 to Week 12, regardless of dietary treatment. Reported FA in Table 5 are  $>1 \mu\text{g}/\text{mg}$ ; the full profile of all reported FA for the muscle tissue is available in Table S1.

### 3.4 | Fatty acid content of liver tissue

Overall, the fatty acid content of liver tissue differed based on dietary treatment (Table 6). ALA was higher in trout that were fed the LCO and HCO diets in comparison to FO diets ( $p < .001$ ). LNA was higher in trout fed LCO and HCO diets in comparison to trout fed FO diets ( $p = .003$ ). Total n-6 stored was higher in trout fed LCO and HCO diets compared to trout fed FO diets ( $p < .0001$ ). The n-3/n-6 ratio was highest in trout fed FO diets in comparison to trout fed LCO and HCO diets ( $p < .0001$ ). There were no differences in LNA ( $p = .295$ ), EPA ( $p = .722$ ), DHA ( $p = .087$ ), total SFA ( $p = .052$ ), total MUFA ( $p = .765$ ), and total PUFA ( $p$ -value) among treatments. Total lipid (wet weight and dry weight) of the liver was not different among treatments; however, increased from Week 0 to Week 12, regardless of dietary treatment. Reported FA in Table 5 are  $>1 \mu\text{g}/\text{mg}$ ; the full profile of all reported FA for the muscle tissue is available in Table S2.

**TABLE 5** Fatty acid content ( $\mu\text{g}/\text{mg}$  dry weight), total fat, protein, and dry matter of rainbow trout muscle tissue from Week 0 (initial) and Week 12

Fatty acid	Initial	FO	LCO	HCO	F-value	p-value
14:0	8.9 $\pm$ 4.9	5.8 $\pm$ 2.3 <sup>a</sup>	1.6 $\pm$ 0.7 <sup>b</sup>	1.4 $\pm$ 0.4 <sup>b</sup>	46.45	.000
16:0	51.7 $\pm$ 26.7	32.3 $\pm$ 11.2 <sup>a</sup>	24.7 $\pm$ 7.9 <sup>b</sup>	22.0 $\pm$ 4.2 <sup>b</sup>	6.15	.005
16:1n-7c	15.8 $\pm$ 8.8	8.0 $\pm$ 3.1 <sup>a</sup>	3.7 $\pm$ 1.5 <sup>b</sup>	3.2 $\pm$ 0.8 <sup>b</sup>	24.57	.000
18:0	11.4 $\pm$ 5.8	8.2 $\pm$ 2.8	7.6 $\pm$ 2.6	7.8 $\pm$ 1.8	.19	.831
18:1n-9c	57.6 $\pm$ 31.8	61.2 $\pm$ 23.5 <sup>a</sup>	56.6 $\pm$ 20.9 <sup>ab</sup>	43.4 $\pm$ 11.4 b	3.43	.042
18:1n-7c	8.5 $\pm$ 4.4	5.9 $\pm$ 2.2 <sup>a</sup>	4.1 $\pm$ 1.5 <sup>b</sup>	3.8 $\pm$ 1.0 b	6.60	.003
18:2n-6 (LNA)	22.5 $\pm$ 11.8	25.2 $\pm$ 9.61	33.5 $\pm$ 12.8	31.9 $\pm$ 9.0	2.56	.089
20:1n-9	5.2 $\pm$ 2.8	4.2 $\pm$ 1.6 <sup>b</sup>	10.6 $\pm$ 4.4 <sup>a</sup>	6.21 $\pm$ 1.6 b	20.32	.000
18:3n-3 (ALA)	4.3 $\pm$ 2.3	6.2 $\pm$ 2.4 <sup>b</sup>	23.6 $\pm$ 8.9 <sup>a</sup>	10.9 $\pm$ 2.7c	39.77	.000
18:4n-3	3.0 $\pm$ 1.6	1.4 $\pm$ 0.5 <sup>b</sup>	2.7 $\pm$ 1.0 <sup>a</sup>	1.3 $\pm$ 0.4 b	22.12	.000
20:2n-6	2.1 $\pm$ 1.1	1.8 $\pm$ 0.7 <sup>b</sup>	2.9 $\pm$ 1.0 <sup>a</sup>	2.0 $\pm$ 0.5 b	8.61	.001
20:3n-6	1.1 $\pm$ 0.6	0.7 $\pm$ 0.3	1.5 $\pm$ 0.5	1.6 $\pm$ 0.4	22.13	.000
22:1n-11	8.9 $\pm$ 5.5	2.9 $\pm$ 1.0 <sup>a</sup>	2.4 $\pm$ 1.0 <sup>ab</sup>	2.1 $\pm$ 0.6b	3.37	.044
22:1n-9	2.5 $\pm$ 1.1	0.6 $\pm$ 0.2 <sup>b</sup>	1.8 $\pm$ 0.7 <sup>a</sup>	0.8 $\pm$ 0.2 b	32.74	.000
20:3n-3	0.2 $\pm$ 0.1	0.4 $\pm$ 0.2 <sup>c</sup>	1.8 $\pm$ 0.6 <sup>a</sup>	1.1 $\pm$ 0.2 b	47.10	.000
20:4n-6 (ARA)	0.1 $\pm$ 0.1	1.6 $\pm$ 0.5 <sup>b</sup>	1.7 $\pm$ 0.4 <sup>b</sup>	3.0 $\pm$ 0.6 a	38.19	.000
24:0	8.9 $\pm$ 4.9	2.6 $\pm$ 3.4	1.4 $\pm$ 1.3	2.9 $\pm$ 2.7	1.40	.258
20:5n-3 (EPA)	0.7 $\pm$ 0.4	5.8 $\pm$ 4.8 <sup>a</sup>	2.1 $\pm$ 1.3 <sup>b</sup>	3.5 $\pm$ 2.7ab	4.74	.014
22:5n-3	3.6 $\pm$ 1.8	3.0 $\pm$ 0.9 <sup>b</sup>	1.3 $\pm$ 0.4 <sup>c</sup>	3.7 $\pm$ 0.8 a	44.28	.000
22:6n-3 (DHA)	44.0 $\pm$ 19.9	27.6 $\pm$ 7.9 <sup>a</sup>	15.4 $\pm$ 2.8 <sup>b</sup>	22.7 $\pm$ 4.8 a	18.28	.000
$\Sigma$ SFA	74.1 $\pm$ 38.4	50.6 $\pm$ 17.5 <sup>a</sup>	37.2 $\pm$ 12.2 <sup>b</sup>	36.2 $\pm$ 8.7 <sup>b</sup>	5.48	.008
$\Sigma$ MUFA	113.6 $\pm$ 61.8	87.6 $\pm$ 32.9 <sup>a</sup>	82.3 $\pm$ 28.0 <sup>ab</sup>	62.2 $\pm$ 16.1 <sup>b</sup>	3.80	.030
$\Sigma$ PUFA	101.0 $\pm$ 48.0	74.5 $\pm$ 24.8	88.0 $\pm$ 28.7	83.8 $\pm$ 18.8	1.20	.312
$\Sigma$ n-3	70.9 $\pm$ 32.8	44.4 $\pm$ 14.3	46.9 $\pm$ 13.6	43.3 $\pm$ 8.28	.35	.707
$\Sigma$ n-6	30.2 $\pm$ 15.5	30.1 $\pm$ 11.2 <sup>a</sup>	40.9 $\pm$ 15.0 <sup>b</sup>	40.4 $\pm$ 11.0 <sup>b</sup>	3.56	.037
n-3/n-6	2.4 $\pm$ 0.4	1.5 $\pm$ 0.2 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>b</sup>	1.1 $\pm$ 0.1 <sup>b</sup>	27.14	.000
<b>Total lipid, protein, dry matter (g/kg)</b>						
Lipid ww	34 $\pm$ 10	48 $\pm$ 18	52 $\pm$ 17	45 $\pm$ 11	.81	.452
Lipid dw	150 $\pm$ 40	205 $\pm$ 73	218 $\pm$ 66	191 $\pm$ 44	.67	.515
Protein dw	765 $\pm$ 117	714 $\pm$ 41	692 $\pm$ 42	717 $\pm$ 39	1.67	.200
Dry matter	226 $\pm$ 9.0	234 $\pm$ 11	239 $\pm$ 8.0	234 $\pm$ 7.3	1.14	.330

Note: Data expressed as  $\mu\text{g}$  FAME/mg (dry weight), values are means ( $n = 3$  per treatment)  $\pm$  standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. The full fatty acid profile can be found in the Table S1. Reported fatty acids are  $>1 \mu\text{g}/\text{mg}$ .

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; dw, dry weight; EPA, eicosapentaenoic acid; FO, fish oil; HCO, high camelina oil; LCO, low camelina oil; LNA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; ww, wet weight..

### 3.5 | Fatty acid content of brain tissue

There were significant differences in brain FA based on dietary treatment (Table 7). LNA was higher in trout fed LCO diets in comparison to trout fed HCO or FO diets ( $p = .007$ ). ALA was highest in trout fed the LCO diet compared to trout fed

**TABLE 6** Fatty acid content ( $\mu\text{g}/\text{mg}$  dry weight), total lipid, protein, and dry matter of rainbow trout liver tissue from Week 0 (initial) and Week 12

Fatty acid	Initial	FO	LCO	HCO	F-value	p-value
14:0	3.0 $\pm$ 2.0	1.1 $\pm$ 0.2 <sup>a</sup>	0.4 $\pm$ 0.1 <sup>b</sup>	0.5 $\pm$ 0.2 <sup>b</sup>	52.19	.000
16:0	41.3 $\pm$ 22.4	18.5 $\pm$ 2.2 <sup>a</sup>	15.8 $\pm$ 2.1 <sup>b</sup>	16.6 $\pm$ 2.9 <sup>ab</sup>	4.89	.012
16:1n-7c	7.7 $\pm$ 5.5	1.1 $\pm$ 0.3 <sup>a</sup>	0.6 $\pm$ 0.2 <sup>b</sup>	0.8 $\pm$ 0.3 <sup>b</sup>	13.50	.000
18:0	14.7 $\pm$ 7.3	7.3 $\pm$ 1.4	7.2 $\pm$ 1.2	7.0 $\pm$ 1.4	.09	.916
18:1n-9c	37.3 $\pm$ 21.8	11.7 $\pm$ 2.9	12.3 $\pm$ 2.3	12.8 $\pm$ 4.3	.46	.637
18:1n-7c	6.4 $\pm$ 3.5	1.5 $\pm$ 0.4 <sup>a</sup>	1.2 $\pm$ 0.2 <sup>b</sup>	1.3 $\pm$ 0.4 <sup>ab</sup>	4.05	.025
18:2n-6 (LNA)	6.8 $\pm$ 4.1	5.4 $\pm$ 1.6 <sup>b</sup>	7.3 $\pm$ 1.5 <sup>a</sup>	7.6 $\pm$ 2.3 <sup>a</sup>	6.54	.003
18:3n-3 (ALA)	0.9 $\pm$ 0.6	1.1 $\pm$ 0.5 <sup>b</sup>	2.7 $\pm$ 1.0 <sup>a</sup>	2.7 $\pm$ 1.7 <sup>a</sup>	9.16	.000
20:2n-6	2.2 $\pm$ 1.2	0.8 $\pm$ 0.3 <sup>b</sup>	1.2 $\pm$ 0.3 <sup>ab</sup>	1.4 $\pm$ 0.8 <sup>a</sup>	6.57	.003
20:3n-6	2.6 $\pm$ 1.0	0.6 $\pm$ 0.2 <sup>b</sup>	1.6 $\pm$ 0.5 <sup>a</sup>	1.4 $\pm$ 0.6 <sup>a</sup>	15.29	.000
20:4n-6 (ARA)	7.8 $\pm$ 4.4	4.2 $\pm$ 1.4 <sup>b</sup>	5.5 $\pm$ 1.1 <sup>a</sup>	5.7 $\pm$ 1.4 <sup>a</sup>	5.75	.006
24:0	0.0 $\pm$ 0.1	1.8 $\pm$ 2.4	1.3 $\pm$ 1.7	1.0 $\pm$ 1.8	.64	.530
20:5n-3 (EPA)	11.8 $\pm$ 6.7	4.4 $\pm$ 2.6	3.8 $\pm$ 2.3	3.8 $\pm$ 2.4	.33	.722
22:5n-3	4.0 $\pm$ 2.2	2.0 $\pm$ 0.4	1.8 $\pm$ 0.5	2.1 $\pm$ 0.7	.96	.390
22:6n-3 (DHA)	100.0 $\pm$ 54.0	32.1 $\pm$ 7.9	27.0 $\pm$ 4.6	29.0 $\pm$ 5.6	2.59	.087
$\Sigma\text{SFA}$	60.5 $\pm$ 31.6	29.6 $\pm$ 5.3	25.5 $\pm$ 3.9	26.1 $\pm$ 5.1	3.17	.052
$\Sigma\text{MUFA}$	66.4 $\pm$ 38.5	17.3 $\pm$ 4.2	17.9 $\pm$ 3.3	18.7 $\pm$ 6.8	.27	.761
$\Sigma\text{PUFA}$	140.9 $\pm$ 75.9	51.5 $\pm$ 11.7	52.6 $\pm$ 8.2	55.1 $\pm$ 10.3	.51	.605
$\Sigma\text{EPA \& DHA}$	4.1 $\pm$ 2.2	36.6 $\pm$ 8.4	30.8 $\pm$ 5.2	32.8 $\pm$ 6.3	2.80	.072
$\Sigma\text{n-3}$	118.1 $\pm$ 63.9	40.1 $\pm$ 9.1	36.5 $\pm$ 5.8	38.6 $\pm$ 7.0	.90	.415
$\Sigma\text{n-6}$	22.8 $\pm$ 12.4	11.4 $\pm$ 3.1 <sup>b</sup>	16.1 $\pm$ 2.8 <sup>a</sup>	16.5 $\pm$ 4.3 <sup>a</sup>	10.29	.000
n-3/n-6	5.2 $\pm$ 0.7	3.6 $\pm$ 0.5 <sup>a</sup>	2.3 $\pm$ 0.2 <sup>b</sup>	2.4 $\pm$ 0.5 <sup>b</sup>	37.08	.000
<b>Total lipid, protein, dry matter (g/kg)</b>						
Lipid ww	44 $\pm$ 1.3	34 $\pm$ 0.8	34 $\pm$ 1.0	31 $\pm$ 8.0	.70	.501
Lipid dw	186 $\pm$ 58	136 $\pm$ 20	140 $\pm$ 39	126 $\pm$ 24	.92	.406
Dry matter	236 $\pm$ 35	252 $\pm$ 43	245 $\pm$ 31	245 $\pm$ 8.0	.24	.786

Note: Data expressed as  $\mu\text{g}$  FAME/mg (dry weight), values are means ( $n = 3$  per treatment)  $\pm$  standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. Reported fatty acids are  $>1$   $\mu\text{g}/\text{mg}$ ; the full fatty acid profile can be found in the Table S2.

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; dw, dry weight; EPA, eicosapentaenoic acid; FO, fish oil; HCO, high camelina oil; LCO, low camelina oil; LNA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; ww, wet weight.

either the HCO or FO diets ( $p < .0001$ ). Arachidonic acid (ARA; 20:4n-6) was higher in trout fed LCO and HCO compared with trout fed FO ( $p < .001$ ). Total MUFA was highest in trout fed LCO diets in comparison to HCO diets; however, total MUFA in fish fed both LCO and HCO diets was not different than FO ( $p = .024$ ). Total PUFA was found to be highest in trout fed LCO diets in comparison to FO and HCO diets ( $p = .012$ ). Total n-3 in trout fed LCO diets was higher than for fish fed HCO diets; however, total n-3 in fish fed both LCO and HCO diets was not different than fish fed the FO diet ( $p = .039$ ). Total n-6 was highest in trout fed LCO diets in comparison to FO and HCO ( $p = .001$ ). Trout fed FO diets had a higher n-3/n-6 ratio in the brain in comparison to trout fed the LCO and HCO diets ( $p < .001$ ). There were no differences depending on treatment in EPA ( $p = .280$ ), DHA ( $p = .207$ ), and total SFA ( $p = .093$ ). Reported FA in Table 5 are  $>1$   $\mu\text{g}/\text{mg}$ ; the full profile of all reported FA for the muscle tissue is available in Table S3.

### 3.6 | Fatty acid content of eye tissue

Overall, rainbow trout eye tissue showed differences in FA content depending on treatment (Table 8). LNA was significantly higher in eye tissue in fish fed HCO and LCO diets in comparison to the FO control ( $p < .001$ ). ALA was higher in eye tissue of fish fed LCO diets in comparison to fish fed HCO and FO diets ( $p < .001$ ). EPA was higher in trout fed HCO and FO diets in comparison to LCO ( $p < .0001$ ). DHA was higher in eye tissue of fish fed HCO diets in comparison to fish fed the FO and LCO diets ( $p < .001$ ). Total PUFA was higher in trout fed the HCO and LCO diets when compared to fish fed FO ( $p < .001$ ). Total SFA ( $p = .365$ ), n-3/n-6 ratios ( $p = .264$ ), and MUFA ( $p = .385$ ) were not different among treatments. Reported FA in Table 5 are  $>1 \mu\text{g}/\text{mg}$ ; the full profile of all reported FA for the muscle tissue is available in Table S4.

### 3.7 | Multivariate analyses of fatty acid data

PERMANOVA results indicated that the spatial dispersion of groups was not equivalent, indicating a difference in FA profiles depending on both diet ( $F = 5.9257$ ;  $p = .002$ ) and tissue type ( $F = 2.9426$ ;  $p = .001$ ). This was apparent in the PCoA plot (Figure 1), with 91.6% of the variation was accounted for, mainly in PCO1 (74.6%), where strong distinction among tissue type was evident along the PCO1 axis. PCO2 (17%) also shows distinction among treatments, although not as distinctly separated as tissue type.

### 3.8 | Compound-specific stable isotope analysis

The  $\delta^{13}\text{C}$  value for DHA in FO ( $-27.3$ ) was isotopically different than the transgenic camelina oil ( $-34.10$ ) used in this study. Consequently, the  $\delta^{13}\text{C}$  values for DHA for the HCO diet ( $-32.85$ ) and FO diet ( $-28.91$ ) were also isotopically different. The  $\delta^{13}\text{C}$  values for DHA in muscle tissue were significantly different between the trout fed the HCO diet compared with the FO diet (Figure 2). The  $\delta^{13}\text{C}$  for DHA in muscle tissue of trout fed HCO ( $-30.8 \pm 1.12$ ) was isotopically lighter when compared to trout fed FO ( $-26.75 \pm 0.84$ ;  $p = .001$ ).

### 3.9 | Sensory properties

There was no difference in fillet texture among treatments ( $p = .377$ ; Table 9). Based on the color analysis, there were no differences in the Hunter lab scale parameters L ( $p = .066$ ), a ( $p = .895$ ), or b scores ( $p = .581$ ) depending on treatment.

Considering the results of the sensory panel, or the triangle test, nine out of 24 panelists chose the correct sample as the "odd" fillet compared with the other two, which is not significant (13/24 assessors are required to give correct judgment at the 5% level). For the hedonic test, participants detected a significant difference in fillet texture, where HCO fillets were more elastic in comparison to FO fillets, which was noted to be slightly firmer (Table 9). There was no significant difference in appearance or odor based on the hedonic test. For the QDA, fillets from the HCO treatment were found to be more orange in color ( $p = .012$ ) and firm in texture ( $p = .001$ ) compared with fillets from the FO control (Figure 3). No significant differences were found in fillet surface moistness, marine odor, vegetable odor, rancid odor, texture, and brightness between salmon fed the HCO and FO diets.

## 4 | DISCUSSION

One of the primary issues faced by aquaculture is the need to identify sustainable sources of EPA and DHA for use in aquafeeds meet the nutritional demands of cultured fish and for the consumers of fish. As health-related benefits

**TABLE 7** Fatty acid content ( $\mu\text{g}/\text{mg}$  dry weight), total lipid and dry matter of rainbow trout brain tissue after 12 weeks of feeding

Fatty acid	FO	LCO	HCO	F-value	p-value
14:0	3.5 $\pm$ 1.6 <sup>a</sup>	2.0 $\pm$ 0.8 <sup>b</sup>	1.4 $\pm$ 0.2 <sup>b</sup>	15.03	.000
16:0	72.3 $\pm$ 21.2	80.7 $\pm$ 22.5	64.7 $\pm$ 13.2	2.36	.107
16:1n-7c	7.3 $\pm$ 2.5 <sup>a</sup>	6.9 $\pm$ 1.7 <sup>ab</sup>	5.3 $\pm$ 0.9 <sup>b</sup>	4.49	.017
18:0	30.3 $\pm$ 7.3	34.0 $\pm$ 8.7	27.5 $\pm$ 5.2	2.81	.072
18:1n-9c	104.6 $\pm$ 26.6 <sup>ab</sup>	123.3 $\pm$ 32.5 <sup>a</sup>	95.7 $\pm$ 15.9 <sup>b</sup>	4.13	.023
18:1n-7c	9.9 $\pm$ 2.4	10.4 $\pm$ 2.3	8.8 $\pm$ 1.5	2.05	.142
18:2n-6 (LNA)	11.3 $\pm$ 5.5 <sup>b</sup>	17.5 $\pm$ 8.4 <sup>a</sup>	10.4 $\pm$ 3.3 <sup>b</sup>	5.57	.007
20:1n-9	7.6 $\pm$ 2.1 <sup>b</sup>	12.3 $\pm$ 3.0 <sup>a</sup>	8.5 $\pm$ 1.9 <sup>b</sup>	15.50	.000
18:3n-3 (ALA)	3.6 $\pm$ 1.4 <sup>b</sup>	11.9 $\pm$ 5.3 <sup>a</sup>	4.5 $\pm$ 0.9 <sup>b</sup>	29.40	.000
18:4n-3	0.8 $\pm$ 0.6 <sup>b</sup>	1.7 $\pm$ 1.2 <sup>a</sup>	0.6 $\pm$ 0.4 <sup>b</sup>	7.38	.002
20:2n-6	1.6 $\pm$ 0.5 <sup>b</sup>	2.7 $\pm$ 0.7 <sup>a</sup>	1.7 $\pm$ 0.4 <sup>b</sup>	18.15	.000
22:3n-3	2.3 $\pm$ 0.6	2.8 $\pm$ 0.7	2.5 $\pm$ 0.7	2.21	.123
20:3n-6	0.9 $\pm$ 0.3 <sup>c</sup>	2.5 $\pm$ 0.7 <sup>a</sup>	1.3 $\pm$ 0.1 <sup>b</sup>	49.19	.000
22:1n-9	1.9 $\pm$ 0.4 <sup>b</sup>	2.7 $\pm$ 0.6 <sup>a</sup>	2.0 $\pm$ 0.5 <sup>b</sup>	9.71	.000
20:3n-3	1.0 $\pm$ 0.3 <sup>c</sup>	2.8 $\pm$ 0.7 <sup>a</sup>	1.6 $\pm$ 0.3 <sup>b</sup>	65.93	.000
20:4n-6 (ARA)	4.9 $\pm$ 1.1 <sup>b</sup>	8.0 $\pm$ 2.0 <sup>a</sup>	7.2 $\pm$ 1.4 <sup>a</sup>	16.46	.000
24:0	1.1 $\pm$ 0.3	1.2 $\pm$ 0.3	1.0 $\pm$ 0.2	1.77	.183
20:5n-3 (EPA)	19.2 $\pm$ 4.3	19.6 $\pm$ 4.9	17.3 $\pm$ 2.7	1.31	.280
24:1n-9	22.1 $\pm$ 5.1	24.5 $\pm$ 7.3	20.9 $\pm$ 5.2	1.29	.286
22:4n-6	2.6 $\pm$ 3.3	3.8 $\pm$ 5.2	4.4 $\pm$ 5.2	.55	.583
22:5n-3	8.2 $\pm$ 2.2	8.2 $\pm$ 1.8	7.9 $\pm$ 1.9	.11	.896
22:6n-3 (DHA)	85.0 $\pm$ 20.7	94.9 $\pm$ 25.7	80.0 $\pm$ 19.8	1.64	.207
$\Sigma$ SFA	110.3 $\pm$ 29.8	120.4 $\pm$ 31.8	97.1 $\pm$ 19.1	2.53	.093
$\Sigma$ MUFA	162.5 $\pm$ 40.3 <sup>ab</sup>	189.0 $\pm$ 47.4 <sup>a</sup>	147.7 $\pm$ 24.6 <sup>b</sup>	4.11	.024
$\Sigma$ PUFA	142.2 $\pm$ 33.7 <sup>b</sup>	177.8 $\pm$ 43.4 <sup>a</sup>	140.3 $\pm$ 28.3 <sup>b</sup>	4.97	.012
$\Sigma$ EPA and DHA	104.2 $\pm$ 24.6	114.5 $\pm$ 30.3	97.2 $\pm$ 22.2	1.57	.221
$\Sigma$ n-3	117.8 $\pm$ 27.6 <sup>ab</sup>	139.1 $\pm$ 33.7 <sup>a</sup>	111.8 $\pm$ 24.1 <sup>b</sup>	3.52	.039
$\Sigma$ n-6	22.0 $\pm$ 8.1 <sup>b</sup>	35.8 $\pm$ 13.0 <sup>a</sup>	26.0 $\pm$ 4.8 <sup>b</sup>	8.55	.001
n-3/n-6	5.7 $\pm$ 1.2 <sup>a</sup>	4.1 $\pm$ 1.0 <sup>b</sup>	4.3 $\pm$ 0.7 <sup>b</sup>	11.32	.000
<b>Total lipid and dry matter (g/kg)</b>					
Lipid ww	79 $\pm$ 27	62 $\pm$ 26	79 $\pm$ 9.0	2.97	.062
Lipid dw	404 $\pm$ 132	329 $\pm$ 151	418 $\pm$ 42	2.42	.102
Dry matter	195 $\pm$ 9.0	194 $\pm$ 19	191 $\pm$ 11	.31	.733

Note: Data expressed as  $\mu\text{g}$  FAME/mg (dry weight), values are means ( $n = 3$  per treatment)  $\pm$  standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. Reported fatty acids are  $>1$   $\mu\text{g}/\text{mg}$ ; the full fatty acid profile can be found in the Table S3.

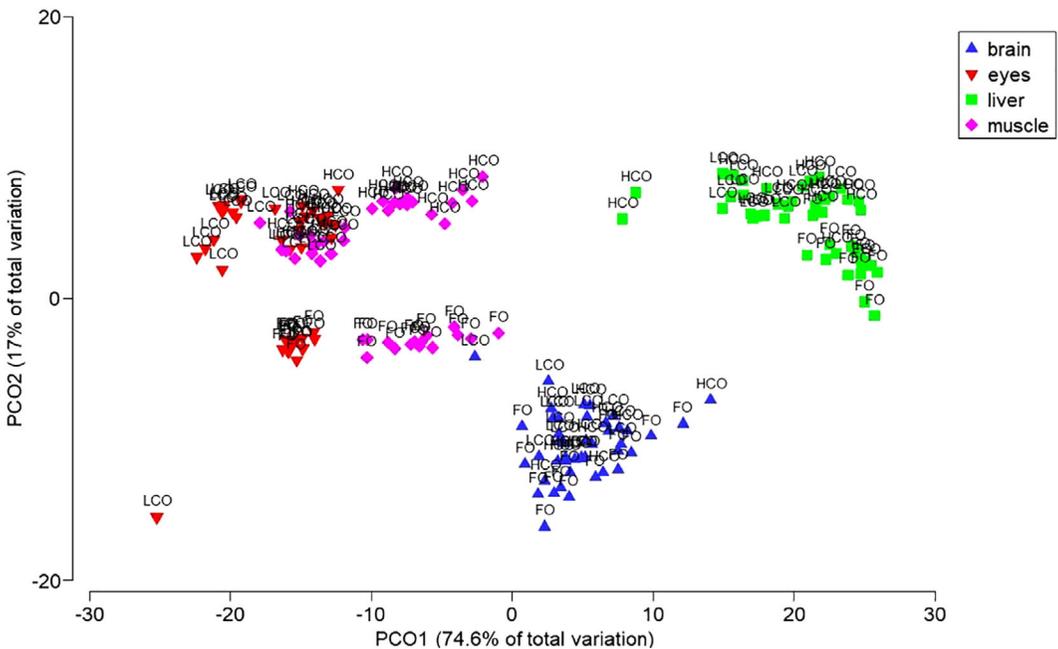
Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; dw, dry weight; EPA, eicosapentaenoic acid; FO, fish oil; HCO, high camelina oil; LCO, low camelina oil; LNA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; ww, wet weight.

**TABLE 8** Fatty acid content ( $\mu\text{g}/\text{mg}$  dry weight), total lipid and dry matter of rainbow trout eye tissue after 12 weeks of feeding

Fatty acid	FO	LCO	HCO	F-value	p-value
14:0	29.4 $\pm$ 7.8 <sup>a</sup>	12.7 $\pm$ 2.8 <sup>b</sup>	13.1 $\pm$ 3.1 <sup>b</sup>	50.67	.000
15:0	2.1 $\pm$ 0.5 <sup>a</sup>	1.1 $\pm$ 0.2 <sup>b</sup>	1.2 $\pm$ 0.2 <sup>b</sup>	39.18	.000
16:0	133.9 $\pm$ 38.1	121.3 $\pm$ 25.9	120.5 $\pm$ 29.6	.83	.445
16:1n-7c	42.7 $\pm$ 10.3 <sup>a</sup>	27.0 $\pm$ 6.0 <sup>b</sup>	27.5 $\pm$ 6.6 <sup>b</sup>	18.72	.000
17:0	2.0 $\pm$ 0.5 <sup>a</sup>	1.1 $\pm$ 0.3 <sup>b</sup>	1.3 $\pm$ 0.2 <sup>b</sup>	29.16	.000
18:0	31.0 $\pm$ 7.6 <sup>b</sup>	35.2 $\pm$ 5.9 <sup>ab</sup>	39.8 $\pm$ 8.7 <sup>a</sup>	5.19	.010
18:1n-9c	323.3 $\pm$ 91.0	358.3 $\pm$ 74.6	319.1 $\pm$ 77.1	1.00	.375
18:1n-9t	1.2 $\pm$ 0.5 <sup>ab</sup>	0.9 $\pm$ 0.1 <sup>b</sup>	1.3 $\pm$ 0.3 <sup>a</sup>	5.23	.009
18:1n-12c	3.2 $\pm$ 1.1	8.1 $\pm$ 14.1	4.8 $\pm$ 1.1	1.39	.262
18:1n-7c	27.3 $\pm$ 6.0	24.1 $\pm$ 3.4	25.1 $\pm$ 4.9	1.61	.212
18:2n-6 (LNA)	127.1 $\pm$ 34.4 <sup>b</sup>	200.9 $\pm$ 33.5 <sup>a</sup>	215.2 $\pm$ 47.9 <sup>a</sup>	21.61	.000
20:0	2.1 $\pm$ 0.5 <sup>c</sup>	5.1 $\pm$ 0.8 <sup>b</sup>	7.0 $\pm$ 1.6 <sup>a</sup>	76.04	.000
18:3n-6	1.7 $\pm$ 0.5 <sup>c</sup>	4.9 $\pm$ 1.7 <sup>b</sup>	7.6 $\pm$ 2.1 <sup>a</sup>	54.66	.000
20:1n-9	19.7 $\pm$ 6.2	30.2 $\pm$ 28.3	30.0 $\pm$ 17.1	1.46	.245
18:3n-3 (ALA)	30.6 $\pm$ 8.6 <sup>c</sup>	136.7 $\pm$ 24.9 <sup>a</sup>	71.0 $\pm$ 15.8 <sup>b</sup>	134.38	.000
18:4n-3	7.8 $\pm$ 2.0 <sup>b</sup>	16.6 $\pm$ 3.5 <sup>a</sup>	10.2 $\pm$ 2.6 <sup>b</sup>	40.48	.000
20:2n-6	8.3 $\pm$ 2.0 <sup>c</sup>	15.5 $\pm$ 2.2 <sup>a</sup>	12.1 $\pm$ 2.5 <sup>b</sup>	37.57	.000
22:3n-3	0.3 $\pm$ 0.1 <sup>c</sup>	1.0 $\pm$ 0.2 <sup>a</sup>	0.6 $\pm$ 0.1 <sup>b</sup>	73.92	.000
22:0	1.0 $\pm$ 0.2 <sup>b</sup>	1.4 $\pm$ 0.3 <sup>a</sup>	1.6 $\pm$ 0.4 <sup>a</sup>	15.57	.000
20:3n-6	3.0 $\pm$ 0.6 <sup>c</sup>	6.5 $\pm$ 0.9 <sup>b</sup>	8.9 $\pm$ 2.0 <sup>a</sup>	76.81	.000
22:1n-9	2.7 $\pm$ 0.5 <sup>c</sup>	8.2 $\pm$ 2.7 <sup>a</sup>	4.5 $\pm$ 0.9 <sup>b</sup>	42.64	.000
20:3n-3	2.0 $\pm$ 0.7 <sup>c</sup>	9.2 $\pm$ 1.2 <sup>a</sup>	6.8 $\pm$ 1.3 <sup>b</sup>	156.80	.000
20:4n-6 (ARA)	4.9 $\pm$ 1.1 <sup>b</sup>	5.3 $\pm$ 0.9 <sup>b</sup>	14.6 $\pm$ 3.1 <sup>a</sup>	112.13	.000
22:2n-6	0.9 $\pm$ 0.2 <sup>b</sup>	1.1 $\pm$ 0.2 <sup>a</sup>	1.2 $\pm$ 0.3 <sup>a</sup>	9.41	.000
20:5n-3 (EPA)	34.7 $\pm$ 9.0 <sup>a</sup>	15.3 $\pm$ 3.0 <sup>b</sup>	39.8 $\pm$ 9.2 <sup>a</sup>	40.67	.000
24:1n-9	2.4 $\pm$ 0.6 <sup>b</sup>	3.7 $\pm$ 0.6 <sup>a</sup>	2.8 $\pm$ 0.6 <sup>b</sup>	16.93	.000
22:4n-6	1.3 $\pm$ 0.8 <sup>b</sup>	0.9 $\pm$ 0.5 <sup>b</sup>	3.1 $\pm$ 1.4 <sup>a</sup>	21.52	.000
22:5n-3	9.4 $\pm$ 1.8 <sup>b</sup>	4.7 $\pm$ 0.9 <sup>c</sup>	16.5 $\pm$ 3.1 <sup>a</sup>	109.42	.000
22:6n-3 (DHA)	60.7 $\pm$ 12.3 <sup>b</sup>	40.3 $\pm$ 7.6 <sup>c</sup>	74.0 $\pm$ 15.1 <sup>a</sup>	28.07	.000
$\Sigma$ SFA	202.5 $\pm$ 55.2	179.1 $\pm$ 35.3	185.4 $\pm$ 43.4	1.03	.365
$\Sigma$ MUFA	450.3 $\pm$ 123.1	496.0 $\pm$ 109.7	439.4 $\pm$ 111.6	.98	.385
$\Sigma$ PUFA	292.9 $\pm$ 72.4 <sup>b</sup>	444.7 $\pm$ 94.5 <sup>a</sup>	481.7 $\pm$ 101.6 <sup>a</sup>	18.35	.000
$\Sigma$ EPA and DHA	95.4 $\pm$ 21.2 <sup>b</sup>	55.6 $\pm$ 10.1 <sup>c</sup>	113.9 $\pm$ 23.9 <sup>a</sup>	33.51	.000
$\Sigma$ n-3	145.2 $\pm$ 34.0 <sup>b</sup>	222.7 $\pm$ 35.7 <sup>a</sup>	218.3 $\pm$ 44.6 <sup>a</sup>	18.96	.000
$\Sigma$ n-6	147.3 $\pm$ 38.4 <sup>b</sup>	221.0 $\pm$ 67.0 <sup>a</sup>	262.8 $\pm$ 57.7 <sup>a</sup>	16.72	.000
n-3/n-6	1.0 $\pm$ 0.0	1.4 $\pm$ 1.7	0.8 $\pm$ 0.0	1.30	.264
<b>Total lipid and dry matter (g/kg)</b>					
Lipid ww	235 $\pm$ 69	227 $\pm$ 55	199 $\pm$ 25	1.90	.162
Lipid dw	739 $\pm$ 192	744 $\pm$ 131	694 $\pm$ 67	.60	.553
Dry matter	315 $\pm$ 26	302 $\pm$ 52	286 $\pm$ 23	2.28	.115

Note: Data expressed as  $\mu\text{g}$  FAME/ $\text{mg}$  (dry weight), values are means ( $n = 3$  per treatment)  $\pm$  standard deviation. Means with different superscripts indicate significant differences based on Tukey's post-hoc test following a one-way ANOVA. Reported fatty acids are  $>1$   $\mu\text{g}/\text{mg}$ ; the full fatty acid profile can be found in the Table S4.

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; dw, dry weight; EPA, eicosapentaenoic acid; FO, fish oil; HCO, high camelina oil; LCO, low camelina oil; LNA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; ww, wet weight.

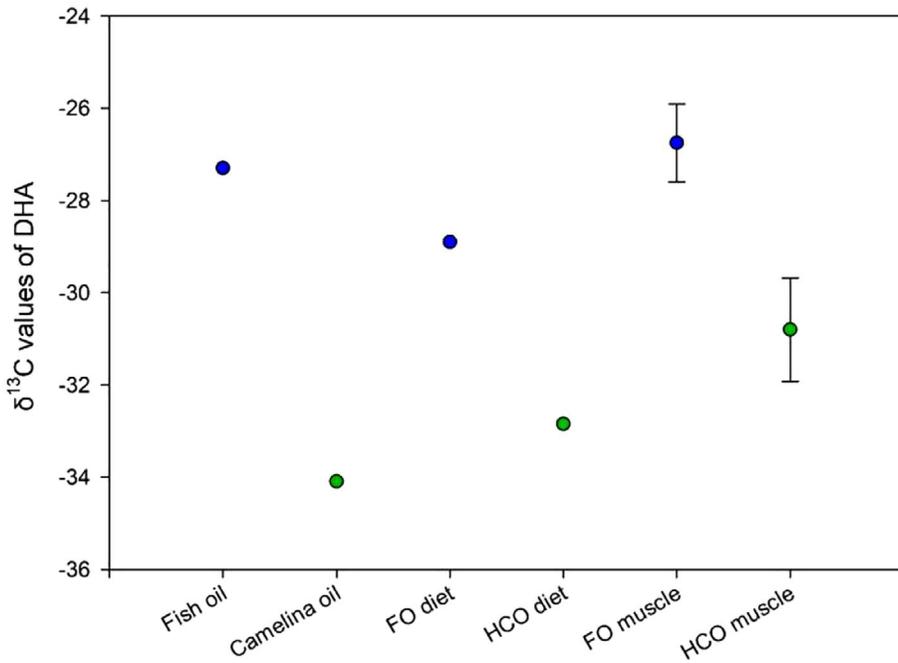


**FIGURE 1** Principal co-ordinate ordination plot of fatty acid profiles of individual rainbow trout tissues (brain, eye, liver, and muscle) using a Bray–Curtis similarity matrix, where three dietary treatments are represented (fish oil [FO], high camelina oil [HCO], and low camelina oil [LCO]), with  $n = 9$  per treatment

associated with n-3 LC-PUFA are well documented, it is crucial that alternative forms of FO contain n-3 LC-PUFA to ensure that the nutritional needs of both farmed fish and consumers are maintained (Betancor et al., 2018). Transgenic lipid sources, such as camelina, present a unique and novel opportunity to eliminate and/or significantly reduce FO in aquafeeds as a source of n-3 LC-PUFA (Osmond & Colombo, 2019). Previous studies that have tested transgenic camelina oil in diets for Atlantic salmon (Betancor et al., 2015, 2017; Betancor, Sprague, Sayanova, et al., 2016) and gilthead seabream (Betancor, Sprague, Montero, et al., 2016) suggest that transgenic camelina oil could be implemented as a viable dietary lipid substitute for FO. In the present study, transgenic camelina oil (with EPA and DHA) was evaluated as a dietary lipid source for freshwater rainbow trout as a replacement for FO. HCO and FO diets had similar EPA and DHA quantities, indicating that transgenic camelina oil must be supplied at a high inclusion rate (>50% of the total dietary oils) to provide enough EPA and DHA equivalent to that of FO.

#### 4.1 | Impact on fatty acid content on growth performance

Overall, rainbow trout in this study responded positively to the transgenic camelina oil treatments and growth performance was comparable to trout fed FO diets, suggesting that this oil was digested and utilized. This is in agreement with studies showing that both a high-EPA oil and an EPA+DHA oil from transgenic camelina included in feeds for post-smolt Atlantic salmon and gilthead seabream had no detrimental effects on fish performance, metabolic responses, or the nutritional quality of fillet (Betancor, Sprague, Montero, et al., 2016; Betancor, Sprague, Sayanova, et al., 2016). In the present study, juvenile rainbow trout fed diets with transgenic camelina oil (high or low inclusion) had a higher final weight and were longer in fork length, with the same FCR as trout fed FO. Weight gain, although not statistically different, was numerically and biologically higher in trout fed LCO and HCO diets compared to trout fed FO. Since total PUFA were higher in the CO diets, fish may have metabolized the FA more efficiently compared



**FIGURE 2**  $\delta^{13}\text{C}$  values of DHA in fish oil (‰), transgenic camelina oil, and rainbow trout muscle tissue fed fish oil (FO) and high camelina oil (HCO) diets. Values for muscle samples are means  $\pm$  standard deviation ( $n = 9$ ) and were significantly different ( $p = 0.001$ ). The value for fish oil was obtained from Hixson, Parrish, and Anderson (2014). Data points in blue are from fish oil sources and data points in green are from camelina sources

to fish fed the FO diet, which was higher in SFA. It is possible that n-3 LC-PUFA in transgenic camelina oil may be more bioavailable (i.e., digestible and better utilized) or contain other minor compounds (i.e., choline) as compared to FO, which may explain the slightly better growth performance in rainbow trout fed LCO and HCO compared to trout fed FO. Notably, the final VSI was greater in fish fed LCO diets compared to those fed the FO and HCO diets, indicating additional fat accumulation in the viscera. Increased fat deposition in fish tissues appears to be a common effect of dietary plant oil inclusion (Betancor, Sprague, Sayanova, et al., 2016). This increase in VSI may be attributed to elevated quantities of plant-associated FA (ALA and LNA) in LCO diets. If ALA and LNA are not used as substrates toward n-3 and n-6 LC-PUFA, and if not oxidized, the FA are stored in adipose tissue. Similarly, in salmon fed non-transgenic camelina oil, more lipid was stored surrounding the viscera than salmon fed a FO-based diet (Hixson, Parrish, & D.M., 2014). The interaction between plant oil and protein has also been found to increase visceral adipose tissue in salmon (Torstensen, Espe, Stubhaug, & Lie, 2011). As it is not advantageous, from an aquaculture perspective, to accumulate visceral fat as opposed to somatic growth, HCO diets may offer an advantage over LCO diets as a result of higher n-3 LC-PUFA and lower ALA and LNA, particularly when fish are fed for longer periods of time.

## 4.2 | Impact on tissue fatty acid content

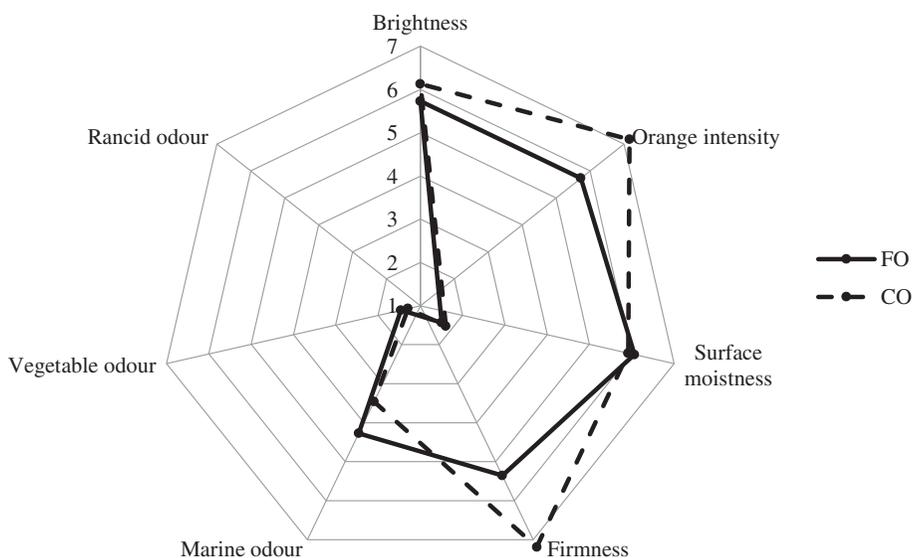
Muscle tissue acts as the primary site of dietary FA deposition in salmonids (Hixson, Parrish, & D.M., 2014; Katan, Caballero-Solares, Taylor, Rise, & Parrish, 2019; Polvi & Ackman, 1992; Sprague et al., 2016). With the inclusion of novel dietary lipids, the potential influence on tissue fatty acid profiles is an important consideration (Bell, Henderson, Tocher, & Sargent, 2004). When FO was replaced by transgenic camelina oil (both high and low inclusion levels),

**TABLE 9** Instrumental analyses of color and texture and sensory evaluation of rainbow trout muscle tissue after 12 weeks of feeding experimental diets

Sensory parameter	FO	LCO	HCO	F- or T-value	p-value
Instrumental analysis <sup>a</sup>					
Color score, L	52.86 ± 3.3	56.29 ± 3.7	55.45 ± 2.0	3.06	.066
Color score, a	21.2 ± 2.5	20.5 ± 3.6	21.2 ± 3.7	.11	.895
Color score, b	31.7 ± 1.9	30.9 ± 3.1	30.5 ± 2.5	.56	.581
Texture (force)	215.9 ± 58.9	200.3 ± 29.6	184.8 ± 45.8	1.02	.377
Qualitative descriptive analysis <sup>b</sup>					
Brightness	5.729 ± 2.533	-	6.125 ± 1.825	-.76	.451
Orange intensity	5.729 ± 2.430b	-	7.167 ± 2.099a	-2.60	.012
Surface moistness	6.063 ± 2.254	-	5.917 ± 1.909	.29	.775
Firmness	5.354 ± 1.995b	-	7.188 ± 1.774a	-3.96	.001
Marine odor	4.271 ± 2.727	-	3.458 ± 2.963	1.13	.267
Vegetable odor	1.458 ± 2.010	-	1.292 ± 2.010	.33	.742
Rancid odor	0.375 ± 1.104	-	0.250 ± 0.532	.65	.519
Hedonic test <sup>b</sup>					
Appearance	3.688 ± 1.764	-	3.604 ± 1.700	.20	.846
Texture	2.375 ± 0.809a	-	1.771 ± 1.063b	2.45	.019
Odor	2.229 ± 0.893	-	2.563 ± 0.681	-1.76	.083

<sup>a</sup>Values are means ± standard deviation (n = 9). Means with different lowercase letters indicate significant difference as determined by one-way ANOVA (F-value in table).

<sup>b</sup>Values are means ± standard deviation (FO, n = 48; HCO, n = 24). Sensory evaluation conducted with untrained panelists on FO (fish oil) and HCO (high camelina oil) treatments only. Means with different lowercase letters indicate significant difference as determined by t-test (T-value in table).

**FIGURE 3** Radial plot of quantitative descriptive analysis sensory comparative analysis of Week 12 rainbow trout muscle tissue sampled from fish oil (FO) and high camelina oil (HCO) diets

significant changes were observed in the quantities of individual FA in trout muscle. After 12 weeks, EPA and DHA stored in muscle tissue of rainbow trout fed the HCO diet was similar to that of fish fed the FO diet. This is similar to the results of Betancor et al. (2017), where Atlantic salmon fed transgenic camelina had the highest EPA and DHA in comparison to salmon fed diets with non-transgenic camelina and FO. This is an important result, since generally when plant-based lipid alternatives to FO are used, a major concern is reduced levels of n-3 LC-PUFA in the fillet tissue that impacts the human consumer (Sprague et al., 2016; Turchini et al., 2018). Previous studies have also reported high n-3 bioconversion capabilities of trout and the ability to maintain high stored EPA and DHA within the fillet when the fish are provided with a diet that is high in ALA (Hixson, Parrish, & Anderson, 2014; Turchini et al., 2018). However, in our study with juvenile rainbow trout, the fish fed the LCO diet stored significantly less EPA and DHA in muscle tissue, and instead accumulated ALA without further bioconversion. This likely indicates that the LCO diet provided sufficient dietary levels of EPA and DHA for health and growth, but the amounts of EPA and DHA were not low enough to trigger biosynthetic action toward production of surplus EPA and DHA, even if high amounts of ALA were supplied. Despite the accumulation of ALA, there was no difference total lipid stored in the fillet among treatments. The n-3/n-6 ratio was lower in muscle tissue of trout fed diets containing transgenic camelina oil. Changes to n-3/n-6 fatty acid ratios could directly impact eicosanoid production; however, the quantity of total n-3 FA stored within the muscle tissue in trout fed HCO were similar to trout fed diets containing FO. This is an important consideration from both a consumer health and fish health perspective as the consumption of increased n-3 LC-PUFA assists in the production of anti-inflammatory eicosanoids. Trout fed the HCO diet also had an increased quantity of ARA in their muscle tissue, which could be attributed to the presence of ARA (and LNA) in the transgenic camelina oil used in this study (Table 1; as in Han et al., 2020) and the HCO diet.

For trout fillets from both HCO and LCO treatments, the quantitative amount of EPA+DHA both meet the human nutritional requirement for these essential FA. According to the World Health Organization (WHO), the daily requirement for EPA+DHA is 250 mg (WHO, 2008); although other regulatory bodies suggest higher intake levels. The recommended serving of fish is 100 g (~3.5 oz) of cooked fish (American Heart Association, 2017). The sum of EPA+DHA (wet weight) in one serving of rainbow trout muscle for fish fed FO is 823, 652, and 433 mg in trout fed FO, HCO, and LCO, respectively. Therefore, consuming one serving of rainbow trout fillet that was fed either HCO or LCO is more than sufficient to meet the daily requirement (250 mg) as recommended by the WHO.

Liver tissue is a primary site of lipid metabolism, oxidation, and LC-PUFA synthesis (Caballero, Izquierdo, Kjørsvik, Fernández, & Rosenlund, 2004). As imbalances or changes in dietary FA could influence the functioning of the liver, it is important to consider the impact on this organ as an indicator of changes in lipid and fatty acid metabolism (Caballero et al., 2004) in the fish. Overall, the inclusion of dietary transgenic camelina oil significantly influenced profiles of individual FA in the liver; however, the liver appeared to be more resistant to changes in dietary FA profile compared to muscle tissue (see Figure 1). EPA and DHA in the liver of fish fed transgenic camelina oil was found to be similar to that of fish fed FO. This result agrees with previous findings in which transgenic camelina oil fed to Atlantic salmon resulted in high levels of EPA and DHA in the liver in comparison to the FO diet (Betancor et al., 2015; Betancor et al., 2016; Betancor et al., 2017). The conservation of DHA in the liver in fish fed the HCO diet compared to those fed the FO diet was expected, as the HCO diet contained transgenic camelina oil with EPA and DHA proportions that are equivalent to that of FO. ALA and LNA stored in the liver tissue were also greater in fish fed LCO and HCO diets compared to the FO diet. In fingerling salmon fed diets with transgenic canola oil (high DHA, but low EPA), the liver stored elevated levels of ALA and LNA (Ruyter et al., 2019).

Because DHA is required for neural development in vertebrates, brain and eye tissues are areas of DHA localization and it has been consequently been proposed that these tissues may be relatively resistant to changes in the fatty acid profile of the diet (Brodtkorb, Rosenlund, & Lie, 1997; Stoknes, Økland, Falch, & Synnes, 2004). Nevertheless, we propose that marked changes in diet FA composition diet (in particular, with respect to the n-3 PUFA content) have the potential to change FA profiles in these tissues. We assayed brain and eye tissues to check if there are changes in FA profiles of these tissues as a result of changes in diet. Not surprisingly, the brain appeared to be most resistant to dietary change, compared to the other tissues in this study (see Figure 1). For example, despite different amounts of

dietary EPA and DHA among diets in this study, after 12 weeks of feeding, the brain still showed similar amounts of EPA and DHA among treatments, which likely suggests selective retention. It could also be possible that trout in the LCO group were metabolizing ALA toward EPA and DHA synthesis, and subsequently transporting these n-3 LC-PUFA to the brain. However, excess amounts of ALA stored in the muscle and liver suggest that this may not be the case. Higher ALA (and LNA) amounts were found stored in the brain tissue of rainbow trout fed transgenic camelina diets compared to trout fed the FO control diet. Interestingly, higher amounts of ARA were stored in the brain in trout fed LCO and HCO diets compared to the FO treatment, despite the fact that ARA levels in the feed were similar for FO and LCO diets (although about four times higher in the HCO diet). The ARA in transgenic camelina oil may perhaps be more bioavailable (e.g., accessible for efficient digestion and utilization) than in FO. Approximately 5% of ARA is stored in phosphatidylcholine in transgenic camelina (Ruiz-Lopez et al., 2014), which would be highly digestible and bioavailable to fish. It is also possible that the surplus LNA in the LCO diet went toward ARA production, which was stored in the brain, compared to the trout fed FO. Feeding oils rich in LNA has resulted in elevated ARA in membrane phospholipids in Atlantic salmon (Bell, Dick, & Sargent, 1993). This is relevant as ARA has important functions in the brain and since plant-based oils are typically devoid of ARA, its bioavailability is important for brain function in fish that are fed plant-based diets (Oxley et al., 2010). Major groups of FA remained the same among treatments, notably total SFA, MUFA, PUFA, n-3, and n-6, suggesting FA conservation in the brain, to prioritize critical maintenance of brain function. Similar findings have been reported by Betancor, Sprague, Sayanova, et al. (2016); Betancor, Sprague, Montero, et al. (2016); Betancor et al. (2017) in which both Atlantic salmon and gilthead seabream fed diets containing transgenic camelina oil were noted to have less dietary impact noted in fatty acid composition of brain tissue sampled from fish as there were no differences in total lipid contents of brain tissue among fed dietary treatments. The robustness of brain tissue, in its ability to maintain levels of n-3 LC-PUFA, regardless of dietary source, that was observed in our study has also been noted in studies with other fish species, (Benedito-Palos, Navarro, Kaushik, & Pérez-Sánchez, 2010; Nayak, Saha, Pradhan, Samanta, & Giri, 2017; Silva-Brito et al., 2016).

The presence of neural tissue within the eye prioritizes the specific retention of n-3 LC-PUFA such as DHA, to assist in optimizing the integrity of the retina and maintaining visual acuity. In the vertebrate eye, DHA contributes to 50–60% of the total fatty acid content found within the rod outer segment of photoreceptors (Querques, Forte, & Souied, 2011). As such, retinal tissue within the eye is used as an indicator for specific changes in n-3 fatty acid storage related to dietary influences or deficiencies. Compared to the brain, the eye tissue was more reflective of the diet, especially for juvenile rainbow trout fed the LCO treatment. The most distinct examples of this are higher amounts of ALA and LNA and lower amounts of EPA and DHA stored in eye tissue of trout fed LCO versus HCO and FO diet groups. It is unknown whether this level of change in eye tissue FA profile can eventually impact vision of trout fed LCO diets because of reduced EPA and DHA. Interestingly, DHA in eye tissue of trout fed HCO was higher than in the FO group, despite having similar amounts of DHA in the diet. Again, this may perhaps suggest superior bioavailability of LC-PUFA in transgenic camelina. Navarro et al. (1997) noted that various dietary inclusion levels of DHA fed to seabass larvae significantly influenced the composition of lipids within eye tissue. In contrast, changes in dietary DHA inclusion when fed to Atlantic salmon had no significant impacts on DHA levels in eye tissue (Brodtkorb et al., 1997). Clearly the species, diet, duration of feeding, life stage of the fish, etc., can impact the outcome of FA storage in tissues. However, few studies have evaluated the impact of dietary on eye tissue in general. Thus, we suggest that more research needs to be done to better understand the relationship between dietary DHA and its impact on neural tissues in fish.

The PCoA provides a visualization of the effect of diet and tissue type on the FA outcome of this study (see Figure 1). Each tissue type is clustered, regardless of diet, which was confirmed by PERMANOVA, that is, tissue type is a significant factor in the variation of FA profiles. Most of the variation is explained by PC1 (~75%), which clearly separated groups of tissues, rather than dietary groups. This indicates that tissue type is more of a defining factor in determining FA composition than the impact of diet. The clustering of individual samples is obvious for brain tissue, but treatment effect is virtually undistinguishable within the cluster of brain data points, again, indicating that brain tissue has a distinct FA profile, that is largely independent of dietary FA. In contrast, muscle tissue in salmonids is

known to be quite plastic with respect to its fatty acid profile in relation to FA supplied in the diet, and as such, retains the FA signature of the diet. There are clearly two separate groups along PC2 dividing the muscle tissue data points (and eye tissue), clearly separating FO groups from HCO and LCO groups. Since only muscle and eye are distinctly separated along PC2 (and less of the variation explained, ~17%), diet is less of an explanatory factor considering rainbow trout tissues together.

### 4.3 | $\delta^{13}\text{C}$ values for DHA

With the inclusion of novel dietary oils, such as transgenic camelina, it is important to observe whether stored FA is accumulated from the new transgenic oil source, or selectively retained from the fish meal that was also provided in each of the diets. To confirm this, CSIA was used to determine the DHA isotopic signature in the muscle tissue. With CSIA, the origin of carbon and its specific contribution to tissues can be determined, as  $^{13}\text{C}/^{12}\text{C}$  ratios stay relatively unchanged as they pass through the food web, they can be used to evaluate nutrient transfer (Hixson, Parrish, & Anderson, 2014). As such, we can determine if the DHA stored in muscle is from fish meal origin or stored directly from GE camelina. The DHA in transgenic camelina oil and FO were isotopically distinct, with DHA in FO being more isotopically enriched (-27.3‰) than in camelina oil (-34.1‰), as camelina is a C3 plant and has  $\delta^{13}\text{C}$  values between -25 and -35‰ (O'Leary, 1988) and marine origins are isotopically heavier (i.e., enriched) with  $^{13}\text{C}$  (Phillips, Newsome, & Gregg, 2005). This was reflected in the muscle tissue of juvenile rainbow trout in our study. Different  $\delta^{13}\text{C}$  values for DHA in muscle of trout fed HCO likely indicate direct storage of DHA from transgenic camelina, which resulted in isotopically lighter DHA (-30.8‰) compared to trout fed FO (-26.75‰), a difference of 4.05‰. The  $\delta^{13}\text{C}$  of a consumer is assumed to be equivalent to the weight proportion of the  $\delta^{13}\text{C}$  of all dietary components, therefore, the significant difference in DHA  $\delta^{13}\text{C}$  represents the difference in terrestrial vs. marine isotopic signatures, and therefore suggests that the DHA stored in muscle tissue in trout was directly from dietary transgenic camelina, rather than selectively retained from the DHA in fish meal only. Although, since there was a slight shift, it is likely that some DHA is from marine origin in trout fed HCO.

### 4.4 | Sensory properties of fillets

Applied aquaculture research should, ideally, be complemented with the findings offered by sensometrics (Calanche, Beltrán, & Hernández Arias, 2020). There were minor, yet significant, differences in the sensory properties of rainbow trout fed diets containing high levels of transgenic camelina compared with traditional FO. Interestingly, instrumental analyses of color and texture revealed no significant difference in fillets between HCO and FO treatments; however, untrained consumers reported a difference in color and texture based on their perception. Untrained panelists are "consumers," who represent a valuable source of information with regards to collecting sensory information based on consumer preference, which has gained increasing significance and relevance in aquaculture and seafood assessment (Calanche et al., 2020). The triangle test indicated that panelists could not distinguish between fillets from trout that were fed diets containing transgenic camelina versus fillets from trout that were fed a typical commercial (FO-based) diet, but when asked to rate fillets according to fillet color, texture, odor, etc. in the QDA and hedonic test, panelists found slight but significant differences. The observed higher orange intensity in trout fed HCO could be attributed to pigment in transgenic camelina oil. Non-transgenic camelina can contain up to 112 mg of  $\beta$ -carotene/kg oil (Raczyk, Popis, Kruszewski, Ratusz, & Rudzinska, 2016). Astaxanthin is the major carotenoid pigment responsible for the pink color in the flesh of wild rainbow trout, while both astaxanthin and canthaxanthin are used for pigmentation farmed trout (Storebakken & No, 1992).  $\beta$ -carotene is also one of the carotenoids responsible for the orange and red pigmentation of fish (Keleştemur & Çoban, 2016). For example,  $\beta$ -carotene supplementation in juvenile rainbow trout diets has been shown to improve growth performance and skin carotene concentration at

70 mg/kg  $\beta$ -carotene (Keleştemur & Çoban, 2016). However, it is important to note that the color differences observed by the sensory panel in the present study were not detected by instrumental analysis (i.e., L, a, b color scores; see Table 9), which highlights a discrepancy between the sensory panel and analytical methods. An increase in firmness may be attributed to the incorporation of a vegetable oil lipid substitute as these can impact the sensory characteristic of fish (Martínez-Llorens, Vidal, Moñino, Torres, & Cerdá, 2007). Forty percent of studies that investigated plant product inclusion in aquafeeds stated that texture was significantly affected, particularly in larger or higher quantity substitutions (Gatlin et al., 2007). Also, fillet texture is multifactorial, with complex biological interactions (Mørkøre et al., 2020). Plant-based oils have been found to increase lipid levels stored within the fillet, leading to changes within the sensory properties of the fillet. However, in this study, total lipid, crude protein, and dry matter were not different between fillets from trout fed FO versus HCO diets, so this likely did not impact the texture. These results have often been found to be contradictory to each other. For example, in previous studies, in which non-transgenic camelina oil or FO was fed to Atlantic salmon there were no significant differences in sensory properties including brightness and texture of raw and cooked fillets (Hixson et al., 2017; Hixson, Parrish, & D.M., 2014). Perception of raw fish fillets (which is influenced by color and texture) is recognized as an important factor related to consumer satisfaction (Veiseth-Kent et al., 2010). Because our sensory analysis represents preliminary results (raw fillets of juvenile trout, and therefore not necessarily directly market-applicable), we suggest that a sensory analysis on cooked fillets of market size fish would be pertinent prior to using transgenic camelina commercially in the grow out phase.

## 5 | CONCLUSIONS

We found transgenic camelina oil, at a high inclusion level (>50% of the total dietary oil) and as a full replacement of FO, to be an effective substitute for FO as a dietary lipid source of n-3 LC-PUFA in diets for rainbow trout. Fish fed high levels of transgenic camelina oil enriched with EPA and DHA (HCO diet) had FA profiles that were generally similar to those of fish fed FO. Inclusion of lower levels of transgenic camelina (LCO diet) resulted in similar growth performance as trout fed FO; however, the FA profile was more impacted, particularly in the muscle tissue. This evidence informs that greater quantities of transgenic camelina oil in the feed are warranted in order to effectively replace FO in aquafeed (full replacement of FO and >50% of the total dietary oil). We conclude that dietary inclusion of transgenic camelina oil is effective in meeting the nutritional requirements of juvenile rainbow trout as their growth and development were not affected by the addition of this oil to the feed. Relative to fish health, the inclusion of transgenic plant-based oil must be further examined to evaluate possible impacts of novel oils on both fish health and lipid profiles, which could also further impact fillet product quality and consumer health. Future research is needed to evaluate transgenic camelina at grow out stages closer to market size (i.e., finishing diets), or for a full production cycle.

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## SUPPORTING INFORMATION

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

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