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Research Article

Algal and Vegetable Oils as Sustainable Fish Oil Substitutes in Rainbow Trout Diets: An Approach to Reduce Contaminant Exposure

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The aim of this study was to replace 75% of total fish oil (FO) and alter digestible protein/digestible energy (DP/DE) in rainbow trout feeds to minimize potentially hazardous environmental contaminant exposure. Two diets differing in DP/DE ratios (18 and 25) were combined with soybean (SO), canola (CO), and a blend of canola oil and *Schizochytrium* sp. (COS). Dietary lipids and DP/DE ratios did not affect apparent digestibility, growth, and somatic parameters. The *n*-3/*n*-6 levels decreased significantly in the growth trial, especially for the SO groups. A short washout trial restored *n*-3/*n*-6 levels for the CO and COS groups, irrespective of the DP/DE ratio, but not for the SO groups. At all sampling events, contaminant concentrations in fish flesh were lower than limits set by regulatory agencies in Canada, the US, and Europe. Contaminants were lower in the oil replacement diets compared to FO for toxaphenes, organochlorine pesticides, and PCBs but not flame retardants during the growth phase. At the end of the washout phase, no differences were detected. Thus, this study revealed that replacing 75% of total fish oil in rainbow trout feed by CO and COS, combined with a 25 DP/DE ratio, with a washout period seems to be the most efficient approach in terms of maximizing the total FO replacement and contribute to reducing POPs exposure.

1. Introduction

The contamination of aquaculture products by persistent organic pollutants (POPs) and heavy metals has become an issue of public concern, particularly after the publications reporting the contaminant levels of farmed salmonids [1, 2]. The main source of contaminants is from pelagic fish species used to produce fish meal and oil as ingredients in salmonid feeds [1–3]. To reduce the reliance on fish oils and address contaminant issues, efforts can be directed on reducing fish oil percentage in fish feed by partially replacing it with vegetable oils. Also, feeding diets having higher protein/lipid ratios at similar digestible energy levels can be a means to reduce the content of lipid-soluble contaminants. A number of studies have shown that a significant percentage of fish oil in salmonids feed can be replaced without affecting survival, growth, and feed efficiency [4–9]. However, vegetable oils have a different fatty acid profile than fish oils. When included at high dietary levels, they influence flesh fatty acid composition and reduce the concentration of omega-3 long-chain polyunsaturated fatty acids (*n*-3 LC-PUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), well-known for their beneficial cardiovascular and cognitive properties [10].

Strategies to restore the levels *n*-3 LC-PUFA in farmed fish flesh include a washout period whereby fish oils are re-fed at higher levels following the growth period or the dietary inclusion of algal concentrates high in *n*-3 LC-PUFA. Indeed previous work has attempted to demonstrate the relevance of using a washout feed containing 100% fish oil to restore beneficial fatty acid levels with gilt-head seabream (*Sparus aurata*) [11, 12], Atlantic salmon (*Salmo salar*) [13, 14], and Nile tilapia (*Oreochromis niloticus*) [15]. Although promising, no study was able to sufficiently restore the *n*-3 LC-PUFA levels in the flesh because fish have a limited capacity of producing these fatty acids. However, by feeding vegetable oil-based feeds, combined with a high *n*-3 LC-PUFA source (*Schizochytrium* sp.), we hypothesize that initial *n*-3 LC-PUFA levels will be maintained so the washout period could be shorter than in previous studies. Bell et al. [16] concluded that replacing fish oil in the aquaculture feed by vegetable oils (ratio of 1 : 1 canola and linseed oils) plus a washout period is efficient in reducing persistent organic pollutants (POPs) accumulation in Atlantic salmon. Although vegetable oils are excellent candidates to replace marine oils because of their low contaminant concentrations, further investigation must be done to maintain beneficial *n*-3 LC-PUFA levels in the fillets.

The aim of this study was to determine the loading of several POPs and fatty acid profiles in flesh, when replacing 75% of total fish oil with a blend of vegetable oils and a high *n*-3 LC-PUFA algal source (*Schizochytrium* sp.), and altering the dietary digestible protein/energy (DP/DE) levels in rainbow trout (*Oncorhynchus mykiss*) feeds. A growth trial (9 months) was followed by a washout trial (3 months) to compare POPs and mercury concentrations, while evaluating the restoration of fatty acid profiles. During the growth period, we hypothesize that replacing fish oil with vegetable oils and modifying protein/lipid levels in fish feed will result in significantly lower contaminant levels in the flesh, while maximizing the utilization of lipids as a source of energy. For the washout period, we predict that feeding fish with a "finishing feed" containing high levels of *n*-3 LC-PUFA will permit restoration of these beneficial components in the flesh.

2. Materials and Methods

2.1. Fish and Feeding. Rainbow trout (*Oncorhynchus mykiss*) weighing 43.6 ± 1.0 g at the beginning of growth trial were randomly allocated in 24 tanks of 340 L in a fresh water flow-through system at the Alma Aquaculture Research Station (AARS) in Elora (ON). Water temperature was a constant 8.5°C, incoming flow rate 12 l/min, and oxygen was maintained at >80% saturation. Photoperiod was set at a 12/12 light/dark cycle. Tanks were stocked with 45 fish per tank in order not to exceed a density of 60 kg/m^3 per tank at the end of the trial, based on known growth rates. Each diet was assigned to three replicate tanks (n = 3) in a completely randomized design to limit any environmental effects resulting from lighting intensity or floor traffic. Prior to the beginning of growth trial, fish were acclimated for two weeks. On a weekly basis, fish were hand-fed until apparent satiation two days and then at 75% satiation the next five days with belt feeders, twice a day. Pellet size varied according to fish body size, from 3 to 6 mm [17]. All aspects of the trial were approved by the *Comité de Protection des Animaux de l'Université Laval*.

2.2. Feeds

2.2.1. Growth Trial. Isoenergetic diets were formulated according to the NRC requirements for rainbow trout (1993). Two digestible protein/digestible energy ratios (18 and 25 DP/DE), based on those employed by Azevedo et al. [18], were formulated with four different sources of lipids. The lipid sources were herring oil (FO), soybean oil (SO), canola oil (CO), and a blend of canola oil and *Schizochytrium* sp. biomass (COS) (Table 1). *Schizochytrium* sp. biomass is a dried marine algae preparation rich in docosahexaenoic acid (DHA; 22:6w3). The COS blend was formulated to provide an equivalent level of DHA versus FO, while providing equivalent energy to other FO replacements via CO. The following diets were formulated: 18 DP/DE with 100% FO (18 FO); 18 DP/DE or replaced with 75% SO (18 SO); 18 DP/DE with 75% CO (18 CO); 18 DP/DE with 75% of a blend of CO and *Schizochytrium* sp. (18 COS); 25 DP/DE with 100% FO (25 FO); 25 DP/DE with 75% SO (25 SO); 25 DP/DE with 75% CO (25 CO); and 25 DP/DE with 75% of a blend of CO and *Schizochytrium* sp. (25 COS). Algae biomass was added to approximate *n*-3 LC-PUFA concentration in fish oil. Fatty acid profile of the experimental diets is shown in Table 2. Herring oil was purchased from Corey Aquafeeds (Fredericton, NB), soybean oil from Soya Excel (Beloeil, QC) and canola oil from Bunge (Montreal, QC). *Schizochytrium* sp. (S-Type Gold Fat) was supplied by Advanced BioNutrition (Columbia, MD). All dry ingredients were mixed, after which the mixture was blended with either fish oil or a blend of fish oil and the alternate source of lipid (10% of total oil was reserved for top-coating pellets). Pellets were steam pelleted with a California Pellet Mill (model CPM CL-5, California Laboratory Pellet Mill Co., Crawfordsville, IN), dried under forced air at room temperature for 24 h and then sieved. Diets were then coated with 10% of appropriate oil.

2.2.2. Washout Period. To restore beneficial *n*-3 LC-PUFA levels in fillets, fish oil-based diets were fed for a three-month washout period. During this period, fish were fed diets containing 100% fish oil.

2.3. Sampling Procedures and Evaluation of Growth Parameters

2.3.1. Growth Trial. Fish were weighed at the beginning of the experiment and then every 28 days. At time 0 (beginning of the experiment), 15 fish were removed from the initial population. Thereafter, 5 fish per tank were sampled every 3 months at month 3 (84 days), month 6 (168 days), and month 9 (252 days). At each sampling event, fish were killed with an overdose of tricaine methanesulphonate (MS-222).

Table 1: Feed components and chemical composition of experimental diets (g/kg dry matter).

	18 FO	18 SO	18 CO	18 COS	25 FO	25 SO	25 CO	25 COS
Ingredient composition								
Fish oil, herring	21.3	2.7	2.7	2.8	13.5	0.0	0.0	0.0
Soybean oil	0.0	18.6	0.0	0.0	0.0	13.5	0.0	0.0
Canola oil	0.0	0.0	18.6	15.0	0.0	0.0	13.5	10.5
Schizochytrium sp.	0.0	0.0	0.0	9.0	0.0	0.0	0.0	6.5
Herring meal	22.0	22.0	22.0	22.0	32.5	32.5	32.5	32.5
Blood cell meal								
AP301	7.0	7.0	7.0	7.0	10.0	10.0	10.0	10.0
Corn gluten meal	21.0	21.0	21.0	20.0	34.0	34.0	34.0	33.0
Wheat middlings	13.0	13.0	13.0	10.5	4.0	4.0	4.0	2.3
Whey	11.4	11.4	11.4	9.5	2.8	2.8	2.8	2.0
Sipernat™	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CaHPO4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin/mineral premix[1]	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Carophyll pink premix	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Chemical composition								
Dry matter (g/kg)	92.0	92.0	91.2	92.8	92.5	93.1	92.6	92.0
Crude protein	45.2	44.6	45.4	46.4	61.6	61.7	62.1	61.7
Total lipid	21.6	21.4	21.2	22.5	15.3	15.4	15.9	16.2
Ash	7.5	7.5	7.7	8.1	8.5	8.4	8.4	8.9
Gross energy (MJ/kg DM)	24.4	24.3	24.4	24.5	24.1	23.9	24.1	24.0
Calculated *P*/*E* ratio (g DM/MJ)[2]	18.5	18.3	18.6	18.9	25.5	25.8	25.8	25.7

18 FO, 18 DP/DE diet with 100% fish oil; 18 SO, 18 DP/DE diet with 75% soybean oil; 18 CO, 18 DP/DE diet with 75% canola oil; 18 COS, 18 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; 25 FO, 25 DP/DE diet with 100% fish oil; 25 SO, 24 DP/DE diet with 75% soybean oil; 25 CO, 25 DP/DE diet with 75% canola oil; 25 COS, 25 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; DM, dry matter; DP/DE, digestible protein/digestible energy (lipids). Canola oil, soybean oil, and *Schizochytrium* sp algae were devoid of PCBs, pesticides, toxaphenes, and flame retardants. Herring meal was found to contain the following: PCBs: 6.5 ppb; pesticides: 2.45 ppb; toxaphenes: 0.41 ppb; flame retardants: 0 ppb. Herring oil was found to contain the following: PCBs: 386 ppb; pesticides: 147 ppb; toxaphenes: 46.8 ppb; flame retardants: 49.9 ppb. [1]Salmonid vitamin/trace mineral premix from Corey (Fredericton, NB). [2]*P*/*E* ratio = protein/energy ratio; calculation based on crude protein and gross energy values determined for the experimental diets.

Body, heart, liver, viscera, and gonads (if they were undergoing sexual maturation) were weighed. Fork length was measured, and color was evaluated with a SalmoFan™. Each fillet was taken from the carcass, and skin was removed and stored in a Ziploc bag at −20°C for further analysis.

The effects on growth were determined by evaluating growth performance indices, such as weight gain, thermal growth unit coefficient (TGC), feed conversion ratio (FCR), and survival percentage. Somatic parameters including condition factor (CF) and hepatosomatic index (HSI) were also calculated using the following formulae [5]:

$$\begin{aligned} \mathrm{CF} &= 100 \times \frac{\text{body weight (g)}}{\text{length at fork (mm)}^3}, \\ \mathrm{HSI} &= 100 \times \frac{\text{liver weight (g)}}{\text{body weight (g)}}, \\ \mathrm{FCR} &= \frac{\text{feed intake (g)}}{\text{weight gain (g)}}, \\ \mathrm{TGC} &= 100 \times \frac{\left(\text{final body weight (g)}^{1/3} - \text{initial body weight (g)}^{1/3}\right)}{(\text{temperature} \times \text{days})}. \end{aligned} \tag{1}$$

2.3.2. Washout Period. Five fish per tank were sampled every 28 days, at time 9 (252 days), 10 (280 days), 11 (308 days), and 12 (336 days). Measurements and sample procedure were same as for the growth period of the experiment.

2.4. Chemical Composition. Subsamples of 10 g wet weight/relative tank weight were weighed from each fillet of the same tank, and then pooled together. Samples were taken from the middle of the upper part of the fillet. A pool of different feed sizes was collected for the analyses of the feeds. Analyses for dry matter, ash, crude protein, total lipid, gross energy, and fatty acid profile were performed. Fillet dry matter was obtained by first drying the pools in a lyophilizator for seven days, then in a forced air oven at 105°C overnight. Sample weight was recorded before and after drying, followed by cooling in a desiccator [19]. Feed dry matter was obtained by drying only in a forced-air oven. Ash content was obtained by dry ashing in porcelain crucibles in a muffle furnace at 500°C overnight and expressed as dry weight [19]. Gross energy was performed by bomb calorimetry (Parr Instrument Company, Inc., Moline, IL) and calculated as percentage of dry matter. Crude protein was evaluated using a LECO FP-2000 instrument (LECO Corporation, St. Joseph, MI), then a nitrogen (N) conversion factor of N × 6.25, expressed as dry weight.

Total lipid composition was performed with a Soxhlet HT-TECATOR® extractor (Soxtec System HT12, Foss Tecator AB; Hoganas, Sweden), the solvent being diethyl

Table 2: Fatty acid profile of experimental diets (% of total fatty acids).

	18 FO	18 SO	18 CO	18 COS	25 FO	25 SO	25 CO	25 COS
Fatty acid								
14 : 0	3.4	0.0	0.0	3.2	5.3	0.0	0.0	5.3
16 : 0	14.7	13.1	8.4	12.6	13.2	14.4	9.5	12.0
18 : 0	2.6	3.7	2.6	2.2	2.1	4.1	2.9	1.7
Total SFA[1]	20.7	16.8	11.0	17.9	20.6	18.5	12.4	20.6
16 : 1	0.0	0.0	0.0	1.6	6.2	0.0	1.8	6.3
18 : 1	36.3	18.3	51.4	37.8	12.0	19.0	49.7	11.2
20 : 1	0.0	2.7	3.3	2.8	12.9	0.0	1.8	15.3
22 : 1	0.0	4.4	4.3	3.3	25.0	0.0	0.0	29.3
24 : 1	0.0	0.0	0.0	0.0	1.9	0.0	0.0	1.9
Total MUFA	36.3	25.4	58.9	45.5	58.1	19.0	53.3	64.0
18 : 2*n*-6	16.7	47.0	20.0	14.8	8.9	47.5	21.0	7.4
20 : 2*n*-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 : 3*n*-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 : 4*n*-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22 : 5*n*-6	4.2	0.0	0.0	4.0	0.0	0.0	0.0	0.0
Total *n*-6[2]	20.9	47.0	20.0	18.7	8.9	47.5	21.0	7.4
18 : 3*n*-3	4.2	7.2	6.1	4.5	0.0	7.0	5.8	0.0
20 : 5*n*-3	5.0	1.8	1.9	2.0	4.2	4.7	4.4	3.1
22 : 6*n*-3	13.0	1.8	2.1	11.3	6.2	3.3	3.1	5.0
Total *n*-3	22.1	10.9	10.1	17.8	10.4	15.1	13.3	8.1
Total PUFA	43.0	57.8	30.1	36.5	19.3	62.5	34.3	15.5
n-3/*n*-6	1.1	0.2	0.5	1.0	1.2	0.3	0.6	1.1

18 FO, 18 DP/DE diet with 100% fish oil; 18 SO, 18 DP/DE diet with 75% soybean oil; 18 CO, 18 DP/DE diet with 75% canola oil; 18 COS, 18 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; 25 FO, 25 DP/DE diet with 100% fish oil; 25 SO, 24 DP/DE diet with 75% soybean oil; 25 CO, 25 DP/DE diet with 75% canola oil; 25 COS, 25 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; DM, dry matter; DP/DE, digestible protein/digestible energy (lipids); TFA, total fatty acid; TL, total lipid; SFA, saturated fatty acid; MUFA, monounsaturated acid; PUFA, polyunsaturated fatty acid. [1]Includes 15 : 0, 17 : 0, 20 : 0, and 22 : 0. [2]Includes 18 : 3*n*-6.

ether at 100°C. Total lipids were expressed as dry weight. To determine the fatty acid composition of the diets and fillets, the AOAC FAME procedure [20] was used. Samples were analysed at the *Centre de Recherche en Biotechnologies Marines*, Rimouski (QC), on a GC FID gas chromatograph from Hewlett-Packard model 5890 series II (Palo Alto, CA) equipped with a CP-Sil 88 capillary column (100 m × 0.25 mm). Oven temperature was held at 80°C for 1 min before being raised to 215°C (2°C min^{-1}) for 30 min and maintained for 98 min. Injector temperature was 220°C, and detector temperature was 230°C. Fatty acid peaks were identified, quantified, and the gas chromatograph calibrated using pure methyl ester standards (Nu Chek Prep; Elysian, MN). Individual components were identified by comparing retention times with those of the standards and quantified by calculating area under the curve with the ChemStation Rev: A 10.01 program (Agilent Technologies; Santa Clara, CA).

2.5. Digestibility Measurements. To determine the digestibility of the experimental diets, Sipernat 50™ (Jefo Nutrition, Inc., Saint-Hyacinthe, QC) was included as an inert marker of acid insoluble ash (AIA) to determine apparent nutrient digestibility coefficients (ADCs). The digestibility trial was performed with rainbow trout (222.6 ± 17.2 g) randomly allocated in 24 tanks (160 L) (n = 3) in a recirculating system at the *Laboratoire de Recherche en Sciences Aquatiques* of *Université Laval* (Quebec, QC). Tanks were filled with 20 fish, reaching approximately 28 kg/m^3 biomass per tank. Water temperature was maintained at 12.6 ± 0.4°C, and other environmental parameters remained within limits recommended for salmonids [17].

Fish were acclimated three days to the feed and temperature before beginning of digestibility trial. Fish were hand-fed until apparent satiation twice daily (9 : 00 and 16 : 00). Feces were collected once a day for nine days via a modified Guelph system based on Cho et al. [21], before the morning meal. They were stored at −20°C until the end of the digestibility trial, and then thawed in a refrigerator at 4°C. Feces were centrifuged to remove excess water and freeze dried for seven days prior to analysis to determine ADC for the nutrients and energy of test and reference diets [21, 22]:

$$\text{ADC} = 1 - \left(\frac{F}{D} \times \frac{D_i}{F_i}\right), \quad (2)$$

where D = % nutrient (or kJ/g gross energy) of diet; F = % nutrient (or kJ/g gross energy) of feces; D_i = % indigestible indicator (AIA) of diet; and F_i = % indigestible indicator (AIA) of feces.

2.6. Contaminant Analyses. Levels of POPs and mercury were evaluated at times 0, 9 (252 days), and 12 (336 days). Each fillet from the same tank was pooled by taking a subsample of 2 g wet weight/relative tank weight from the median dorsal region of the fillet; tissue pools were then homogenized. Experimental diets were also analysed for contaminant content. PBCs, organochlorine pesticides,

toxaphenes, flame retardants, and mercury concentrations were evaluated by the toxicology laboratory of the *Institut National de Santé Publique du Québec* (INSPQ) in Quebec City (QC). Tissue samples were enriched with internal standards, mixed with dichloromethane and chemically dried with sodium sulfate. Organohalogenated compounds were then extracted from the matrix by ultrasound sonification. A portion of the organic solvent was used to determine the lipid weight in the sample. The residual fraction was concentrated and purified by gel permeation chromatography and florisil treatment. Extracts were analysed by gas chromatography mass spectrometry (gas chromatograph: Agilent, #6890; mass detector: Agilent, #5973; network and automatic sampler with automatic injector: Agilent, #7683; Agilent Technologies, Mississauga, ON). Ions generated were measured after negative chemical ionization. Analyte concentrations were evaluated by considering the % recovery of labeled internal standards (Agilent MSD CHEM #G1701CA; Agilent Technologies, Missisauga, ON). Mercury was analysed by cold vapour atomic absorption spectrometry (Mercury Monitor Model 100, Pharmacia; Piscataway, NJ) with an application range from 0.05 μg/g (detection limit to 10 μg/g for a sample weight of 0.5 g of wet tissue). Tissues were digested using concentrated nitric acid in pressurized Teflon vessels. An aliquot of the digest was then introduced into the system's reaction chamber (containing a reducing solution of cadmium chloride and stannous chloride). The mercury vapour was generated and detected followed by, aqueous calibration.

For PCBs, 14 congeners were analysed: 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, and 187. The following organochlorine pesticides were analysed: β-HCH, α-chlordane, γ-chlordane, *cis*-Nonachlor, *p*,*p*'-DDE, *p*,*p*'-DDT, hexachlorobenzene, mirex, oxychlordane, and *trans*-Nonachlor. Five toxaphene congeners were analysed: parlar no. 26 (T2), parlar no. 32, parlar no. 50 (T12), parlar no. 62 (T20), and parlar no. 69. PBDE 47, PBDE 99, PBDE 100, PBDE 153, and PBB 153 were the flame retardants analysed.

2.7. Statistical Analyses. Experimental values for growth performance, chemical composition, and fatty acid profile for the growth and washout periods were compared, as well as contaminant exposure and digestibility, via analysis of variance (ANOVA) in a complete randomized design, with each tank being the experimental unit (3 replicates per treatment combinations of DP/DE ratio and source of lipid). Values expressed in percentage were transformed with arcsin prior to the analysis. Normality was evaluated with Kolmogorov–Smirnov test. Differences between means were evaluated for significant differences with Tukey's multiple range test. The significance level was set at $p < 0.05$. Statistical analyses were performed using SAS 8.0 (SAS Institute, Inc., Cary, NC).

3. Results and Discussion

3.1. Growth Period. The 18 DP/DE diet had a greater percentage of oil and contained less fish meal than the 25 DP/DE diets. While the level of fish meal was not altered in vegetable oil-based diets, total fish oil was reduced by 75%. However, these diets must contribute in maintaining growth and somatic parameters, as well as the high quality of the flesh in terms of fatty acid content. The average fish mass increased continuously through the experiment without mortality, and no effects of dietary treatments on weight gain, TGC, condition factor, and hepatosomatic index were observed in this study (Table 3). Diet 18 FO had a significantly higher FCR (1.19), while 25 COS was lower than other experimental diets (0.98). Growth performance and somatic parameter data in this study indicate that soybean and canola oils (with or without the algal biomass) can replace 75% of total fish oil in rainbow trout feed, without affecting growth performance and survival.

With regards to fillet composition, no significant differences were observed for dry matter and ash content among the dietary groups tested. However, crude protein, total lipid, gross energy, and fatty acid composition were significantly different between DP/DE ratios and dietary lipid sources (Table 4). As the composition of dietary fatty acids has an effect on the muscle fatty acids, the high accumulation of 18 : 2*n*-6 in the flesh, especially the SO groups, could be due to the direct absorption of dietary fatty acids in the muscle [23, 24]. Moreover, these percentages could be explained by the great affinity of 18 : 2*n*-6 and the acyltransferases that synthesize phospholipids containing this fatty acid [5]. Also, the accumulation 20 : 3*n*-6, the product of the Δ6 desaturation and elongation of 18 : 2*n*-6, reveals a high activity of these enzymes when fish are fed high percentages of soybean oil [25]. With Atlantic salmon fed high levels of sunflower oil, Bell et al. [26] observed similar comparisons in macrophage phospholipids. When comparing the 18 : 2*n*-6 availability in the diets with the percentage in the fillets, an important decrease was observed. With the exception of the SO groups, no metabolic intermediates were found for the desaturation and elongation of 18 : 2*n*-6 to 22 : 5*n*-6 suggesting that 18 : 2*n*-6 was necessary for other physiologic requirements, for example, catabolizing energy [27, 28]. The 22 : 5*n*-6 in the COS groups appears to come from the direct absorption of DPA from the algal biomass.

It seems no competition was present between 18 : 3*n*-3 and 18 : 2*n*-6 for the desaturation and elongation by the Δ6 desaturases, even when dietary 18 : 2*n*-6 levels were high (32.0% for 18 SO and 26.2% for 25 SO, Table 5). Similar results have been recorded for Atlantic salmon fed sunflower oil [26] and rainbow trout fed different vegetable oils [5]. Bell et al. [26] noted, however, a competition between these fatty acids when the diet was rich in 18 : 3*n*-3 (31.4%) from linseed oil, resulting in an inhibition of the bioconversion of 18 : 2*n*-6 to 22 : 5*n*-6.

Results of this study reveal that the composition of the flesh reflects a selective incorporation of essential fatty acids (EFAs), especially for EPA and DHA. These EFAs are probably adjusted to a narrowly defined physiological level into triacylglycerols and phospholipids [28–30]. In this regard, the preferential retention of EFA seems to influence the fatty acid content of the muscle [5]. Therefore DHA, the major PUFA in phospholipids, is affected to a lesser extent

Table 3: Effect of fish oil replacement with soybean oil, canola oil, and a blend of canola oil and *Schizochytrium* sp. biomass on rainbow trout growth, nutritive utilization, and somatic parameters after growth trial.

	18 FO	18 SO	18 CO	18 COS	25 FO	25 SO	25 CO	25 COS	Pooled SEM
Initial weight (g)	43.6	44.1	44.0	43.2	44.0	43.3	43.2	43.7	0.2
Final weight (g)	611.7	591.7	570.2	583.0	591.0	572.3	535.7	574.1	6.5
Weight gain (g)	568.1	547.6	526.2	539.7	547.0	529.1	492.5	530.4	6.4
TGC	0.23	0.23	0.22	0.23	0.23	0.22	0.21	0.22	0.00
FCR	1.19^{a}	$1.06^{b,c,d}$	$1.14^{a,b}$	$1.09^{b,c}$	$1.07^{b,c,d}$	$1.02^{c,d}$	$1.05^{b,c,d}$	0.98^{d}	0.01
CF	1.61	1.61	1.59	1.62	1.49	1.47	1.58	1.51	0.02
HSI	1.28	1.12	1.15	1.12	1.18	1.24	1.28	1.21	0.02
Survival (%)	97.8	100.0	100.0	99.3	100.0	98.5	99.3	98.5	0.1

18 FO, 18 DP/DE diet with 100% fish oil; 18 SO, 18 DP/DE diet with 75% soybean oil; 18 CO, 18 DP/DE diet with 75% canola oil; 18 COS, 18 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; 25 FO, 25 DP/DE diet with 100% fish oil; 25 SO, 25 DP/DE diet with 75% soybean oil; 25 CO, 25 DP/DE diet with 75% canola oil; 25 COS, 25 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; DM, dry matter; DP/DE, digestible protein/digestible energy (lipids); TGC, thermal growth coefficient; FCR, feed conversion ratio; CF, condition factor; HSI, hepatosomatic index. Data represent the mean ± standard error means of three replicates, except for survival data presented as mean ± standard deviation of three replicates. Within the same line, different superscripts indicate significant differences due to the diet ($p < 0.05$).

Table 4: Effects of fish oil replacement with soybean oil, canola oil, and a blend of canola and *Schizochytrium* sp. biomass on fillet composition (g/kg DM) and fatty acid profile (% of total fatty acids) after growth period.

	18 FO	18 SO	18 CO	18 COS	25 FO	25 SO	25 CO	25 COS	Pooled SEM
Fatty acid									
14 : 0	4.2^{a}	0.0^{c}	0.0^{c}	$2.2^{a,b}$	3.7^{a}	$0.5^{b,c}$	$0.5^{b,c}$	$2.2^{a,b}$	0.3
16 : 0	$16.5^{b,c,d}$	$16.3^{c,d}$	13.0^{e}	14.9^{d}	$18.3^{a,b}$	19.0^{a}	$16.6^{b,c}$	$17.2^{a,b,c}$	0.4
18 : 0	3.0^{f}	4.7^{b}	$3.9^{c,d}$	$3.4^{e,f}$	$3.7^{d,e}$	5.5^{a}	4.6^{b}	$4.3^{b,c}$	0.2
Total SFA[1]	23.8^{a}	21.0^{b}	16.8^{c}	20.5^{b}	25.7^{a}	25.0^{a}	21.8^{b}	23.8^{a}	0.6
16 : 1	6.7^{a}	$1.2^{c,d}$	$1.7^{b,c}$	0.6^{d}	6.6^{a}	2.6^{b}	3.0^{b}	2.6^{b}	0.5
18 : 1	17.4^{c}	18.2^{c}	43.5^{a}	34.8^{b}	20.8^{c}	20.7^{c}	40.5^{a}	34.6^{b}	2.1
20 : 1	12.2^{a}	2.4^{b}	3.8^{b}	3.2^{b}	9.3^{a}	0.4^{c}	2.5^{b}	2.3^{b}	0.8
22 : 1	15.9^{a}	1.7^{c}	0.0^{d}	2.6^{c}	11.2^{b}	0.0^{d}	0.0^{d}	0.0^{d}	1.1
24 : 1	2.0^{a}	0.0^{c}	0.0^{c}	0.0^{c}	1.8^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.2
Total MUFA	54.1^{a}	23.5^{e}	$49.0^{a,b}$	$41.1^{c,d}$	$49.6^{a,b}$	23.7^{e}	$46.0^{b,c}$	39.5^{d}	2.3
18 : 2*n*-6	5.5^{e}	32.0^{a}	13.6^{c}	11.3^{d}	6.0^{e}	26.2^{b}	$12.3^{c,d}$	11.0^{d}	1.9
20 : 2*n*-6	0.0^{b}	2.0^{a}	0.0^{b}	0.0^{b}	0.0^{b}	2.0^{a}	0.0^{b}	0.0^{b}	0.2
20 : 3*n*-6	0.0^{b}	2.3^{a}	0.0^{b}	0.0^{b}	0.0^{b}	2.3^{a}	0.5^{b}	0.0^{b}	0.2
20 : 4*n*-6	0.0^{b}	1.3^{a}	0.0^{b}	0.0^{b}	0.0^{b}	2.0^{a}	0.0^{b}	0.0^{b}	0.2
22 : 5*n*-6	0.0^{c}	0.0^{c}	0.0^{c}	3.7^{a}	0.0^{c}	0.0^{c}	0.0^{c}	3.1^{b}	0.3
Total *n*-6[2]	5.5^{e}	37.5^{a}	$13.6^{c,d}$	15.0^{c}	6.0^{e}	32.4^{b}	12.9^{d}	$14.1^{c,d}$	2.3
18 : 3*n*-3	0.0^{d}	3.8^{a}	3.5^{a}	3.1^{b}	0.0^{d}	2.9^{b}	2.6^{c}	2.4^{c}	0.3
20 : 5*n*-3	$2.5^{a,b,c}$	$2.1^{b,c}$	4.2^{a}	1.2^{c}	$2.7^{a,b}$	$2.5^{a,b}$	$2.7^{a,b}$	$2.7^{a,b}$	0.2
22 : 6*n*-3	$14.2^{b,c}$	12.1^{c}	12.8^{c}	19.1^{a}	$16.0^{a,b,c}$	$13.4^{b,c}$	$14.1^{b,c}$	$17.6^{a,b}$	0.6
Total *n*-3	16.7^{c}	$17.9^{b,c}$	$20.5^{a,b,c}$	23.3^{a}	$18.7^{a,b,c}$	$18.8^{a,b,c}$	$19.4^{a,b,c}$	$22.7^{a,b}$	0.5
Total PUFA	22.2^{d}	55.5^{a}	$34.2^{b,c}$	38.4^{b}	24.7^{d}	51.3^{a}	32.2^{c}	$36.8^{b,c}$	2.3
n-3/*n*-6	3.1^{a}	0.5^{c}	1.5^{b}	1.6^{b}	3.1^{a}	0.6^{c}	1.5^{b}	1.6^{b}	0.2
Chemical composition									
Dry matter (g/kg)	25.5	26.6	25.1	26.0	25.0	24.4	26.1	26.3	0.4
Crude protein	86.2^{b}	$88.6^{a,b}$	$89.0^{a,b}$	$88.7^{a,b}$	$89.0^{a,b}$	91.1^{a}	90.2^{a}	$89.0^{a,b}$	0.4
Total lipid	10.6^{a}	$8.1^{a,b}$	$7.8^{a,b}$	$8.6^{a,b}$	$7.7^{a,b}$	5.9^{b}	$6.9^{a,b}$	$8.0^{a,b}$	0.4
Ash	5.7	5.8	5.8	6.0	5.7	6.0	5.8	5.8	0.0
Gross energy (MJ/kg DM)	23.8^{a}	$23.3^{a,b}$	$23.3^{a,b}$	$23.3^{a,b}$	$23.2^{a,b}$	22.8^{b}	$23.0^{a,b}$	$23.3^{a,b}$	0.1

Data represent the mean ± standard error means of three replicates. Within the same line, different superscripts indicate significant differences ($p < 0.05$).
[1]Includes 15 : 0, 17 : 0, 20 : 0, and 22 : 0. [2]Includes 18 : 3*n*-6.

by the DHA content in the diet. Similar observations have been reported for brown trout (*Salmo trutta* L.) fed canola oil, poultry fat, pork lard, and oleine oil (Turchini et al., 2003). Elongation and desaturation of EFA was observed for all experimental groups, mainly for fish fed lower *n*-3 diets, and levels of *n*-3/*n*-6 were higher in the muscle than in the diet. In comparison with results from Greene and Selivonchick [28] where conversion of 18 : 3*n*-3 in EPA and DHA reached its limit at 14% of total muscle lipids, it seems that our experimental groups reached their limit at 20.3% (18 COS and 25 COS), irrespective of the total dietary *n*-3 available. Regardless of the initial content of EPA in the diets, experimental groups were able to accumulate and/or produce equivalent percentages of EPA versus the FO diets. The 18 CO groups even had a higher content of EPA than its control FO group. DHA accumulation in the muscle also

Table 5: Effects of feeding with a washout diet of herring oil on fillet composition (g/kg DM) and fatty acid profile (% of total fatty acids).

	18 FO	18 SO	18 CO	18 COS	25 FO	25 SO	25 CO	25 COS	Pooled SEM
Fatty acid									
14 : 0	4.4^{a}	3.1^{b}	3.3^{b}	3.3^{b}	$3.4^{a,b}$	2.8^{b}	2.8^{b}	3.0^{b}	0.1
16 : 0	17.1	17.5	16.6	15.8	18.0	19.7	18.5	19.3	0.4
18 : 0	3.1^{c}	$4.1^{a,b}$	$3.5^{b,c}$	3.2^{c}	$3.7^{b,c}$	4.8^{a}	$4.3^{a,b}$	$4.3^{a,b}$	0.1
Total SFA[1]	24.6	24.7	23.4	22.3	25.2	27.3	25.6	26.6	0.5
16 : 1	6.1^{a}	$3.7^{b,c}$	$4.3^{a,b,c}$	3.0^{c}	$5.0^{a,b}$	$4.1^{b,c}$	$4.2^{b,c}$	$3.9^{b,c}$	0.3
18 : 1	18.4^{c}	20.0^{c}	$30.6^{a,b}$	$27.8^{a,b,c}$	$26.8^{a,b,c}$	$21.8^{b,c}$	31.8^{a}	$26.1^{a,b,c}$	1.3
20 : 1	11.5^{a}	$6.7^{b,c}$	7.7^{b}	$6.4^{b,c}$	7.5^{b}	5.2^{c}	5.4^{c}	5.2^{c}	0.5
22 : 1	15.1^{a}	8.5^{b}	9.2^{b}	$7.8^{b,c}$	8.7^{b}	$5.7^{c,d}$	$5.3^{c,d}$	5.2^{d}	0.7
24 : 1	1.9	0.8	1.1	0.0	0.8	0.0	0.0	0.0	0.2
Total MUFA	53.0^{a}	$36.9^{c,d}$	53.0^{a}	$44.9^{a,b,c,d}$	$48.8^{a,b}$	36.8^{d}	$46.7^{a,b,c}$	$40.5^{b,c,d}$	1.3
18 : 2*n*-6	5.5^{b}	18.2^{a}	8.0^{b}	8.9^{b}	7.6^{b}	17.2^{a}	9.7^{b}	8.1^{b}	1.0
20 : 2*n*-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 : 3*n*-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20 : 4*n*-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22 : 5*n*-6	0.0^{b}	0.0^{b}	0.0^{b}	2.2^{a}	0.0^{b}	0.0^{b}	0.0^{b}	2.1^{a}	0.2
Total *n*-6[2]	5.5^{d}	18.2^{a}	$8.0^{c,d}$	$11.1^{b,c}$	$7.6^{c,d}$	$17.2^{a,b}$	$9.7^{c,d}$	$10.2^{c.d}$	1.0
18 : 3*n*-3	0.0	2.2	1.3	2.0	1.0	1.9	1.2	0.0	0.2
20 : 5*n*-3	2.3	2.1	2.4	1.7	2.5	2.2	2.1	2.5	0.1
22 : 6*n*-3	14.6	13.1	15.4	18.0	14.9	14.6	14.7	20.2	0.6
Total *n*-3	16.8	17.3	19.1	21.7	18.4	18.7	18.0	22.7	0.6
Total PUFA	22.4^{c}	35.6^{a}	$27.2^{b,c}$	$32.8^{a,b}$	$26.0^{b,c}$	35.9^{a}	$27.7^{b,c}$	$32.9^{a,b}$	1.1
n-3/*n*-6	3.0^{a}	1.0^{b}	$2.4^{a,b}$	$2.0^{a,b}$	$2.4^{a,b}$	1.1^{b}	$1.8^{a,b}$	$2.2^{a,b}$	0.2
Chemical composition									
Dry matter (g/kg)	26.6	26.1	25.6	26.1	25.2	25.5	25.5	25.0	0.1
Crude protein	88.0	87.7	90.2	92.8	89.3	90.4	90.1	93.0	0.6
Total lipid	12.0	10.5	9.3	10.1	8.8	8.0	8.8	6.2	0.5
Ash	5.8	5.5	5.3	5.6	5.3	5.7	5.2	5.6	0.1
Gross energy (MJ/kg DM)	24.4	23.7	23.9	23.9	23.6	23.6	23.6	23.3	0.1

Data represent the mean ± standard error means of duplicates replicates. Within the same line, different superscripts indicate significant differences ($p < 0.05$). [1]Includes 15 : 0, 17 : 0, 20 : 0, and 22 : 0. [2]Includes 18 : 3*n*-6.

followed this trend, and the overall muscle composition in DHA tended to be similar than for fish oil-fed trout. With this capacity of synthesizing DHA to maintain its concentration in the flesh, fish demonstrate the importance of this structural lipid for its own physiological requirements [23, 30]. In addition, fish fed with a blend of canola and algae had the greatest accumulation of DHA. When comparing CO and COS groups, it is clear that *Schizochytrium* sp. contributes to the overall DHA content in the muscles. As a result, total *n*-3 fatty acids were maintained or higher than for the control FO groups.

It is now well documented that, in terms of human health, it is essential to increase the daily consumption of *n*-3, mainly EPA and DHA [31] and to reduce *n*-6 daily intake, particularly 18 : 2*n*-6 [32]. For the growth phase alone, however, *n*-3/*n*-6 ratios were too low compared with the FO groups because of the high content of *n*-6 in the flesh. Thus, canola and the blend of canola and algae were good-quality candidates for 75% fish oil replacement in rainbow trout feeds, irrespective of the DP/DE ratio used. However, a washout phase is required to balance beneficial *n*-3 levels in the final product.

3.2. Washout Period. With a switch to a diet comprised of 100% fish oil, TGC values (mean 0.16) dropped about 27% between growth and washout trials (Table 6). As fish grow, their potential for rapid growth diminishes, because nutrient utilization is aimed for physiological changes that come with maturity, for example reproduction and maintenance [33–35]. This shift in nutrient partitioning could be responsible for the decrease of TGC values in the washout phase because individuals were larger than in the growth phase, and some were already mature. FCR values (mean 1.23) were similar to 50% (1.25) and 100% (1.18) rapeseed oil feeds observed by Bell et al. [16].

In this study, the washout period represented 33% of the growth period, which is important when determining the time required to restore beneficial fatty acid levels in rainbow trout. The aim is to shorten the washout period to maintain low POP levels and minimize costly fish by-product use in the whole production cycle, while allowing EFA restoration. The DP/DE ratios did not significantly influence the fatty acid accumulation in the flesh for the 25 DP/DE groups (Table 5). In the 18 DP/DE groups, significant differences were observed between the FO and the SO groups. Whereas the flesh *n*-3 profile was equivalent across all diet treatments after the washout period, and the *n*-3/*n*-6 ratios differed. Even after a washout period, fish previously fed with soybean oil demonstrated elevated *n*-6 fatty acid levels; therefore, the inclusion of this oil in rainbow trout feed should be questioned, in terms of desirable *n*-3/*n*-6 ratios profiles.

Table 6: Effect of feeding with a washout diet of herring oil on rainbow trout growth, nutritive utilization, and somatic parameters.

	18 FO	18 SO	18 CO	18 COS	25 FO	25 SO	25 CO	25 COS	Pooled SEM
Initial weight (g)	617.1	596.9	570.2	577.8	580.1	561.3	535.7	549.7	7.7
Final weight (g)	916.6	949.8	906.4	938.7	815.8	776.2	797.5	782.4	19.0
Weight gain (g)	299.5	352.9	336.2	220.7	235.7	214.9	261.7	232.8	14.9
TGC	0.17	0.20	0.19	0.13	0.14	0.13	0.16	0.14	0.01
FCR	1.33	1.16	1.23	1.29	1.16	1.17	1.29	1.19	0.03
CF	$1.58^{a,b}$	$1.64^{a,b}$	1.65^{a}	$1.55^{a,b}$	$1.58^{a,b}$	1.47^{b}	$1.64^{a,b}$	$1.58^{a,b}$	0.02
HSI	1.06	1.22	1.16	1.17	1.22	1.11	1.21	1.26	0.02
Survival (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0

18 FO, 18 DP/DE diet with 100% fish oil; 18 SO, 18 DP/DE diet with 75% soybean oil; 18 CO, 18 DP/DE diet with 75% canola oil; 18 COS, 18 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; 24 FO, 24 DP/DE diet with 100% fish oil; 25 SO, 25 DP/DE diet with 75% soybean oil; 25 CO, 25 DP/DE diet with 75% canola oil; 25 COS, 25 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; DM, dry matter; DP/DE, digestible protein/digestible energy (lipids); TGC, thermal growth coefficient; FCR, feed conversion ratio; CF, condition factor; HSI, hepatosomatic index. Data represent the mean ± standard error means of duplicates, except for survival data presented as mean ± standard deviation of duplicates. Within the same line, different superscripts indicate significant differences due to the diet ($p < 0.05$).

In the growth phase, we observed that trout fed soybean oil accumulated high levels of *n*-6 in the flesh, reducing the quality for human consumption. During the washout phase, fish previously fed with vegetable oils, particularly soybean oil, significantly reduced their 18 : 2*n*-6 content, consequently, total *n*-6 levels were lowered. For the SO groups, this reduction of *n*-6 concentration was around 50%. For the other groups, the switch to lower *n*-6 fatty acids content was less apparent because of reduced *n*-6 accumulation during the growth phase than the SO groups. Irrespective of the DP/DE ratio, fish from the CO and COS groups restored *n*-6 levels, but the SO groups still had higher levels in the flesh. A longer washout period could have contributed in the total restoration of beneficial fatty acids for the SO groups, but it also could have raised the POP levels.

While *n*-6 levels were decreased in the washout period, *n*-3 levels remained as they were in the growth period. 18 : 3*n*-3 levels were lowered for all vegetable oil-fed groups, but DHA levels were increased, balancing the total *n*-3 content. EPA percentages remained the same for overall diets. For the 18 CO group, however, EPA levels significantly fell to reach approximate final 18 FO levels. In summary, *n*-3 levels were restored in all experimental groups, although a tendency of higher accumulation of DHA in the COS groups can be distinguished. The washout trial significantly reduced PUFA levels by lowering total *n*-6 and had a direct effect to increase *n*-3/*n*-6 ratios. Trout from the CO and COS groups restored their fatty acid levels, but the SO groups accumulated a higher *n*-6 level. Further analysis would be needed to understand the restoration profile of fatty acids to determine the shortest washout period required to restore beneficial fatty acids levels. In addition, after a short washout period that contributes in restoring beneficial EFA levels, canola oil and the blend of canola oil plus *Schizochytrium* sp. are high-quality candidates for replacing 75% of fish oil in rainbow trout diet, irrespective of the DP/DE ratio in the diets.

3.3. Contaminant Loading. Levels of POPs in flesh were well under recommendations for mercury (1 ppm), PCBs (2.0 ppm), toxaphenes (1.0 ppm), and organochlorine pesticides (5.0 ppm) (European Committee, 2007) [36, 37]. The majority of contamination exposure comes from two ingredients in the feed: fish meal and to a larger extent, fish oil (Table 1). None of the vegetable oils contained POPs or mercury, nor did the algal biomass. Of all feeds, the 18 FO diet had the highest concentration of fish oil and displayed the highest levels of PCBs, organochlorine pesticides, and toxaphenes (Figure 1). Bell et al. [16] replaced 17% and 35% fish oil by a 1 : 1 combination of linseed and canola oil fed to Atlantic salmon. The growth period was of 115 weeks; afterwards a washout feed of 35% fish oil was given for 24 weeks. The dioxin and dioxin-like PCB concentrations in the flesh followed the pattern of the present study, with bioaccumulation being greater in fish feed high percentages of fish oil. More recent research demonstrated that Atlantic salmon produced using diets with 75% of the supplemental anchovy oil (61% of total dietary lipid) replaced by cold-pressed flaxseed oil had 61% lower levels of PCBs compared to fish grown on diets with 100% fish oil without compromising growth performance [38]. In this respect, the replacement of fish oil by vegetable oils is an excellent alternative for reducing contaminant exposure for rainbow trout. Since these oils alter the quality of the flesh by increasing *n*-6 and *n*-9 levels while diminishing *n*-3 levels, a washout feed should be considered to restore beneficial *n*-3 LC-PUFA levels in the flesh.

POP levels for initial fillets, growth, and washout phase fillets are shown in Figure 2. In growth trial fillets, PCBs were significantly higher for 18 FO than other groups and were the lowest for the 25 SO, CO, COS groups (Figure 2(a)). Organochlorine pesticides were highest in the 18 FO group and the lowest in the 25 SO and CO groups (Figure 2(b)). For the toxaphenes levels, the highest levels were found in the 18 FO and 25 FO groups and were lower in all other experimental diets (Figure 2(c)). Flame retardants were not significantly different for the growth trial (Figure 2(d)). Results from the growth trial in Figure 2 outline the greater accumulation of POPs in the FO groups, particularly for the 18 FO-fed fish. Vegetable oil sources contributed in reducing the POP levels, irrespective of the DP/DE ratios.

When comparing experimental results for the washout phase, a number of tendencies were noted; a switch to a 100% fish oil feed increased the accumulation of PCBs,

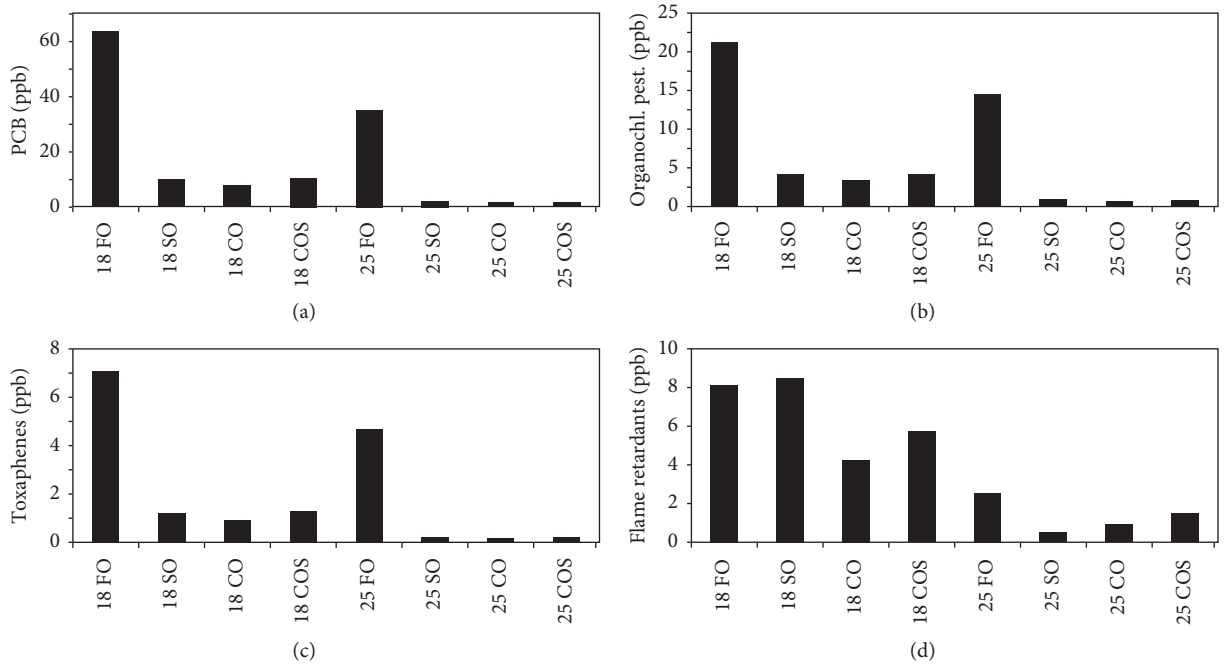

Figure 1: Concentrations of (a) PCBs (ppb), (b) organochlorine pesticides (ppb), (c) toxaphenes (ppb), and (d) flame retardants (ppb) in experimental diets. 18 FO, 18 DP/DE diet with 100% fish oil; 18 SO, 18 DP/DE diet with 75% soybean oil; 18 CO, 18 DP/DE diet with 75% canola oil; 18 COS, 18 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; 25 FO, 25 DP/DE diet with 100% fish oil; 25 SO, 25 DP/DE diet with 75% soybean oil; 25 CO, 25 DP/DE diet with 75% canola oil; 25 COS, 25 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; DM, dry matter; DP/DE, digestible protein/digestible energy (lipids).

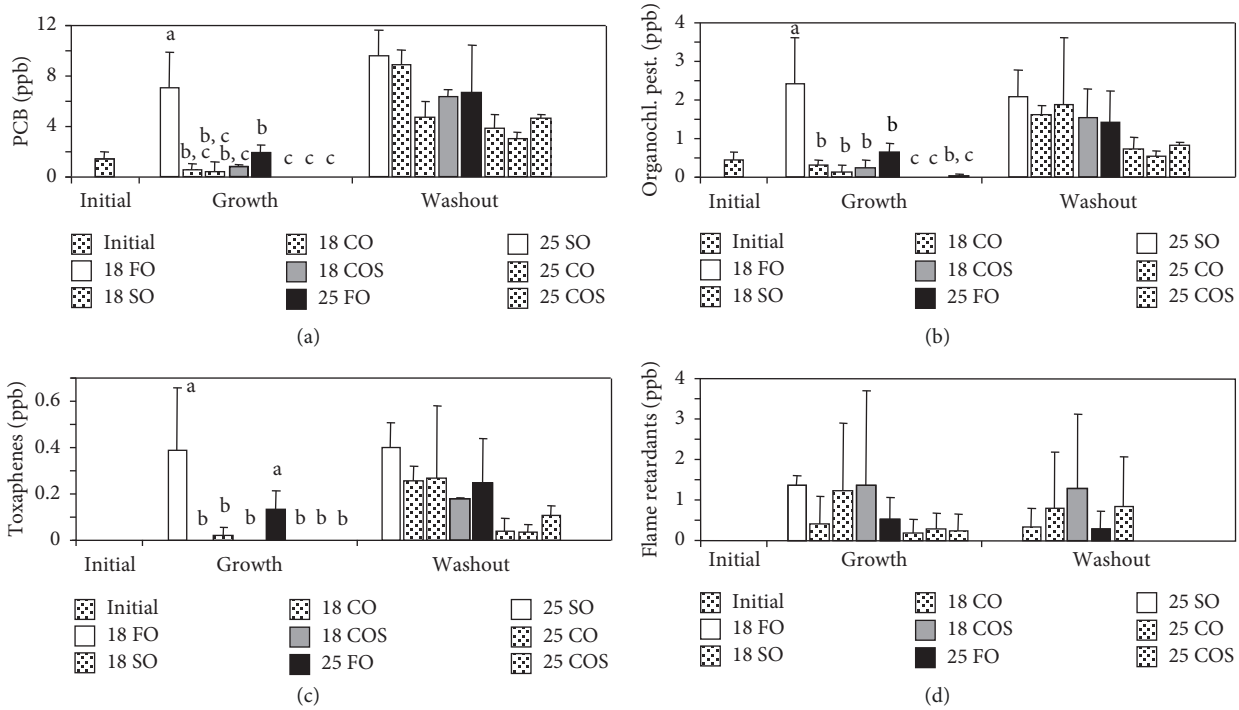

Figure 2: Concentrations of (a) PCBs (ppb), (b) organochlorine pesticides (ppb), (c) toxaphenes (ppb), and (d) flame retardants (ppb) in initial fish, at the end of growth period, and at the end of washout period. 18 FO, 18 DP/DE diet with 100% fish oil; 18 SO, 18 DP/DE diet with 75% soybean oil; 18 CO, 18 DP/DE diet with 75% canola oil; 18 COS, 18 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; 25 FO, 25 DP/DE diet with 100% fish oil; 25 SO, 25 DP/DE diet with 75% soybean oil; 25 CO, 25 DP/DE diet with 75% canola oil; 25 COS, 25 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; DM, dry matter; DP/DE, digestible protein/digestible energy (lipids). Data represent the mean of three replicates for initial data and growth trial; duplicates for washout trial. The error bars represent standard errors. Within the growth period, different superscripts indicate significant differences ($p < 0.05$).

Table 7: Apparent digestibility coefficients (ADC) of experimental diets (g/100g TL).

	18 FO	18 SO	18 CO	18 COS	25 FO	25 SO	25 CO	25 COS	Pooled SEM
Dry matter	72.7^{b}	$76.2^{a,b}$	$77.2^{a,b}$	$77.5^{a,b}$	79.3^{a}	81.4^{a}	82.0^{a}	81.3^{a}	0.7
Crude protein	90.4	91.1	91.4	90.6	90.9	91.6	91.5	91.7	0.2
Total lipid	78.9^{c}	$90.3^{a,b}$	89.0^{b}	$89.9^{a,b}$	89.4^{b}	93.7^{a}	$92.8^{a,b}$	$91.5^{a,b}$	0.9
Gross energy	80.9^{e}	$82.1^{d,e}$	$85.2^{c,d,e}$	$85.5^{b,c,d,e}$	$87.4^{a,b,c}$	$86.3^{a,b,c,d}$	90.3^{a}	$90.0^{a,b}$	0.7

18 FO, 18 DP/DE diet with 100% fish oil; 18 SO, 18 DP/DE diet with 75% soybean oil; 18 CO, 18 DP/DE diet with 75% canola oil; 18 COS, 18 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; 25 FO, 25 DP/DE diet with 100% fish oil; 25 SO, 25 DP/DE diet with 75% soybean oil; 25 CO, 25 DP/DE diet with 75% canola oil; 25 COS, 25 DP/DE diet with 75% blend of canola oil and *Schizochytrium* sp.; DM, dry matter; DP/DE, digestible protein/digestible energy (lipids); TL, total lipids. Data represent the mean ± standard deviation of three replicates. Within the same line, different superscripts indicate significant differences due to the diet ($p < 0.05$).

organochlorine pesticides, and toxaphenes in the flesh. This statement is more obvious with the vegetable oil-feed groups, where POPs were accumulated to a larger extent, but still lower than in the fish oil-feed groups. Once again, a tendency of lower POP concentration is noticeable for the 25 DP/DE group, but is not significant, due to high variability between individuals. Very little information is available regarding the accumulation of POPs in the flesh during a washout period; Bell et al. [16] obtained similar results. Vegetable oil-fed fish accumulated contaminants during the washout phase. Flame retardant accumulation in the flesh does not follow the path of PCBs, toxaphenes, and organochlorine pesticides (Figure 2(d)). As fish oil has a greater percentage of flame retardant levels than vegetable oils and fish were fed 100% FO diets, these data are unexpected. However, overall levels are very low (less than 1.5 ppb). In terms on POP exposure, all vegetable oil-based feeds have ability to reduce the accumulation in the flesh, in the growth phase. Even if levels were increased for PCBs, toxaphenes, and organochlorine pesticides in the washout period of this study, final concentrations were still lower than recommended levels (European Committee, 2007) [36, 37].

3.3.1. Digestibility of Experimental Diets. Results show that the reduction of FO content in the feed (18 FO vs 25 FO) improves ADC of dry matter and lipids (Table 7). Apparent digestibility of dry matter is in accordance with results from Windell et al. [39] for medium (ADC of 72%) and large (74%) rainbow trout fed fish meal at three different temperatures (7, 11, and 15°C). Cho et al. [40] had an ADC in a range of 70 to 77% with rainbow trout fed marine oils and different protein sources. However, Olsen et al. [41] fed 500 g Atlantic salmon with *Calanus finmarchicus* at 10°C seawater and obtained an ADC of 95% while our results range between 73 and 82%. Apparent digestibility was the highest for 25 CO and the lowest for 18 FO diet. Digestibility of crude protein was between 70 and 100% [39, 40, 42, 43]. Our results are not significantly different and are in accordance with these authors. Our results are within the range described by the NRC [17] where digestibility for fish oil usually varies in a range of 75 to 95%. In multiple digestibility trials with rainbow trout using feather meal, meat and bone meal, poultry by-product meal, and blood meal, Bureau et al. [44] observed digestibilities between 68 and 99% for gross energy. Cho and Kaushik [42] had an average of 76% and Cho et al. 79% [40]. In the current study, the digestibility of gross energy for experimental diets was therefore similar, ranging between 81% and 90%.

According to Caballero et al. [5], high lipid ADC for the vegetable oil-based diets is related to their high PUFA content, consequently, their low melting point. Results from Cho and Kaushik [42] support this observation with apparent digestibility of rainbow trout reared in temperatures varying from 5 to 15°C. When fed rapeseed, soybean, linseed or fish oils, ADC was high (between 80 and 95%). Conversely, when trout were fed lard and tallow, which have high melting points because of high SFA concentrations, ADC decreased compared to other lipids sources. Therefore, the melting point of lipids influences their digestibility for rainbow trout. However, this theory can not explain the variations in digestibility obtained in this study, as fish oil and vegetable oils have a similar melting point. Bureau et al. [45] reported a high ADC for tallow fed to rainbow trout, and suggested that ADC is not only a matter of the lipid's melting point but also of the "synergistic effect" of PUFA on the digestibility of SFA. Differences in results may be due to the methodology used by Cho and Kaushik [42] where experimental diets were formulated with a reference diet that was low in lipids (<5%). The addition of oils rich in PUFA would have increased the ADC of SFA, and thereby the ADC of total lipids. Overall, the balance between PUFA and SFA in the diet is crucial to obtain a high ADC. The 18 FO diet, which has the highest percentage in fish oil and total fish by-products, has the lowest digestibility. This diet also has a high SFA/PUFA ratio (1.07) compared to vegetable oil diets (mean 0.38), confirming previous suggestions on lipid digestibility, where the SFA/PUFA ratio of the diet is crucial in the digestibility of a diet. Therefore, our results are in accordance with previous studies and Caballero et al.'s study [5] with rainbow trout reared at 12°C (ADC = 79.2 to 92.5%). In conclusion, apparent digestibility of nutrient and gross energy of the eight experimental diets, irrespective of the DP/DE ratio and the lipid source, are highly suitable for the industry, and these diets are appropriate for rainbow trout.

4. Conclusions

The replacement of 75% total fish oil by vegetable oils and the alteration of DP/DE ratios did not affect apparent digestibility, growth performance, and somatic parameters for trout. However, *n*-3/*n*-6 levels were negatively affected, especially for the SO groups. During the washout trial,

muscle fatty acids were partially restored for the two CO and COS groups, but the SO groups still had high *n*-6 content. In the growth trial, POPs were lower in vegetable oil-fed trout of the 18 and 25 DP/DE groups. The washout trial increased POP percentages, but levels are below recommendation levels. Thus, replacing 75% of total fish oil in rainbow trout feed by CO and COS, combined with a 25 DP/DE ratio, seems to be the most efficient in terms of maximizing the total fish by-product replacement, while reducing POP exposure.

Data Availability

Data have been summarized by the project team for the present publication. Raw data are stored on Université Laval's server.

Disclosure

Pallab K. Sarker is currently at Environmental Studies Program, Dartmouth College, Hanover, NH 03755, USA.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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