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The effect of total replacement of fish oil with DHA-Gold® and plant oils on growth and fillet quality of rainbow trout (*Oncorhynchus mykiss*) fed a plant-based diet

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Abstract

This study investigated the effect of the replacement of fish oil (FO) with DHA-Gold (DHA-G)-supplemented plant oils (PO) in rainbow trout fed plant-protein-based diets. Five diets (450 mg g^{-1} digestible protein and 150 mg g^{-1} crude lipid) were fed to rainbow trout (initial weight 37 ± 0.5 g) for 12 weeks in a 15 °C recirculating water system. The lipid inclusion types and levels were FO, PO and PO with DHA-G supplemented at 30 mg g^{-1}, 60 mg g^{-1} or 90 mg g^{-1} of the diet replacement for corn oil. Fish fed 90 mg g^{-1} DHA-G were significantly larger and consumed more feed than fish-fed PO or FO (218 g and 2.6% bwd^{-1} versus 181 g and 2.4% and 190 g and 2.3%, respectively). Feed conversion ratio was significantly increased in fish fed 90 mg g^{-1} DHA-G (0.99) as compared to fish-fed FO (0.90) and 30 mg g^{-1} DHA-G (0.91). Panellists found trout fillets from fish fed the 90 mg g^{-1} DHA-G diet to have significantly fishier aroma and flavour than fish fed the FO diet. Fatty acid analysis demonstrated that 60 mg g^{-1} or 90 mg g^{-1} DHA-G supplementation increased PO fed fish fillet DHA to fatty acid levels equivalent or higher than those fish fed a FO diet.

KEY WORDS: aquaculture, docosahexaenoic acid, fatty acids, fish oil, plant oil, rainbow trout

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Introduction

Feeding of carnivorous fish species like rainbow trout (*Oncorhynchus mykiss*) requires substantial amounts of dietary lipid for the supply of energy and for the production of a high quality fish fillet containing long-chain essential fatty acids: polyunsaturated fatty acids (PUFA). Due to the short-fall from wild-harvested marine fish stocks for the production of fish oil (FAO 2010), alternative lipid sources for the production of fish feed are essential to meet the demand of the growing aquaculture industry (Bureau *et al.* 2008; Tacon & Metian 2008).

Changes in dietary lipid composition not only affect growth and health, but also product quality in farmed fishes (Tocher *et al.* 2004; Karalazos *et al.* 2011; Turchini *et al.* 2013a,b). Product quality of farmed fishes is defined by customer's needs and expectations, and often includes the long-chain n-3 PUFA content of the fillet (Liu *et al.* 2004; Torstensen *et al.* 2005). In a recent study conducted with brown trout (*Salmo trutta*) by Kenari *et al.* (2011), concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the fish fillet were affected by dietary lipid sources. Concentrations of serum glucose, total cholesterol, triglycerides, and very low-density lipoprotein were significantly higher in fish fed diets with lower concentrations of n-3 PUFA. Studies in gilthead seabream (Benedito-Palos *et al.* 2008), pikeperch (Kowalska *et al.* 2011) and Atlantic salmon (Bell *et al.* 2003; Torstensen *et al.* 2004) reported similar disorders of lipid metabolism and alterations in fatty acid profiles of the fillets when diets containing fish oil-alternative dietary lipid sources were fed. Studies examining replacement of fish oil in diets of rainbow trout have not always observed reduced growth but have documented altered liver fatty acid metabolism and muscle n-3 PUFA content (Caballero *et al.* 2002; Turchini *et al.* 2013a). Consequently, the fillet was deemed less acceptable by consumers due to inability to supply substantial amounts of long-chain n-3 PUFA (Caballero *et al.* 2002; Turchini *et al.* 2013b).

To date, research indicates that up to 80–90% of fish oil can be replaced by vegetable oils and other fat sources in rainbow trout diets without affecting growth. The fillet n-3 PUFA content, however, can be affected (Caballero *et al*. 2002; Turchini *et al*. (2013a), and thus, the fillet quality that is of importance to consumers can be altered (Turchini *et al*. 2013b). For this reason, total replacement of fish oil with vegetable oil for many species, including rainbow trout, is still considered impossible due to the resultant modification of the fatty acid composition of cultured fish. Previous studies have recommended the use of diets containing fish oil during a finishing period to restore the fatty acid composition of the fish previously fed the alternative lipid sources (Mourente & Bell 2006; Trushenski & Boesenberg 2009; Thanuthong *et al*. 2011). However, restoration of muscle fatty acid in carnivorous species requires large quantities of fish oil due to the high dietary levels needed for market size fish (Jobling 2004; Trushenski & Boesenberg 2009). Thus, identification of suitable alternative oils is critical for sustainability of the aquaculture industry.

DHA-Gold® is a dry algae powder containing 530 mg g^{-1} crude lipid on a dry-weight basis, 460 mg g^{-1} of which is DHA, 22:6n-3. The benefit of this microalga (*Crypthecodinium cohnii*) in human and animal nutrition has been investigated (Spolaore *et al*. 2006). In fish species, the potential of a DHA-algae meal was investigated in juvenile cobia as complete replacement for fishmeal and fish oil by Salze *et al*. (2010). The authors reported that 94% replacement of fishmeal is possible when DHA-algae meal is combined with soy products. Subsequent studies recapitulated the beneficial effects of DHA-algae meal supplementation when fish oil is replaced with soy oil in cobia diets (Trushenski *et al*. 2012, 2013; Woitel *et al*. 2014). Improved growth and feeding behaviour was also observed when DHA-algae meal was included in juvenile barramundi diet (Glencross & Rutherford 2011). However, the potential of DHA-Gold® oil to maintain growth and fillet quality in rainbow trout–fed fishmeal and fish oil–free diets has not been previously investigated. Therefore, the purpose of this study was to examine the effects of DHA-Gold® (DHA-G) inclusion as a replacement for dietary fish oil on rainbow trout growth characteristics, fillet fatty acid profiles, texture and consumer acceptance.

Materials and methods

Determination of DHA-G digestibility

The nutritional value of DHA-G was evaluated by determining the apparent digestibility coefficients (ADCs) of protein, lipid and energy, as well as amino acid availability (AACs) in extruded diets. Nutrient and energy availability was determined using the methods described by Cho *et al*. (1982) and Bureau *et al*. (1999) to estimate apparent digestibility coefficients. All diets were double labelled using chromic oxide and yttrium oxide as the inert markers. A complete reference diet meeting or exceeding all known nutritional requirements for rainbow trout was blended with the test ingredients in a 70:30 ratio (dry-weight basis) to form test diets (Table 1). This diet has been used successfully in several digestibility trials (Gaylord & Barrows 2009; Barrows *et al*. 2011) with subsequent rainbow trout growth trials supporting the digestibility trial results (Barrows *et al*. 2008, 2010).

Diets were manufactured by cooking extrusion (DNDL-44; Buhler AG, Uzwil, Switzerland) with an 18-s exposure to an average of 127 °C in the extruder barrel sections. Pressure at the die head was varied from 275 to 400 bar, depending on test diet. Pellets of 3–4 mm were produced and dried in a pulse-bed drier (CTW; Buhler AG) to

Table 1 Composition of digestibility reference diet (g kg^{-1} dry-weight) fed to rainbow trout

Ingredients	g kg^{-1}
Wheat flour[1]	283
Squid meal[2]	250
Soy protein concentrate[3]	171
Fish oil[4]	134
Corn gluten meal[5]	83
Soybean meal[6]	43
Vitamin premix[7]	10
Chromic oxide[8]	10
Choline chloride[8]	06
Taurine[9]	05
Stay-C 35[10]	02
Trace mineral premix[11]	01
Yttrium oxide[8]	01

[1] Archer Daniels Midland (Decatur, IL, USA) 4 g kg^{-1}protein.
[2] Wilbur-Ellis, 723 g kg^{-1}crude protein.
[3] Solae Profine VP (St. Louis, MO, USA) 693 g kg^{-1}crude protein.
[4] Omega Proteins Inc., Virgina Prime Menhaden oil (Houston, TX, USA).
[5] Cargill Animal Nutrition (Minneapolis, MN, USA), 601.0 g kg^{-1}protein.
[6] Archer Daniels Midland (Decatur, IL, USA), 480 g kg^{-1}protein.
[7] Contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 g: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5; inostitol 600.
[8] Sigma-Aldrich Company (St Louis, MO, USA).
[9] Alliance Nutrition, Twin Falls, ID, USA.
[10] Stay-C, 350 mg g^{-1}, DSM Nutritional Products (Boulder, CO, USA).
[11] Sigma-Aldrich Company (St Louis, MO, USA).Contributed in mg/kg of diet; zinc 40; manganese 13; iodine 5; copper 9.

<100 mg g^{-1} moisture, followed by a 20-min cooling period at ambient temperature. Both diets were top coated with fish oil using a vacuum coater (A.J. Mixing, Oakville, Ontario, Canada) and 24-mm mercury pressure.

Diets were fed to 30 fish (average weight 600 g) per tank in 500-L tanks with three replicate tanks per treatment. Water temperature was maintained at 15 °C, and lighting was maintained on a 13-h light/11-h dark regimen. Fish were fed to satiation by hand twice daily. Faeces from fish in each replicate tank were obtained by manual stripping (Austreng 1978). In brief, all fish in each tank were netted, anaesthetized with MS-222 (Tricane methane sulphonate; Western Chemical Company, Ferndale, WA, USA), dried and gentle pressure was applied to the lower abdominal region to express faecal matter into a plastic weighing pan. Care was taken to exclude urinary excretions from the collection. Faecal samples for a given tank were freeze-dried, ground with a mortar and pestle and stored at −20 °C until chemical analyses were performed.

Digestibility calculations

Apparent digestibility coefficients of each nutrient in the test diet and DHA-G were calculated according to the following equations (Kleiber 1961; Forster 1999):

$$\mathrm{ADCN_{diet}} = 100 \times (\%\ \text{marker in diet} \times \%\ \text{nutrient in faeces})/(\%\ \text{marker in faeces} \times \%\ \text{nutrient in diet}) \quad (1)$$

$$\mathrm{ADCN_{ingredient}} = \{(a+b)\mathrm{ADCN}_t - (a)\mathrm{ADCN}_r\}b^{-1} \quad (2)$$

where, $\mathrm{ADCN_{ingredient}}$ = apparent digestibility coefficients of the nutrient in the test ingredient; ADCN_t = apparent digestibility coefficients of the nutrient in the test diets; ADCN_r = apparent digestibility coefficients of the nutrient in the reference diet; $a = (1-p) \times$ nutrient content of the reference diet; $b = p \times$ nutrient content of the test ingredient; p = proportion of test ingredient in the test diet.

DHA-G feeding trial

Experimental diets Five isonitrogenous and isolipidic experimental diets, based on the digestibility data of DHA-G, were formulated using the same basal ingredients and varying only in their lipid sources (Table 2). The lipid sources and their inclusion are fish oil: FO (100%), Plant oil: PO (Corn oil and flax oil in a 2.5:1 ratio; 100%) and DHA-G: DHA-G at 30 mg g^{-1}, 60 mg g^{-1} or 90 mg g^{-1} supplementation replacing only the corn oil portion of the PO blend on an isolipidic basis). The experimental diets contained soy protein concentrate, corn protein concentrate and soybean meal as the protein sources. The FO and 60 mg g^{-1} DHA-G diets were formulated to ensure equivalent estimated values of 13.8 mg g^{-1} dietary DHA; subsequently, 30 mg g^{-1} and 90 mg g^{-1} DHA-G diets contained lower and higher values of this fatty acid, respectively.

Diets were manufactured (3-mm-diameter pellets) by cooking extrusion using a twin-screw extruder (Buhler AG) and dried in a pulse-bed drier to <100 mg g^{-1} moisture. Lecithin and DHA-G were included in the mash prior to extruding and fish oil, corn and flax oil were was top-coated postextrusion using a vacuum coater (A.J. Mixing).

Feeding trial procedures Eggs were obtained from Troutlodge Inc., Sumner, WA, USA and raised on a commercial trout grower diet until an average body weight of 37 ± 0.5 g was attained. The fish were stocked into 200-L tanks in a recirculating culture system at 15 fish per tank. Four tanks of fish were randomly assigned to each dietary treatment. The experimental diets were fed to apparent satiation twice daily (at 8:00 am and 3:00 pm), 6 days a week, for 12 weeks. Apparent satiation was defined as all the feed the fish would consume in a 20-min period. During the experiment, the recirculating system was maintained at optimum levels for rainbow trout culture: 15 °C for water temperature, >6.0 mg L^{-1} dissolved O_2, pH of 7.5, and <0.12 mg L^{-1} ammonia. After 12 weeks of feeding, fish were fasted for 24 h before collection of tissue samples for compositional analyses.

Feeding trial sampling and measurements Ten fish were euthanized with tricane methane sulphonate (MS-222; 200 mg L^{-1} water) prior to the experiment and kept at −20 °C for the determination of initial body composition. Bulk tank weights of fish were measured at the beginning of the experiment and every 2 weeks thereafter with feed intake determined weekly. Three fish per tank were randomly selected and euthanized with tricane methane sulphonate (MS-222; 200 mg L^{-1} water) at the end of 12-week experiment. Individual fish weights, length, visera weight and liver weight were determined for the calculation of weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein retention efficiency (PRE), energy retention efficiency (ERE), condition factor (CF), hepatosomatic index (HSI) and viscerosomatic index (VSI). Blood samples were obtained from each of the three fish

Table 2 Ingredients and proximate composition of the experimental plant-based diets with different lipid sources

Ingredients (g kg^{-1}dry weight)	PO	30 mg g^{-1} DHA-Gold	60 mg g^{-1} DHA-Gold	90 mg g^{-1} DHA-Gold	FO
Fish oil [1]	0.0	0.0	0.0	0.0	152.0
DHA-Gold[2]	0.0	30.0	60.0	90.0	0.00
Soy lecithin[3]	30.0	30.0	30.0	30.0	30.0
Flax oil	40.0	40.0	40.0	40.0	40.0
Corn oil	109.0	93.0	76.0	59.0	0.0
Soy protein concentrate[4]	236.4	236.4	236.4	236.4	236.4
Corn protein concentrate[5]	175.4	175.4	175.4	175.4	175.4
Soybean meal[6]	133.0	133.0	133.0	133.0	133.0
Wheat flour	188.5	174.8	160.8	151.8	185.8
Lysine HCL	20.5	20.5	20.5	20.5	20.5
Methionine	5.5	5.5	5.5	5.5	5.5
Threonine	3.0	3.0	3.0	3.0	3.0
Taurine	5.0	5.0	5.0	5.0	5.0
Magnesium Oxide	0.5	0.5	0.5	0.5	0.5
Sodium Chloride	2.8	2.8	2.8	2.8	2.8
Trace min premix[6]	1.0	1.0	1.0	1.0	1.0
Vitamin premix 702[7]	10.0	10.0	10.0	10.0	10.0
Natu-rose[8]	10.0	10.0	10.0	10.0	10.0
Guar gum	3.0	3.0	3.0	3.0	3.0
Ascorbic acid	2.0	2.0	2.0	2.0	2.0
Estimated DHA from DHA-G[7]	0.00	6.9	13.8	20.7	13.8
Analysed composition[9]					
Protein (N* 6.25) (mg g^{-1})	452 ± 0.1	447 ± 0.4	451 ± 0.1	461 ± 0.2	439 ± 0.4
Fat	164 ± 0.3	182 ± 0.3	191 ± 0.2	175 ± 0.1	154 ± 0.4
Energy (kJ g^{-1})	22.5 ± 0.2	22.8 ± 0.1	22.6 ± 0.1	22.4 ± 0.1	22.2 ± 0.2

[1] Omega Proteins Inc., Virgina Prime Menhaden oil (Houston, TX, USA).
[2] DSM Nutritional Products Inc.
[3] ALCOLEC® S, 62% Acetone Insolubles, (American Lecithin Company, Oxford, CT, USA).
[4] Solae Profine VP 693 g kg^{-1}crude protein.
[5] Cargill Corn Milling, Empyreal (Cargill, Inc., Blair, Nebraska, USA).
[6] Contributed in mg/kg of diet; zinc 40; manganese 13; iodine 5; copper 9.
[7] Contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 g: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5; inostitol 600.
[8] Natu-rose, Cyanotech Corporation (Kona, HI, USA).
[9] Means of duplicate analyses of an as-fed basis.

using a 3-mL syringe fitted with 25 gauge, 3-mm needle. Blood was transferred into a 1.5-mL heparinized centrifuge tube and centrifuged at 83 Hz for 5 min. Plasma from each of the tubes was withdrawn and stored in individually coded microcentrifuge tubes at −80 °C until analyses. Fillet and liver samples were frozen with liquid nitrogen immediately after collection and were transferred and stored at −80 °C until proximate composition and fillet fatty acid analyses. Fillets were obtained from the remaining fish in the tanks following approved human food processing protocols of slaughter and filleting; fillets were skinned, rinsed with tap water, packed in ice and placed into coded bags and stored at −20 °C prior to sensory analysis and tenderness score determinations.

Feeding trial calculations The following equations were used in calculating each of the body indexes parameters:

$$\mathrm{WG} = \left[\frac{(\text{final weight} - \text{initial weight})}{(\text{initial weight})}\right] \times 100 \quad (3)$$

$$\mathrm{FCR} = \frac{\text{kg body mass}}{\text{kg diet consumed}} \quad (4)$$

$$\mathrm{PRE} = \left[\frac{(\text{final body mass} - \text{initial body mass})}{(\text{amount of protein given})}\right] \times 100 \quad (5)$$

$$\mathrm{ERE} = \left[\frac{(\text{final body mass} - \text{initial body mass})}{(\text{amount of energy given})}\right] \times 100 \quad (6)$$

where protein and energy retention efficiencies were calculated as weight gain (kg) for each kg protein consumed and kcal energy consumed, respectively.

$$\%\text{Heptatosomatic index} = (\text{liver mass}/\text{body mass}) \times 100 \quad (7)$$

$$\%\text{Viscerosomatic index} = (\text{gut mass}/\text{body mass}) \times 100 \quad (8)$$

$$\%\text{Survival rate} = \left[\frac{(\text{final number of fish} - \text{initial number of fish})}{(\text{initial number of fish})}\right] \times 100 \quad (9)$$

Chemical analysis of diets, faecal samples, fillets and whole body composition

Dry matter was determined according to AOAC (1995). Crude Protein ($N \times 6.25$) values were determined by Dumas method on a Leco TruSpec nitrogen determinator. Gross energy was determined using isoperibol bomb calorimetry (Parr 1281; Parr Instrument Co. Inc., Moline, IL, USA). Lipid content was determined by petroleum ether extraction using an Ankom XT 10 according to AOAC (1995). Yttrium and phosphorus were determined in diets and faeces by inductively coupled plasma atomic absorption spectrophotometry following nitric acid digestion (Anderson 1996). Amino acids were quantified following acid hydrolysis utilizing a Beckman 7300 amino acid analyser and postcolumn derivitization with ninhydrin (AAA Laboratory, Mercer Island, WA, USA).

Feeding trial blood chemistry analyses

The plasma samples were analysed for the concentrations of triglyceride (TG), total cholesterol (TC) and activities of lipase enzyme (LP) using Vitros chemistry system DT6011 (Rochester, NY, USA), a modified method of Rouser (1970).

Diet and fillet fatty acid methyl ester preparation and analyses

Fatty acid profiles were determined in triplicate for each of the experimental diets. Frozen fillets were individually thawed, homogenized, and fat was extracted from approximately 0.3 g samples using chloroform/ methanol according to Bligh & Dyer (1959). Subsequently, 20 μL of a 1% solution of pentadecanoic acid (15:0) in dissolved methanol was added to each of the sample as an internal standard. Folch organic extraction was performed by adding 3 mL of 0.88% potassium chloride (KCl) and 5 mL of 2:1 dichloromethane (DCM) and centrifuging at 1500 ***g*** for 5 min. The DCM layer was removed and placed into a new clean glass dram vial extraction. This step was repeated thrice, and thereafter, the hydrophobic DCM extract was dried with nitrogen. Fatty acid methyl ester (FAME) analysis was performed by adding 0.5 mL of boron trifluoride methanol (BF3) to the hydrophobic extract and heating at 60 °C for 30 min, followed by addition of 0.5 mL of DDO_2 water and 0.5 μL hexane. Fatty acid methyl esters were separated and quantified by gas chromatography–mass separation (GC–MS) (Donato *et al.* 2003). The FAMEs were quantified by the comparison of retention time and peak area to those of reference standards (GLC-473b; Nu-Check Prep, Elysian, MN, USA).

Fillet shear force measurement

Shear force measurements were conducted on frozen fillet samples following the procedures of Boles *et al.* (2009). Samples initially stored at −20 °C were thawed at 4 °C, and thereafter, three circle cores were removed parallel to the fibre direction. Each raw sample was sheared once perpendicular to the fibre direction with a TMS 30 Food Texturometer (Food Technology Corporation, Sterling, VA, USA) fitted with a Warner–Bratzler shear attachment using a crosshead speed of 100 mm min^{-1} to obtain the shear force (N), distance, and time required to calculate springiness, stringiness, adhesiveness and cohesiveness for each sample. The average of the three samples sheared per fillet was used for statistical analysis. Connective tissue shear force was obtained by measuring the connective tissue peak on the shear deformation curve as described by Moller (1980).

Sensory analysis

Fillets were skinned and individually packaged in 'Ziploc' freezer bags and stored at −23 °C until analysis. Prior to analysis, fillets were partially thawed, just enough to cut with a knife; about 10 mm was removed from each side, and then, 9.0 ± 1.0 g serving portions were sliced across the fillet from dorsal to ventral. Frozen fillet portions (six to nine pieces) were placed in 25 × 30 cm boil-in pouches. Samples in pouches were vacuum-packed using the Ultravac 250 sealer (Koch Equipment, Kansas City, MO, USA). Vacuum-packed fillet samples were immediately returned to −23 °C until sensory analysis. For sensory analyses,

samples were poached at 74 °C for 6 min, then removed from the boiling pouch and held under the radiant heat unit at setting '6.5' to maintain a temperature of not <60 °C for a maximum of 15 min before serving to panellists. Samples were randomly served hot in a consumer test to 25 panellists who were asked to compare four unknown samples (representing each dietary treatment) with an identified control (100% FO), in a room designed for sensory analysis. Untrained sensory panellists were recruited from the Washington State University community. Panellists were from a diverse ethnic background and ranged in age from 18 to 64. Approximately, 50% of the test subjects were under age 35; 61% of test subjects were female. All participants signed an informed consent form, and the project was approved by the Washington State University Institutional Review Board. Sensory testing was conducted over two test dates in the WSU School of Food Science sensory laboratory. The FO control was presented in a pair with each of the test treatments. On Day 1, 24 panellists were presented with the FO versus 30 mg g^{-1} DHA, and in a second tasting flight, FO versus 90 mg g^{-1} DHA. On Day 2, the same 25 panellists were presented with the FO versus 60 mg g^{-1} DHA and versus PO, respectively. Pair comparisons were presented in random order under white light. Just prior to serving, each fillet sample was transferred from the radiant warmer to a 4 oz soufflé cup identified with a 3-digit code, then sealed with a cap to trap aroma. Panellists were provided with reagent grade deionized water and unsalted-top saltine crackers for cleansing the palate. A cuspidor was provided for expectoration. Using a paired difference test and paired preference test, panellists were asked to indicate which sample in a pair had: (i) more fishy aroma, (ii) more fishy flavour, (iii) more grassy flavour and (iv) firm texture, as well as which sample was preferred. Panellists were asked to comment as to why they preferred one sample over the other.

Statistical analysis

All data on growth characteristics, blood chemistry and fatty acids profiles were subjected to one-way ANOVA with the Proc GLM of SAS version 9.1 (SAS Institute Inc., Cary, NC, USA), significant difference was considered at $P < 0.05$. Sensory analysis results were analysed using Compusense® five software (Compusense Inc., Guelph, ON, Canada) using Tukey's HSD and the binomial distribution tables for paired comparison (Roessler *et al.* 1978). Significance is reported as $P \leq 0.05$, ≤ 0.01 or ≤ 0.001, representing minimum agreeing judgments of 18, 19 and 21 of 25 panellists.

Results

DHA-G digestibility results

The analysed proximate composition (g kg^{-1}) of DHA-G was 960 dry matter, 534 crude lipid, 151 crude protein and 28.6 kJ g^{-1}. Calculated dry matter, protein, lipid and energy apparent digestibility coefficients (ADCs) were 61%, 18%, 98% and 73%, respectively (Table 3). Amino acid availability coefficients (AACs) of the DHA-G were low, as were sum AACs.

Analysed proximate composition of diets

Proximate analysis of the diets reflected formulation targets (Table 2), while the dietary fatty acid profile reflected that of the oils used in feed formulation (Table 4). The PO diet had no detectable levels of 14:0, but algal DHA supplementation increased 14:0 levels in a corresponding manner. A similar trend was observed with 18:1n-9 as algal DHA was supplemented in the PO diet. The FO diet was the only diet with 20:5n-3, while inclusion of algal DHA in the PO diet increased the level of 22:6n-3 in the DHA-G diets with the exception of 30 mg g^{-1} DHA-G diet, which was below the level of detection.

Table 3 Proximate composition, ADCs and AACs of DHA-Gold®[1]

	Analysed composition mg g^{-1}	ADCs and AACs (%)
Dry matter	960.0	61
Protein	151.1	18
Lipid	534.4	98
Energy kJ g^{-1}	28.6	73
Phosphorus	2.0	100
Amino Acids (sum)	418.0	13
Ala	6.9	46
Arg	6.4	61
Asp	14.0	26
Glu	14.9	9
Gly	5.6	33
His	1.8	14
Ile	3.9	40
Leu	6.8	18
Lys	3.3	99
Met	2.3	48
Phe	4.4	36
Pro	4.0	1
Ser	3.0	0
Thr	4.1	29
Tyr	2.6	28
Val	5.7	55

[1] Means of duplicate analyses on a dry matter basis (mg g^{-1} sample).

Growth, plasma and condition indexes

Dietary treatments significantly ($P = 0.0343$) affected the weight of rainbow trout and the highest final weight was observed in fish fed the 90 mg g^{-1} DHA-G diet (Table 5). Feed intakes of fish fed the 60 mg g^{-1} and 90 mg g^{-1} DHA-G diets were significantly higher than all other treatments ($P < 0.0001$) and FCR of fish fed the 60 mg g^{-1} and 90 mg g^{-1} DHA-G diets were significantly higher compared to fish fed FO diet or 30 mg g^{-1} DHA-G diet ($P = 0.0025$).

No significant effect of dietary lipid source on PCV, cholesterol or lipase was observed (Table 5). There was a tendency towards increased plasma triglyceride concentration in fish fed PO and DHA-G diets ($P = 0.0562$).

Hepatosomatic index observed in the DHA-G diets averaged 1.0, which was significantly lower than the value obtained from those fish fed the PO diet ($P = 0.0490$; Table 5). However, viscerosomatic index and condition factor were not affected by dietary treatments ($P = 0.2107$ and 0.2389, respectively).

Body and fillet proximate compositions

Whole body moisture ($P = 0.7425$), lipid ($P = 0.4659$) protein ($P = 0.3553$) were not affected by dietary treatments; however, energy was significantly affected by diet ($P = 0.0237$; Table 6). Whole-body energy composition of fish fed the PO diet was significantly higher than in fish fed the 90 mg g^{-1} DHA and FO diets. Both protein retention efficiency (PRE) and energy retention efficiency (ERE) were significantly lower in fish fed 90 mg g^{-1} DHA-G diet than in fish fed FO diet ($P = 0.0007$, $P = 0.0021$, respectively). In contrast, dietary treatments did not significantly alter fillet moisture, lipid, protein and energy levels ($P = 0.7520$, $P = 0.2412$, $P = 0.3806$, and $P = 0.5907$, respectively).

Table 4 Selected fatty acid composition mg g^{-1} of the plant-based diet containing different lipid sources[1]

Fatty acids	PO	30 mg g^{-1} DHA-Gold®	60 mg g^{-1} DHA-Gold®	90 mg g^{-1} DHA-Gold®	FO
9:0	3	3	2	2	2
14:0	–	15	30	37	99
15:0	21	19	16	20	22
16:0	281	282	285	296	303
16:1n-7	–	–	–	–	62
17:0	–	–	–	–	3
18:0	28	26	26	26	30
18:1n-9	141	157	141	126	96
16:2n-4	–	–	–	–	07
18:2n-6	456	423	400	391	292
18:3n-3	71	75	77	62	32
18:3n-6	–	–	–	–	8
18:4n-3	–	–	–	–	5
20:4n-6	–	–	–	–	–
20:5n-3	–	–	–	–	23
22:6n-3	–	4	24	39	17

[1] Mean of triplicate analyses per diet.

Fillet sensory and shear force analyses

Differences in consumer preference were noted when comparing each diet (PO, 30 mg g^{-1}, 60 mg g^{-1} and 90 mg g^{-1} DHA-G) to the control diet (Fig. 1). Panellists found trout fillets from the FO diet to have significantly more fishy aroma ($P < 0.001$) and fishy flavour ($P < 0.05$) when compared to fillets from fish fed the PO diet (Fig. 1a). Fillets from fish fed the PO diet had more grassy flavour ($P < 0.01$) when compared to fillets from fish fed the FO diet (Fig. 1a). When fillets from fish fed the FO diet were compared to fillets from fish fed the 30 mg g^{-1} DHA-G diet, the fillets from fish fed the FO diet were found to have more grassy flavour than fillets from fish fed the 30 mg g^{-1} DHA-G diet ($P < 0.05$; Fig. 1b). When fillets from fish fed the FO diet were compared with fillets from fish fed the 60 mg g^{-1} DHA-G diet, a significant difference in preference was found for fillets from fish fed the FO diet ($P < 0.05$; Fig. 1c). Fillets from trout fed the 90 mg g^{-1} DHA-G diet were perceived by panellists to have more fishy flavour than the fillets from fish fed the FO diet ($P < 0.05$; Fig. 1d).

Although there was a trend ($P < 0.1$) for fillets from FO diet to be considered firmer than PO diet by panellists, shear force analysis displayed no significant effect of dietary lipid source on fillet hardness ($P = 0.4142$) or fracturability ($P = 0.6277$; Table 7). Similarly, other measures of fillet texture including springiness, stringiness, adhesiveness and cohesiveness were not altered by dietary lipid source.

Fillet fatty acid composition

The fatty acid profiles of the fillets reflected dietary fatty acid concentrations (Table 8). The most abundant saturated fatty acid was 16:0, the level of which was not altered by dietary treatment ($P = 0.0051$). The 18:1n-9 level was lower in fish fed the FO diet than those fed the PO and the DHA-G diets ($P = 0.0017$). Moreover, 18:2n6 was significantly higher in fish fed the PO diet than those fed the FO diet. Inclusion of algal DHA in the PO diet inversely affected the level of 18:2n6. Arachidonic acid was highest in fish fed the PO diet ($P = 0.0009$). Eicosapentanoic acid concentration was highest in fish fed the FO diet and the

Table 5 Performance of rainbow trout (initial weight 37 ± 0.5 g) fed a plant-based diet containing different lipid sources[1]

Parameter	PO	30 mg g^{-1} DHA-Gold®	60 mg g^{-1} DHA-Gold®	90 mg g^{-1} DHA-Gold®	FO	*P*-value	SEM[2]
Growth							
Final weight (g)	181^{c}	201^{ab}	200^{ab}	214^{a}	190^{bc}	0.0343	6.7
FI (% BW/d)[3]	2.4^{b}	2.3^{b}	2.5^{a}	2.6^{a}	2.3^{b}	<0.0001	0.04
FCR[4]	0.95^{ab}	0.91^{b}	0.99^{a}	0.99^{a}	0.90^{b}	0.0025	0.02
Plasma							
PCV (%)	48	45	47	46	49	0.3153	1.5
Cholesterol mg dL^{-1}	234	208	191	197	222	0.4736	4.9
Triglycerides mg dL^{-1}	227	228	246	187	156	0.0562	13.6
Lipase units L^{-1}	141	169	142	143	180	0.1743	8.7
Condition indices							
HSI[5]	1.2^{a}	1.0^{b}	1.0^{b}	1.0^{b}	1.1^{ab}	0.0490	0.04
VSI[6]	12.0	12.0	10.8	10.6	11.2	0.2107	0.5

Data on the same row not sharing a common superscript letter are significantly different ($P < 0.05$).
[1] Means of four replicate tanks per diet.
[2] Pooled standard error of the mean.
[3] Feed Intake ratio (g 100 g $fish^{-1}$ day^{-1}), FI = 100 × feed consumption (g)/average biomass (g) × days.
[4] Feed conversion ratio, FCR = feed offered (g)/weight gain (g).
[5] Hepatosomatic index (%) = (liver mass/body mass) × 100.
[6] Viscerosomatic index (%) = (gut mass/body mass) × 100.

Table 6 Whole body and fillet proximate composition of rainbow trout fed a plant-based diet containing different lipid sources[1]

Parameter	PO	30 mg g^{-1} DHA-Gold®	60 mg g^{-1} DHA-Gold®	90 mg g^{-1} DHA-Gold®	FO	*P*-value	SEM[2]
Whole body composition (mg g^{-1})							
Moisture	657	658	660	667	665	0.7425	6
Lipid	148	149	152	139	142	0.4659	5
Protein	165	166	165	171	170	0.3553	3
Energy kJ g^{-1}	10.8^{a}	10.3^{bc}	10.5^{ab}	10.0^{c}	9.9^{c}	0.0237	0.2
PRE (%)[3]	38.9^{bc}	41.4^{ab}	37^{c}	37.9^{c}	43.5^{a}	0.0007	0.9
ERE (%)[4]	54^{a}	53^{a}	49.1^{bc}	47^{c}	52^{ab}	0.0021	1.1
Fillet composition (mg g^{-1})							
Moisture	755	755	750	752	750	0.7520	2
Lipid	27	31	36	38	37	0.2412	4
Protein	206	202	202	200	200	0.3806	2
Energy kJ g^{-1}	6.4	6.0	6.2	6.1	6.2	0.5907	0.2

Data on the same row not sharing a common superscript letter are significantly different ($P < 0.05$).
[1] Means of four replicate analyses per diet.
[2] Pooled standard error of the mean.
[3] Protein retention efficiency.
[4] Energy retention efficiency.

level was significantly lower in all other diets ($P < 0.0001$). The highest concentration of 22:6n-3 was found in fish fed 90 mg g^{-1} DHA-G diet, while the lowest concentration was observed in fish fed the PO ($P = 0.0021$).

Discussion

This study showed the potential of plant-based diets without the inclusion of marine or terrestrial animal ingredients for rainbow trout. Previous studies have investigated plant-based diets as replacement for conventional fishmeal diets in rainbow trout, but those diets contained fish oil (Gaylord *et al.* 2006, 2007; Gaylord & Barrows 2009). In the present study, a plant-based diet with the addition of algal DHA as a supplementary lipid source produced comparable performance with that of fish fed an FO diet. Similarity in rainbow trout performance, particularly in growth and FCR when fish are fed 30 mg g^{-1} DHA-G and FO diets, suggests that PO can successfully replace FO in a plant-based diet when diets are supplemented with a low inclusion level of algal oil. Other studies (Caballero *et al.* 2002; Richard *et al.* 2006) have demonstrated similar

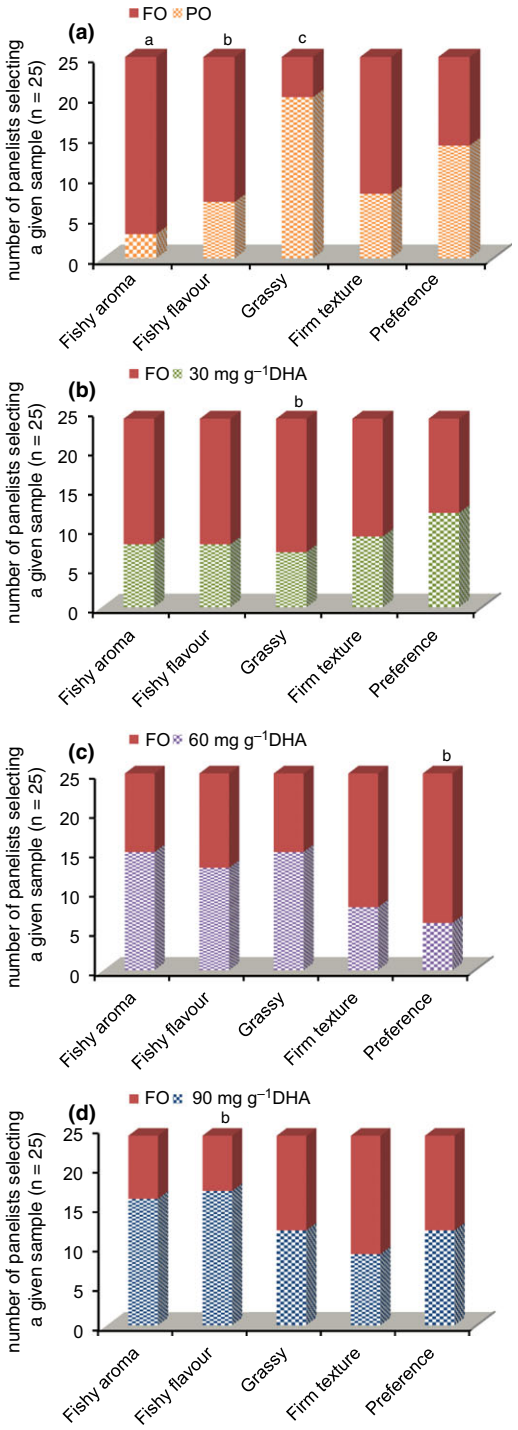

Figure 1 Paired comparison sensory evaluation of fillets from rainbow trout fed a plant-based diet containing different lipid sources. A paired comparison for a given attribute between the control diet and treatment diet is denoted as significant, with "a" being significant at $p < 0.001$, "b" being significant at $p < 0.05$ and "c" being significant at $p < 0.01$.

impact of total replacement of fish oil on growth performance in a fishmeal-based diet.

Lack of consistent sensory characteristic differences between fillets from FO and PO diets in this study indicate the ability of PO, with the inclusion of DHA-G, to also replace fish oil without detrimental effect on sensory properties. Previous studies have reported inconsistent organoleptic evaluation of fish fillets when fed plant ingredients either as protein or as lipid sources (Turchini *et al.* 2009; Stone *et al.* 2011). In this study, the panellists found fillets from fish fed 90 mg g^{-1} DHA-G to have fishier flavour than those fish that were actually fed FO diet. The reason for this observation is likely the increased DHA levels in those fillets. It has previously been reported that organoleptic scores are highly significantly correlated with levels of EPA, docosapentaenoic acid and DHA (Miller & Robisch 1969).

Higher dietary concentrations of 18:3n-3 and 18:2n-6 as compared to fillet concentrations is supported by earlier findings in rainbow trout (Thanuthong *et al.* 2011) and in salmon (Stubhaug *et al.* 2006, 2007). The authors found that both fatty acids are abundantly used for fatty acid β-oxidation when present in high quantities in fish diets. Rinchard *et al.* (2007) reported that rainbow trout only retain high proportion of 18:3n-3 in case of shortage. Bioconversion pathways for the elongation of polyunsaturated fatty acids have been reported (Buzzi *et al.* 1997; Bell *et al.* 2001; Torstensen & Tocher 2010), where elongases are proposed to be responsible such that 18:2n-6, 18:3n-6, 18:3n-3 and 18:4n-3 are elongated to 20:2n-6, 20:3n-6, 20:3n-3 and 20:4n-6, respectively. However, in this study, 20:2n-6 and 20:4n-6 were only detected in significant amounts in fish fed the PO diet, and 20:3n-6 and 20:3n-3 were not detected at all. Although it has been suggested that 18:3n-3 is a preferred substrate for the elongases over that of 18:2n-6 and that the enzymes activities are substrate-dependent, it is difficult to ascertain if in the current study elongase was insufficient or that the high contents of 18:2n-6 and 18:3n-3 in the diets prevented the activities of these enzymes. The concentration of 18:3n-3 in the fillets of fish fed the PO diet was not affected by supplementing the PO diet with DHA-G, but fillet 18:2n-6 and 20:4n-6 levels decreased with increased dietary inclusion of DHA-G. As adequate dietary DHA was supplied, it is possible that there was no need for the Δ-6 desaturase to mobilize 18:2n-6 and 18:3n-3 for elongation as well as subsequent *in vivo* desaturation to 22:6n-3 (Thanuthong *et al.* 2011). Substrate availability does not explain the low amounts of arachidonic acid detected in the fillets of fish fed FO or DHA-

Table 7 Shear force (N) measurements of fillets from rainbow trout fed a plant-based diet containing different lipid sources

Parameters	PO	30 mg g^{-1} DHA-Gold®	60 mg g^{-1} DHA-Gold®	90 mg g^{-1} DHA-Gold®	FO	*P*-value	SEM[2]
Hardness	34.06	40.42	35.21	39.26	39.36	0.4142	5.2
Cohesiveness	0.13	0.16	0.46	0.09	0.05	0.6579	0.38
Adhesiveness	0.015	0.021	0.010	0.036	0.004	0.3201	0.02
Fracturability	18.50	22.83	21.28	22.62	27.0	0.6277	5.2
Springiness	0.63	0.92	0.20	0.81	0.18	0.1929	0.47
Stringiness	3.47	3.26	3.46	3.20	3.15	0.6149	0.15

[1] Means of four replicate analyses per diet.
[2] Pooled standard error of the mean.

Table 8 Fillet fatty acid compositions (mg g^{-1}) of rainbow trout fed a plant-based diet containing different lipid sources[1]

	Dietary treatments							
Fatty acid	Initial	PO	30 mg g^{-1} DHA-Gold®	60 mg g^{-1} DHA-Gold®	90 mg g^{-1} DHA-Gold®	FO	*P*-value	SEM[2]
14:0	167	13^{c}	29^{c}	50^{b}	52^{b}	105^{a}	<0.0001	06.1
15:0	52	57	73	74	66	76	0.5149	08.2
16:0	356	273^{b}	342^{a}	372^{a}	344^{a}	389^{a}	0.0015	16.0
18:0	31	45	39	36	39	39	0.4589	03.4
16:1n-7	135	15^{b}	13^{b}	15^{b}	12^{b}	87^{a}	<0.0001	02.0
18:1n-9	96	199^{a}	161^{b}	147^{bc}	139^{bc}	116^{c}	0.0017	11.3
16:2n-4	10	ND	ND	3^{ab}	ND	6^{a}	0.0359	01.5
18:2n-6	48.	273^{a}	234^{b}	203^{bc}	185^{c}	101^{d}	<0.0001	12.3
20:2n-6	ND	9^{a}	ND	ND	3^{b}	ND	0.0129	01.7
20:4n-6	ND	11^{a}	1^{b}	ND	4^{b}	ND	0.0009	01.6
18:3n-3	08	48^{a}	45^{a}	50^{a}	45^{a}	12^{b}	<0.0001	02.9
18:3n-6	ND	6^{a}	1^{b}	ND	ND	ND	<0.0001	00.4
18:4n-3	ND	6^{a}	ND	ND	ND	ND	<0.0001	00.5
20:5n-3	34	7^{b}	5^{b}	5^{b}	4^{b}	26^{a}	<0.0001	01.9
22:6n-3	66	21^{c}	26^{bc}	46^{a}	60^{a}	43^{ab}	0.0021	06.0

Data on the same row not sharing a common superscript letter are significantly different ($P < 0.05$).
[1] Means of triplicate analyses of three fish per tank, four tanks per treatment.
[2] Pooled standard error of the mean.

supplemented diets. Recent research by Emery *et al.* (2013) suggests that, the activity of Δ-6 desaturase on n-3 and n-6 substrates is independent of substrate availability.

Conclusion

Taken together, these results appear to demonstrate that supplementing a PO diet with algal DHA can meet the dietary requirement of trout for n-3 fatty (NRC, 2011) as no apparent signs of n-3 deficiency such as poor growth or shock syndrome were observed and plasma cholesterol and triglyceride levels were within normal ranges (Kopp *et al.* 2011) and not significantly higher in fish fed diets with lower concentrations of n-3 PUFA as has previously been reported by Kenari *et al.* (2011). Algal DHA supplementation also provided an effective mechanism for increasing long-chain PUFA in fillets of rainbow trout-fed plant proteins and oil without fish meal or fish oil that still allowed fillets to meet the recommended intake for long-chain PUFA (Simopoulos *et al.* 1999). These results may be beneficial to the aquafeed industry by providing effective alternatives to reduce over-dependence on wild-harvested fish products for formulation of fish feed. However, only supplementation levels of 60 mg g^{-1} and 90 mg g^{-1} DHA-G increased fillet DHA levels to levels that were comparable or in excess of fillets from rainbow trout fed the FO diet and at these levels some consumer preference effects were noted. Thus, future studies should address both the increased production cost associated with the high supplementation levels necessary and the potential negative effects of inclusion on consumer preference.

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References

Anderson, K.A. (1996) Micro-digestion and ICP-AES analysis for the determination of macro and micro elements in plant tissues. *At. Spectrosc.*, **17**, 30–33.

Association of Official Analytical Chemists (AOAC). (1995) Official Methods of Analysis, vol. 4, 16th edn. AOAC, Arlington, VA.

Austreng, E. (1978) Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture*, **13**, 265–272.

Barrows, F.T., Gaylord, T.G., Sealey, W.M., Smith, C.E. & Porter, L. (2008) The effect of vitamin premix in extruded plant based and fish meal based diets on growth efficiency and health of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **283**, 148–155.

Barrows, F.T., Gaylord, T.G., Sealey, W.M., Porter, L. & Smith, C.E. (2010) Supplementation of plant-based diets for rainbow trout (*Oncorhynchus mykiss*) with macro-minerals and inositol. *Aquacult. Nutr.*, **16**, 654–661.

Barrows, F.T., Gaylord, T.G., Sealey, W.M. & Rawles, S.D. (2011) Database of nutrient digestiblity's of traditional and novel feed ingredients for trout and hybrid striped bass. USDA-ARS. Accessed at http://www.ars.usda.gov/Main/docs.htm?docid=21905.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J. & Sargent, J.R. (2001) Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J. Nutr.*, **131**, 1535–1543.

Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R. & Crampton, V.O. (2003) Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rape seed oils can be partially restored by a subsequent fish oil finishing diet. *J. Nutr.*, **133**, 2793–2801.

Benedito-Palos, L., Navarro, J.C., Sitjà-Bobadilla, A., Bell, J.D., Kaushik, S. & Pérez-Sánchez, J. (2008) High levels of vegetable oils in plant protein-rich diets fed to gilthead sea bream (*Sparus aurata* L): growth performance, muscle fatty acid profiles and histological alterations of target tissues. *Br. J. Nutr.*, **100**, 992–1003.

Bligh, E.G. & Dyer, W.J. (1959) A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911–917.

Boles, J.A., Boss, D.L., Neary, K.I., Davis, K.C. & Tess, M.W. (2009) Growth implants reduced tenderness of steaks from steers and heifers with different genetic potentials for growth and marbling. *J. Anim. Sci.*, **87**, 269–274.

Bureau, D.P., Harris, A.M. & Cho, C.Y. (1999) Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **180**, 345–358.

Bureau, D.P., Hua, K. & Harris, A.M. (2008) The effect of dietary lipid and long-chain n-3 PUFA levels on growth, energy utilization, carcass quality, and immune function of rainbow trout, *Ocorhynchus mykiss*. *Aquaculture*, **214**, 253–271.

Buzzi, M., Henderson, R.J. & Sargent, J.R. (1997) Biosynthesis of docosahexaenoic acid in trout hepatocytes proceeds via 24-carbon intermediates. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, **116**, 263–267.

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. & Izquierdo, M.S. (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **214**, 253–271.

Cho, C.Y., Slinger, S.J. & Bayley, H.S. (1982) Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. *Comp. Biochem. Physiol.*, **73**, 25–41.

Donato, M., Vilela, M.H. & Bandarra, N.M. (2003) Fatty acids, sterols, alpha-tocopherol and total carotenoids of *Diacronema vlkianum*. *J. Food Lipids*, **10**, 267–276.

Emery, J.A., Hermon, K., Hamin, N.K.A., Donald, J.A. & Turchini, G.M. (2013) Delta 6 desaturase substrate competition: dietary linoleic acid has only trivial effects on linolenic acid bioconversion in the teleost rainbow trout. *PLoS ONE*, **8**, 1–14.

Food and Agriculture Organisation of the United States (FAO) (2010) The State of World Fisheries and Aquaculture 2009 (SOFIA). FAO Fisheries and Aquaculture Department, Rome.

Forster, I. (1999) A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. *Aquacult. Nutr.*, **5**, 143–145.

Gaylord, T.G. & Barrows, F.T. (2009) Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, (*Oncorhynchus mykiss*) feeds. *Aquaculture*, **287**, 180–184.

Gaylord, T.G., Teague, A.M. & Barrows, F.T. (2006) Taurine supplementation of all-plant protein diets for rainbow trout (Oncorhynchus mykiss). *J. World Aquacult. Soc.*, **37**, 509–517.

Gaylord, T.G., Barrows, F.T., Teague, A.M., Johansen, K.A., Overturf, K.E. & Shepherd, B. (2007) Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **269**, 514–524.

Glencross, B. & Rutherford, N. (2011) A determination of the quantitative requirements for docosahexaenoic acid for juvenile barramundi (*Lates calcarifer*). *Aquacult. Nutr.*, **17**, 536–548.

Jobling, M. (2004) Finishing feeds for carnivorous fish and the fatty acid dilution model. *Aquacult. Res.*, **35**, 706–709.

Karalazos, V., Bendiksen, E.A. & Bell, J.G. (2011) Interactive effects of dietary protein/lipid level and oil source on growth, feed utilization and nutrient and fatty acid digestibility of Atlantic salmon. *Aquaculture*, **311**, 193–200.

Kenari, A.B., Mozanzadeh, M.T. & Pourgholam, R. (2011) Effects of total fish oil replacement to vegetable oils at two dietary lipid levels on the growth, body composition, haemato-immunological and serum biochemical parameters in Caspian brown trout (*Salmo trutta caspius* Kessler, 1877). *Aquacult. Res.*, **42**, 1131–1144.

Kleiber, M. (1961) The Fire of Life: An Introduction to Animal Energetics. John Wiley and Sons Inc, New York, NY.

Kopp, R., Mareš, J., Lang, S., Brabec, T. & Zikova, A. (2011) Assessment of ranges plasma indices in rainbow trout (*Oncorhynchus mykiss*) reared under conditions of intensive aquaculture. *Acta univ. agric. et silvic. Mendel. Brun.*, **6**, 181–188.

Kowalska, A., Zakes, Z., Jankowska, B. & Siwicki, A. (2011) Substituting vegetable oil for fish in pikeperch diets: the impact on growth, internal organ histology, blood biochemical parameters, and proximate composition. *Aquacult. Nutr.*, **17**, 148–163.

Liu, K.K.M., Barrows, F.T., Hardy, R.W. & Dong, F.M. (2004) Body composition, growth performance and product quality of rainbow trout (*Oncorhynchus mykiss*) fed diets containing poultry fat, soybean/corn lecithin or menhaden oil. *Aquaculture*, **238**, 309–328.

Miller, D. & Robisch, P. (1969) Comparative effect of herring, menhaden, and safflower oils on broiler tissues fatty acid composition and flavor. *Poult. Sci.*, **48**, 2146–2157.

Moller, A.J. (1980) Analysis of Warner-Bratzler shear pattern with regard to myofibrillar and connective tissue components of tenderness. *Meat Sci.*, **5**, 247–260.

Mourente, G. & Bell, J.G. (2006) Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, **145**, 389–399.

National Research Council (NRC) (2011) Nutrient Requirements of Fish. National Academy Press, Washington.

Richard, N., Kaushik, S., Larroquet, L., Panserat, S. & Corraze, G. (2006) Replacing dietary fish oil by vegetable oils has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss*). *Br. J. Nutr.*, **96**, 299–309.

Rinchard, J., Czesny, S. & Dabrowski, K. (2007) Influence of lipid class and fatty acid deficiency on survival, growth, and fatty acid composition in rainbow trout juveniles. *Aquaculture*, **264**, 363–371.

Roessler, E.B., Pangborn, R.M., Sidel, J.L. & Stone, H. (1978) Expanded statistical tables for estimating significance in paired preference, paired difference, duo-trio and triangle tests. *J. Food Sci.*, **43**, 940–943.

Rouser, L. (1970) Estimation of phospholipids by phosphorous assays. *Lipids*, **5**, 494–496.

Salze, G., McLean, E., Battle, P.R., Schwarz, M.H. & Craig, S.R. (2010) Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. *Aquaculture*, **298**, 294–299.

Simopoulos, A.P., Leaf, A. & Salem, N. (1999) Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Ann. Nutr. Metab.*, **43**, 127–130.

Spolaore, P., Joannis-Cassan, C., Duran, E. & Isambert, A. (2006) Commercial applications of microalgae. *J. Biosci. Bioeng.*, **101**, 87–96.

Stone, D.A.J., Oliveira, A.C.M., Ross, C.F., Plante, S., Smiley, S., Bechtel, P. & Hardy, R.W. (2011) The effects of phase-feeding rainbow trout (Oncorhynchus mykiss) with canola oil and Alaskan pollock fish oil on fillet fatty acid composition and sensory attributes. *Aquacult. Nutr.*, **17**, 521–529.

Stubhaug, I., Lie, Ø. & Torstensen, B.E. (2006) β-oxidation capacity in liver increases during parr-smolt transformation of Atlantic salmon (*Salmo salar* L.) fed vegetable- and fish oil. *J. Fish Biol.*, **69**, 504–517.

Stubhaug, I., Lie, Ø. & Torstensen, B.E. (2007) Fatty acid productive value and b-oxidation capacity in Atlantic salmon (*Salmo salar* L.) fed different lipid sources along the whole growth period. *Aquacult. Nutr.*, **13**, 145–155.

Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture*, **285**, 146–158.

Thanuthong, T., Francis, D.S., Senadheera, S.D., Jones, P.L. & Turchini, G.M. (2011) Fish oil replacement in rainbow trout diets and total dietary PUFA content: I) Effects on feed efficiency, fat deposition and the efficiency of a finishing strategy. *Aquaculture*, **320**, 82–90.

Tocher, D.R., Fonseca-Madrigal, J., Dick, J.R., Ng, W., Bell, J.G. & Campbell, P. (2004) Effects of water temperature and diets containing palm oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes of rainbow trout (*Onchorhynchus mykiss*). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, **137**, 49–63.

Torstensen, B.E. & Tocher, D.R. (2010) The effects of fish oil replacement on lipid metabolism of fish. In: Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds (Turchini, G.M., Ng, W.K. & Tocher, D.R. eds.), pp. 405–438. CRC Press, Taylor & Francis group, Boca Raton, FL.

Torstensen, B.E., Frbyland, L., Ornsrud, R. & Lie, Ø. (2004) Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. *Food Chem.*, **87**, 567–580.

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

Trushenski, J.T. & Boesenberg, J. (2009) Influence of dietary fish oil concentration and finishing duration on beneficial fatty acid profile restoration in sunshine bass *Morone chrysops* ♀ x *M. saxatilis* ♂. *Aquaculture*, **296**, 277–283.

Trushenski, J.T., Schwarz, M., Bergman, A., Rombenso, A. & Delbos, B. (2012) DHA is essential, EPA appears largely expendable, in meeting the n-3 long-chain polyunsaturated fatty acid requirements of juvenile cobia *Rachycentron canadum*. *Aquaculture*, **326**, 81–89.

Trushenski, J.T., Woitel, F., Schwarz, M. & Yamato, F. (2013) Saturated fatty acids limit the effects of replacing fish oil with soybean oil with or without phospholipid supplementation in feeds for juvenile cobia. *N. Am. J. Aquacult.*, **75**, 316–328.

Turchini, G.M., Torstensen, B.E. & Ng, W.K. (2009) Fish oil replacement in finfish nutrition. *Rev. Aquacult.*, **1**, 10–57.

Turchini, G.M., Hermon, K., Cleveland, B.J., Emery, J.A., Rankin, T. & Francis, D.S. (2013a) Seven fish oil substitutes over a rainbow trout grow-out cycle: I) Effects on performance and fatty acid metabolism. *Aquacult. Nutr.*, **19**, 82–94.

Turchini, G.M., Hermon, K., Moretti, V.M., Caprino, F., Busetto, M.L., Bellagamba, F., Rankin, T. & Francis, D.S. (2013b) Seven fish oil substitutes over a rainbow trout grow-out cycle: II) Effects on final eating quality and a tentative estimation of feed related production costs. *Aquacult. Nutr.*, **19**, 95–109.

Woitel, F.R., Trushensiki, J.T., Schwarz, M.H. & Jahncke, M.L. (2014) More judichious use of fish oil in cobia feeds: I. Assessing the relative merits of alternative lipids. *N. Am. J. Aquacult.*, **76**, 222–231.