FINAL REPORT

Balancing Dietary Lipid and Cholesterol to Increase Fillet Omega-3 Deposition in Rainbow Trout Fed a Soy-Based Diet

Report to:

Andy Tauer
Soy Aqua Alliance (SAA)

Tel: 317-644-2862
E-mails: atauer@soyaquaalliance.com

From:

University of Idaho

Project Status:

Principal Investigators:

Brian C. Small, Ph.D. Professor
Ronald W. Hardy, Ph.D. Professor
Kenneth E. Overtuf, Ph.D. Research Geneticist
Jeongwhui Hong, Graduate student

Unit:

University of Idaho
Aquaculture Research Institute
Hagerman Fish Culture Experiment Station
3059F National Fish Hatchery Road
Hagerman, Idaho 83332
Tel: 208-837-9096, ext. 1108
Fax: 208-837-6047
E-mail: bcsmall@uidaho.edu
rhardy@uidaho.edu
Ken.overturf@ars.usda.gov
jhong@uidaho.edu

March 26, 2020

1. Abstract

We hypothesized that soybean farmers could increase the value of U.S. soybean oil by establishing soybean oil as a preferred lipid ingredient to replace fish oil in global aquaculture feeds while lowering costs and improving profits for the aquaculture industry. This hypothesis was based on our primary objective to blend soybean oil with linseed oil and supplemental cholesterol to maintain fish growth and increase de novo production of the omega-3 fatty acids (n-3 FA) EPA and DHA in trout muscle, since EPA and DHA have been identified as beneficial nutrients in human nutrition, and farmed seafood must meet consumer needs and expectations. To accomplish this, we attempted to use soy oil for it's low phytosterol properties in combination with linseed oil for it's n3-FA content, with and without cholesterol to improve utilization. Upon conclusion of the study, our results did not support our hypothesis. Fish fed all-plant based diets containing any amount of soy oil had reduced growth performance. Only fish fed the all-plant diet with 100% linseed oil plus supplemental cholesterol grew as well as the fishmeal/fish oil positive control group. With regard to fillet EPA and DHA content, no improvements were seen with soybean oil. Dietary cholesterol supplementation improved plasma cholesterol levels and numerically increased EPA and DHA levels in fillets when linseed oil was provided above 50 %. Gene expression results support the fatty acid analysis, indicating no significant enhancement by soybean oil or cholesterol. Although, our hypothesis failed and soybean oil was found to be less accepted by the fish, resulting in lower growth and feed intake. We were successful in developing a high soy-protein diet (35%) with all-plant ingredients using linseed oil and supplemental cholesterol that resulted in equal fish performance to fish fed a fishmeal/fish oil control diet. More research is needed to identify a cost-effective EPA/DHA source to meet consumer needs and expectations for n-3 FA in trout fillets.

2. Introduction

Aquaculture is often touted as a solution to ensuring enough fish protein for a rapidly increasing global population (FAO, 2014). A great deal of research in the aquaculture field now emphasizes the need to make fish farming more sustainable (FAO, 2014). A large effort in this regard is to develop feeds that reduce our reliance on capture fisheries for fish meal and fish oil. Success in this regard has brought with it a complication for human health, which is lower omega-3 (n-3) fatty acids (FA) in fish feeds leading to lower n-3 FA in the consumable flesh of the fish produced (Sprague et al., 2016). Omega-3 fatty acids, particularly EPA (20:5n-3) and DHA (22:6n-3) have been identified as beneficial nutrients in human nutrition leading to improved heart and brain health (Calder, 2014). For this reason, farmed seafood must meet consumer needs and expectations. One-for-one substitutions of fish oil with plant oils currently isn't enough to ensure healthful levels of EPA and DHA in farmed fish products. Alternative feeding strategies, coupled with improved plant and fish genetics, must be developed to increase n-3 FA in farmed fish products.

Over the past decade, considerable research has been conducted on fatty acid metabolism in rainbow trout, a model species for other carnivorous fish species and important food fish. This is mostly driven by increased demand, stagnant production, rising costs of fish oil and the inevitable modification of the final n-3 long-chain polyunsaturated fatty acid (LCPUFA) content of fish fillets when fish oil is replaced with more economical and sustainable plant oil alternatives. The thrust of much of the research has been on the capability of fish to

biosynthesize DHA (22:6n-3) through the desaturation and elongation of α-linolenic acid (ALA, 18:3n-3). The problem appears to be adaptation by the fish to abundant DHA in their natural diet leading to reduced or even "dormant" n-3 biosynthetic capacity (Gregory at el., 2016). As such, a better understanding of FA metabolism has evolved from recent research evaluating FA fate in fish fed alternative dietary oil sources (Turchini and Francis, 2009; Gregory at el., 2016; Teoh and Ng, 2016) and in fish selectively bred for improved n-3 biosynthetic capacity (K. Overturf, unpublished).

It is now clear that a lack of dietary n-3 LCPUFA leads to an increase in both elongase and desaturase activity and transcription rates (Gregory at el., 2016). However, research has demonstrated that this compensation in fish is insufficient to increase n-3 LCPUFA tissue concentrations to levels adequate to result in human health benefits. In a study by Turchini and Francis (2009) where rainbow trout were fed two different dietary treatments of fish oil or linseed oil, fish fed the linseed oil diet showed elevated delta-6 (Δ-6) and delta-5 (Δ-5) desaturases and commensurate increases in EPA and DHA. However, tissue levels of EPA and DHA were still 5- and 3-fold lower than,respectively, in fish fed the fish oil diet. In light of recent findings by Gregory et al. (2016), it is important to note that both dietary treatments contained fishmeal in the Turchini and Francis (2009) study. Gregory et al. (2016) concluded that when optimizing aquaculture feeds containing vegetable oils and/or fish oil or fishmeal, one must consider both the amount of dietary ALA and DHA. Their results suggest that dietary DHA has a large negative effect on downregulating both elongases and desaturases, and when no DHA was present in a diet high in ALA, expression levels of Δ-6 and Δ-5 desaturases and elongase-5 and -2 were highest. Cholesterol appears to also play a significant role in regulating FA metabolism by stimulating fatty acid β-oxidation and the conversion of ALA to DHA (Norambuena et al., 2013). Even so, cholesterol use in optimizing fish feed formulations for maximizing n-3 LCPUFA biosynthesis has been limited and not previously reported for fish fed an all-plant based diet replacing fish oil with soy oil. This is important since cholesterol's activity may be affected by phytosterols in plant oils, and phytosterols are known to inhibit intestinal cholesterol absorption (Ostlund, 2004). The deemphasized role of physosterols to date may have confounded much of the research done on FA metabolism and n-3 LCPUFA biosynthesis in trout because the focus has been on maximizing dietary ALA for conversion to DHA by using linseed oil in the diets. While linseed oil is the richest source of ALA among common plant oils, soy oil has less than half the phytosterol content (300 mg/100 g; Verleyen et al., 2002) of linseed oil (700 mg/ 100 g; Schwartz et al., 2008). The objective of the proposed research is to optimize dietary soy oil utilization by providing dietary sources of ALA and cholesterol to rainbow trout fed a fishmeal/fish oil free diet (low in LCPUFA's) and demonstrate increased conversion of ALA to DHA in the edible fillet.

Further development and utilization of soy-based feeds will likely rely on the formulation of diets composed of all plant-derived feed ingredients, including plant oils. However, substituting fish oil with plant oils lowers the levels of EPA and DHA compared to levels in fish fed diets containing fish oil. Omega-3 fatty acids are essential nutrients for fish and inclusion of 5-6% fish oil is sufficient to meet the dietary requirements of salmonids. However, this level of dietary fish oil is not sufficient to ensure levels of EPA and DHA in fillets meet consumer expectations and dietary intake recommendations. Among alternative lipid sources for fish feeds, plant oils high in ALA are of interest because ALA can serve as a precursor for the

biosynthesis of EPA and DHA. ***We hypothesize that the use of a low-phytosterol oil, such as soy-oil, in combination with a high ALA oil, such as linseed oil, and supplemental cholesterol can be used to improve EPA + DHA biosynthesis and fillet content for human consumption.***

Salmonids possess the capacity to synthesize EPA and DHA from ALA, but the rate of bioconversion is extremely low. However, research in our laboratory has shown that improved bioconversion is heritable and selectively bred trout exhibit differential expression of genes involved in FA metabolism (K. Overturf, unpublished). The objective of the proposed research is to optimize dietary soy oil utilization by providing dietary sources of ALA and cholesterol to rainbow trout fed a fishmeal/fish oil free diet (low in LCPUFA's) and demonstrate increased conversion of ALA to DHA in the edible fillet. If successful, the results will have significant implications for salmonid diet formulation and warrant future investigations with non-salmonid species. ***The long-range impact of this research is to increase the use of U.S. soy oil in an all-plant fish feed while improving the human health benefits associated with consuming DHA and EPA in fillets.***

3. Materials and methods

Dietary Treatments and Formulation-

Diets were formulated to contain 48% protein, 21% lipid and 22.5 MJ/kg energy, and meet or exceed the published minimum nutrient requirements for rainbow trout (NRC, 2011). Experimental diets were cold pelleted at the University of Idaho's Hagerman Fish Culture Experiment Station (HFCES) using a laboratory-scale California pellet mill fitted with a 2.4-mm die. Feeds were dried in a forced-air dryer at 35°C to < 10% moisture, then stored at ambient temperature (20-22°C) until being fed. Samples of the diets were collected for chemical analyses. Dietary cholesterol supplementation was at a level of 1.43 mg/g of diet to mimic the amount of cholesterol in a fish oil-based diet.

Experimental diets: 12 experimental diets were formulated as follows (Table 1):

1. Diet 1 (Control): Fishmeal based control diet
2. Diet 2 (F0100): All-plant protein, soy-based diet with 100% fish oil
3. Diet 3 (SO100): All-plant protein, soy-based diet with 100% soy oil
4. Diet 4 (SO100+C): Diet 3 supplemented with 1.43 mg/g diet cholesterol
5. Diet 5 (SO75/25LO): All-plant protein, soy-based diet with 75% soy oil/25% linseed oil
6. Diet 6 (SO75/25LO+C): Diet 5 supplemented with 1.43 mg/g cholesterol
7. Diet 7 (SO50/50LO): All-plant protein, soy-based diet with 50% soy oil/50% linseed oil
8. Diet 8 (SO50/50LO+C): Diet 7 supplemented with 1.43 mg/g cholesterol
9. Diet 9 (SO25/75LO): All-plant protein, soy-based diet with 25% soy oil/75% linseed oil
10. Diet 10 (SO25/75LO+C): Diet 9 supplemented with 1.43 mg/g cholesterol
11. Diet 11 (LO100): All-plant protein, soy-based diet with 100% linseed oil
12. Diet 12 (LO100+C): Diet 11 supplemented with 1.43 mg/g cholesterol

Fish and Feeding Trial-

The fish feeding trial was conducted at the University of Idaho's Hagerman Fish Culture Experiment Station in Hagerman, Idaho. All fish handling and sampling, plus the experimental protocols used in this project were approved in advance by the University of Idaho's Institutional Animal Care and Use Committee (IACUC).

Rainbow trout (initial body weight: 18.8 ± 0.3g) of the USDA-UI strain crossed with Donaldson strain, selected for improved growth on an all-plant protein feed (Diet 2), were stocked into each of 36, 145-L tanks at 25 fish per tank supplied with spring water. Each tank was supplied with 8-10 L/min of constant temperature (15 °C) spring water fed by gravity to the fish rearing laboratory. Each diet was assigned randomly to three tanks in a completely randomized design. Fish were hand-fed to apparent satiation two times per day, six days per week for 12 weeks. Photoperiod were maintained at 14 h light: 10 h dark with fluorescent lights controlled by electric timers. At the end of 12 weeks, 16-hour postprandial, three fish per tank were anesthetized with tricaine methanesulfonate (MS-222, 100 mg/L, buffered to pH 7.0). Plasma was collected from the caudal vessels of fish with 1-ml heparinized syringes fitted with 25G 3/4-inch needle for cholesterol analysis. Upon euthanizing those fish with MS-222, liver and white muscle were excised for gene expression analysis. Another three fish per tank were euthanized to remove fillet for fatty acid analysis. Tissue samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Chemical analyses-

Experimental feeds and whole-body fish samples were analyzed for proximate composition and energy content. Fish samples were pooled by tank and homogenized using an industrial food processor. Samples were dried in a convection oven at 105°C for 12h to determine moisture level according to AOAC (2000). Dried samples were finely ground by mortar and pestle and analyzed for CP (total nitrogen x 6.25) using combustion method with a nitrogen determinator (TruSpec N, LECO Corporation, St. Joseph, MI). Crude lipid was analyzed by subjecting samples to acid hydrolysis using an ANKOM HCL hydrolysis system (ANKOM Technology, Macedon, NY) and extracting them with petroleum ether using an ANKOM XT15 extractor. Ash was analyzed by incineration at 550°C in a muffle furnace for 5h. The energy content of samples was determined using an isoperibol bomb calorimeter (Parr 6300, Parr Instrument Company Inc., Moline, IL). Total cholesterol in plasma was analyzied with total cholesterol assay kit (Cayman Chemical Inc. Ann Arbor, MI). Cholesterol content in feeds was performed by Eurofins Scientific Inc., Des Moines, IA. according to AOAC Official Method 994.10 (Cholesterol in Foods) (AOAC, 1995). The content and composition of plant stanols and plant sterols were determined according to the NMKL 198 procedure utilizing a GC-FID method. All analyses were conducted by Eurofins Scientific Finland Oy, Raisio, Finland.

Fatty Acid Analysis-

Fatty acid analysis of feeds and muscle was conducted using gas chromatography. Lipids were extracted for fatty acid analysis following a modified Folch method (Folch et al.,1957; Clark et al., 1982). Extracted lipids were derivatized to prepare fatty acid methyl esters (FAME) using tert-butyl methyl ether (TBME) and trimethylsulfonium hydroxide in an Agilent 7696A Sample Prep WorkBench (Agilent Technologies (Shanghai) Co. Ltd, Shanghai, China). FAME was then analyzed using an Agilent 7890B GC System. Fatty acids in samples were identified by comparing the retention times with those of commercial fatty acid analytical standards. Results were expressed on a relative percentage basis, then normalized and reported as % of FAME (fatty acid methyl ester).

Real-time qPCR-

Total RNA was isolated from liver and muscle tissue and converted to cDNA following accepted methods. Extracted RNA was treated with DNAse, then 1 µg of total RNA was

reverse-transcribed using the iScript™ cDNA Synthesis kit (BioRad, Hercules, CA). Real-time quantitative PCR was carried out on a CFX96 Real-Time System (BioRad) in a 10 µL total volume reaction using iTaq SYBR Green Supermix (BioRad) and 500 nmol primers according to the protocol provided by the manufacturer. PCR cycling conditions for all genes were as follows: 95 °C for 5 s followed by 55 °C for 30s over 40 cycles with an initial denaturation step of 95 °C for 3 min. For each fish, PCR reactions were run in duplicate on RNA samples. Extracted RNA was quantified and treated with DNAse, and 1 µg were the reverse- transcribed following the methods of the manufacturer (BioRad, Hercules, CA). Relative expression values for genes constituting the FA transport, oxidation, desaturation, elongation, and incorporation, including *delta-5 fatty acyl desaturase* (*d5fad*), *delta-6 fatty acyl desaturase* (*d6fad*), *fatty acid elongase 2* (*elovl2*), *fatty acid elongase 5* (*elovl5*), *fatty acid bind protein 2* (*fabp2*), and *enoyl-coa hydratase and 3-hydroxyacyl coa dehydrogenase* (*ehhadh*) were determined using primers designed from rainbow trout sequences in the NCBI Genbank® database. In addition, a cellular mRNA control was selected from a set of two reference genes (Arp and elf1α). Primer PCR efficiency was calculated by including six serial dilutions of a standard (pooled from each experimental sample for a given tissue) and utilized for PCR correction for all primer pairs (Pfaffl, 2001). Normalized data were analyzed using the relative quantification method described by Pfaffl (2001).

Calculations-

Using the live-weight and feed consumption data, the following indices were calculated.

Weight gain (WG, g/fish)

= (g mean final weight–g mean initial weight)

Specific growth rate (SGR,%/d)

= [(ln mean final weight–ln mean initial weight)/number of days] x 100

Survival (%)

= (number of fish at the end of the trial/number of fish at the beginning) 100

Average feed intake (FI, g/fish)

= g total dry feed intake/number of surviving fish

Feed conversion ratio (FCR)

= g total feed consumed/ (g final biomass – g initial biomass + g dead fish weight)

Tank means were used for statistical analysis. Fish growth and feed utilization indices, physiological parameters, and gene expression data were tested for normality and homogeneity of variance prior to one or two-way factorial Analysis of Variance (ANOVA). If significant differences were found, data were subjected to Tukey's HSD test to separate the means at a significance level of $P<0.05$. IBM SPSS (Version 21 for Window; IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

4. Results

Diets:

The nutrient composition of the twelve experimental diets used in the growth trial is presented in Table 1. Experimental diets were isonitrogenous, isolipidic and isocaloric, and differed for their total cholesterol (55 – 2040 mg/kg), total phytosterol content (1090 – 2350 mg/kg; Table 3) and fatty acid content (Table 2). FM/FO and PM/FO diets contained EPA (6.59 - 6.79%) and DHA (4.98 - 5.32%) and the rest of the diets did not contain EPA and DHA. Linoleic acid (LA) content decreased as soy oil level dropped to 75, 50, 25 and 0 % (49.5 - 16.4%). On the contrary, alpha-linolenic acid (ALA) content increased as linseed oil increased from 25 to 100% (6.87 - 45.1%) (Table 2).

Growth trial:

Rainbow trout juveniles were fed diets containing different ratios of soybean oil and linseed oil and with or without added cholesterol for twelve weeks. The growth performance and feed utilization of the fish are presented in Table 4. The survival rate (93.3% to 100%) and feed conversion ratio (1.02 to 1.09) were similar among dietary treatments groups ($P > .05$). The weight gain of fish fed diet FM/FO, PM/FO or L100+C was the greatest ($P < .05$) compared with the fish fed other diets. Different ratios of SO/LO did not affect trout weight gain; however, the addition of cholesterol to the L100+C diet significantly increased fish weight gain. The interaction between the two main factors (Cholesterol and SO/LO ratio) significantly affected feed intake (Table 6, $P < .05$). Fish fed L100+C showed increased feed intake, but had no significant effects on growth performance or feed utilization ($P > .05$)

Whole-body proximate composition:

Whole-body proximate composition of rainbow trout juveniles fed the experimental diets are presented in Table 5. Dry matter of fish whole-body varied from 31.8% (SO50/LO50+C) to 33.3% (L100+C). Percent crude protein of fish whole-body ranged from 15.3% (FM/FO) to 16.0% (SO25/LO75, L100) on wet basis. Percent crude fat of fish whole-body ranged from 13.7% (SO50/LO50+C) to 15.2% (LO100+C) on wet basis. Ash content of fish whole-body ranged from 1.90% (SO100+C) to 2.20% (PM/FO) on wet basis. Gross energy of fish whole-body ranged from 27.2 MJ/kg (SO100) to 28.9 MJ/kg (L100+C) on wet basis. There were no differences in whole-body proximate composition among the treatment groups. All of these values are within expected ranges for rainbow trout of this size.

Chemical analysis:

The result of the chemical assessment of plasma is presented in Table 7. Total cholesterol in plasma of fish fed diets ranged from 5.47 mmol/L (SO75/LO25) to 8.73 mmol/L (SO50/LO50+C, L100+C) on wet basis. Plasma cholesterol of fish fed L100+C diet was significantly higher than fish fed SO100 and SO75/LO25 diets. Plasma cholesterol of fish fed diet supplemented with cholesterol showed higher level than those were not supplemented with cholesterol.

Fatty acid analysis:

Fatty acid composition of rainbow trout juvenile fed the experimental diets are presented in Table 8 and 9. LA (C18:2n-6) of fish fillet decreased as soy oil level dropped to 75, 50, 25 and 0% (33.0 - 4.66%),while ALA (C18:3n-3) of fish fillet increased as linseed oil increased from 25 to 100% (0.64 – 24.9%). EPA (C20:5n-3) of fish fillet ranged from 0.80% (SO100, SO100+C) to 5.34% (FM/FO). DHA (22:6n-3) of fish fillet varied from 3.68% (SO100+C) to 13.3% (PM/FO). The interaction of the two main factors (Cholesterol x SO/LO ratio) significantly affected DHA content in fish fillet. The interaction significantly increased DHA content in fish fillet. EPA content in fish fillet was also affected but there were no significant differences (*P*=0.051).

Gene expression:

Relative gene expression in the liver and muscle of rainbow trout juvenile fed experimental diets is presented in Fig.1 and 2. *Elovl2* expression in liver and muscle varied from 1.07 (FM/FO) to 1.60 (SO100 and SO50/LO50) and 0.70 (FM/FO) to 1.27 (SO100), respectively. *Elovl5* expression in liver and muscle ranged from 0.84 (PM/FO) to 1.20 (LO100+C) and 0.55 (FM/FO) to 1.63 (SO100 and SO50/LO50+C), respectively. Expression of *elovl2* and *elovl5* was lowest with the control diets (FM/FO and PM/FO) but did not differ among diets containing vegetable oils. Similar trend was observed in *d5fad* and *d6fad* in both tissues. *D5fad* expression in liver and muscle varied from 1.02 (FM/FO) to 1.44 (SO100+C) and 0.68 (PM/FO) to 1.27 (SO100+C), respectively. D6fad expression in liver and muscle ranged from 1.74 (PM/FO) to 3.24 (SO100+C) and 3.26 (FM/FO) to 4.68 (SO75/LO25+C), respectively. On the contrary, expression of fabp2 was highest in the control treatments (FM/FO and PM/FO). Expression of *ehhadh* was not affected by diets (*P* > .05)

5. Conclusions

The results of this study validate the use of supplemental cholesterol to support growth performance in rainbow trout fed all-plant protein diet. At the same time, phytosterol concentration did not affect the fish growth performance. The main hypothesis of this study was that a plant-based diet would be responsible for increased energy expenditure for *de novo* cholesterol biosynthesis, and consequently would have an impact on fish performance and apparent *in vivo* fatty acid metabolism. However, in the present study, only fish fed LO100+C showed better growth performance out of fish fed other plant-based diets. One would expect that rainbow trout require a sufficient level of ALA (C18:3n-3) to boost their growth performance. In the 12-week feeding trial with rainbow trout juveniles, replacing fish oil with different ratios of SO/LO reduced growth rate except for fish fed L100+C diet. No effects of dietary cholesterol supplementation on fish performances were observed in Atlantic salmon fed high fish meal and fish oil based diets (Bjerkeng and Wathne, 1999) and in channel catfish fed casein based diets (Twibell and Wilson, 2004). However, improved growth performance in response to dietary cholesterol supplementation was observed when channel catfish were fed soybean based diets (Twibell and Wilson, 2004). In hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed diets containing abundant fish meal and fish oil no effect of cholesterol supplementation on growth was recorded (Sealey et al., 2004). These studies were all implemented using diets containing abundant levels of fish meal and fish oil, and thus even the control treatments were providing large amounts of dietary cholesterol.

Additionally, It was shown that the weight gain and feed intake in fish fed fishmeal-based diet without plant protein sources was not affected by supplemental cholesterol, as reported in Atlantic salmon (Bjerkeng et al., 1999) and Japanese flounder (Deng et al., 2010). However, in the present study, fish fed LO100+C diet had significantly higher weight gain and feed intake compared with those fish fed other plant-based diets without cholesterol supplementation. Therefore, cholesterol supplementation may be required when fish are fed plant-based protein with 100% of linseed oil.

The effect of supplemental cholesterol was not observed in diets containing soybean oil. This maybe due to the lack of ALA in the diets that are required for bioconversion into EPA and DHA and this study showed that there was interactions between cholesterol and ALA. Fish, like all vertebrates, cannot synthesize PUFA *de novo* as they lack the necessary Δ12 and Δ15 FAD to convert Oleic acid (C18:1n-9) to LA (C18:2n-6) and ALA (C18:3n-3). However, growth performance of fish fed S100 and L100 diet were not significantly different. Similarly, in juvenile Nile tilapia (*Oreochromis niloticus, L.*) sharpsnout seabream (*Diplodus puntazzo*) there was no significant differences in fish growth between fish fed soybean oil and linseed oil treatments (Sezai et al., 2012; Piedecausa et al., 2007). Differences in feed intake and weight gain were significant between soybean oil diets and control groups. The decrease of fish growth of fish fed soybean oil diets can be explained by the absence of n-3 LCPUFA in the feeding behavior of rainbow trout. Jerome et al. (2020) showed that rainbow trout could discriminate between the diets containing different level of n-3 LCPUFA.

In this study, the hypocholesterolemic effect was observed in plasma of fish fed plant-based diet without cholesterol supplementation compared with that fish fed control treatments (FM/FO and PM/FO) and diets supplemented with cholesterol. Plant protein ingredients, such as soybean meal, soy protein concentrate, corn protein concentrate and wheat gluten meal are generally low cost protein sources and the hypocholesterolemic effect was found in plasma of the wide range of fish species fed these ingredients alone or together compared with fish fed fish meal-based diet, such as in rainbow trout (Yamamoto et al., 2007), turbot (Regost et., 1999), gilthead sea bream (Venou et al., 2006), Atlantic cod (Hansen et al., 2007) and parrot (Lim and Lee, 2009).

Another aspect of the present study was to assess if dietary cholesterol had any effect on fatty acid metabolism, as the key enzymes involved this pathway are known to be affected by several physiological and nutritional factors, including dietary fatty acid composition and cholesterol. In rats, dietary supplementation of cholesterol has shown to reduce the expression of *D5fad* and *D6fad* (Muriana et al., 1992). On the contrary, in the Norambuena (2013) study on rainbow trout, positive effect was shown on the expression of *d6fad* and *elovl5* and resulted in the modification of the whole body fatty acid composition in fish fed high cholesterol diet. In the present study, supplementation of cholesterol did not affect the expression of any genes in both tissue liver and muscle; but there was a trend that gene expression of elongase and desaturase increased with cholesterol supplementation when linseed oil was provided above 50%. Had the study contained a higher level of cholesterol, it is likely that this difference would have increased to a statistically significant level.

In conclusion, results of the present study showed that: 1) a plant-based diet without added cholesterol resulted in reduction in growth and hypocholesterolemic effect in plasma in juvenile rainbow trout; 2) Furthermore, fish fed 100% of linseed oil with cholesterol

supplementation had significantly higher weight gain and feed intake compared with other plant-based diets; 3) Cholesterol supplementation numerically increased EPA and DHA levels in fish fillet when linseed oil was provided above 50 %; 4) Growth performance on an all-plant diet high in soy protein (35%) can be achieved by supplementing with cholesterol and using a plant oil high in α-linolenic acid (ALA, 18:3n-3).

Table 1. Ingredient and nutrient composition of the experimental diets fed to rainbow trout juveniles (%, as-fed basis)

Ingredients (%)	**Diets**											
	FM/FO	PM/FO	SO100	SO100 +C	SO75 LO25	SO75 LO25 +C	SO50 LO50	SO50 LO50 +C	SO25 LO75	SO25 LO75 +C	L100	L100 +C
Fishmeal, sardine	30.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Poultry by-product meal	8.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blood meal, spray dried	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Corn protein concentrate	14.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	0.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soy protein concentrate	5.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Wheat gluten meal	5.97	11.60	11.60	11.60	11.60	11.60	11.60	11.60	11.60	11.60	11.60	11.60
Wheat flour	17.90	6.60	6.60	6.46	6.60	6.46	6.60	6.46	6.60	6.46	6.60	6.46
L-Lysine HCL	0.00	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
DL-methionine	0.00	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Threonine	0.00	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix 702	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Stay-C (vitamin C, 35%)[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Trace mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate	0.00	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43
Fish oil	**15.20**	**19.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Soybean oil	**0.00**	**0.00**	**19.00**	**19.00**	**14.30**	**14.30**	**9.51**	**9.51**	**4.76**	**4.76**	**0.00**	**0.00**
Linseed oil	**0.00**	**0.00**	**0.00**	**0.00**	**4.76**	**4.76**	**9.51**	**9.51**	**14.30**	**14.30**	**19.00**	**19.00**
Cholesterol	**0.00**	**0.00**	**0.00**	**0.14**	**0.00**	**0.14**	**0.00**	**0.14**	**0.00**	**0.14**	**0.00**	**0.14**
Nutrients (% as-fed basis)												
Dry matter (%)	92.2	92.5	92.7	92.9	92.1	92.5	92.7	91.8	92.9	91.7	94.3	92.1
Crude protein (%)	47.5	48.1	48.2	48.8	47.9	48.0	47.9	47.1	47.8	47.4	48.1	47.4
Crude fat (%)	21.5	21.3	21.2	21.2	21.4	21.5	21.3	21.3	21.3	21.3	21.3	21.6
Ash (%)	6.79	6.15	5.99	5.86	5.82	6.10	5.93	5.97	5.87	5.75	6.03	5.82
Gross energy (MJ/kg)	22.5	22.5	22.4	22.5	22.4	22.5	22.8	22.3	22.5	22.5	22.7	22.2
Cholesterol (mg/kg)	2040	1430	55	1290	89	1230	93	1220	106	1270	92	1150

[1]Vitamin premix supply the following to the diet (mg/kg diet): D calcium pantothenate, 46.47; pyridoxine (pyridoxine HCl), 13.68; riboflavin, 9.58; niacinamide, 21.78; folic acid, 2.49; thiamine (thiamine mononitrate), 9.1; inositol, 599; biotin, 0.33; vitamin B_{12}, 0.03; menadione sodium bisulfite complex, 1.1; vitamin E (DL α-tocopherol acetate), 131.9 IU; vitamin D_3 (stabilized), 6594 IU; vitamin A (vitamin A palmitate, stabilized), 9641 IU; ethoxyquin, 198.

[2] Trace mineral premix supply the following to the diet (mg/kg diet): Zn (as $ZnSO_4$ $7H_2O$), 50; Mn (as $MnSO_4$), 7.5; Cu (as $CuSO_4$ $5H_2O$), 2.5; I (as KIO_3), 1; selenium, 0.05.

*FM, Fish meal; FO, Fish oil; PM, Plant meal; SO, Soybean oil; LO, Linseed oil; C, Cholesterol.

Table 2. Fatty acid composition (% of total fatty acids) of the experimental diets

Fatty acids (%)	**Diets**											
	FM/FO	PM/FO	SO100	SO100 +C	SO75 LO25	SO75 LO25 +C	SO50 LO50	SO50 LO50 +C	SO25 LO75	SO25 LO75 +C	L100	L100 +C
C14:0	4.30	4.23	0.22	0.08	0.09	0.08	0.07	0.07	0.06	0.06	0.04	0.06
C16:0	14.2	12.7	10.2	9.94	9.00	9.01	7.79	7.86	6.70	6.57	5.43	5.59
C18:0	3.08	2.62	3.53	3.52	3.50	3.52	3.45	3.49	3.47	3.38	3.17	3.48
C16:1n-7	4.68	4.30	0.28	n.d.	0.09	n.d.	0.09	0.10	n.d.	n.d.	0.08	0.08
C18:1n-7	3.16	3.27	1.20	1.11	1.01	1.01	0.89	0.89	0.79	0.76	0.63	0.68
C18:1n-9	16.1	14.3	18.2	18.1	18.2	18.3	18.2	18.4	18.5	18.2	17.8	18.8
C18:2n-6	6.86	6.51	48.8	49.5	41.4	41.9	33.0	33.3	24.7	24.7	19.6	16.4
C18:3n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:3n-3	1.02	1.12	6.87	7.01	15.2	15.8	25.2	25.2	35.2	35.6	43.3	45.1
C20:5n-3	6.59	6.79	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22:6n-3	4.98	5.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total SFA[1]	22.7	20.6	15.9	15.5	14.5	14.6	13.4	13.3	12.2	11.8	10.3	11.1
Total MUFA[2]	24.0	22.0	19.7	19.2	20.5	20.4	19.2	19.4	19.3	18.9	18.5	19.5
Total n-3 PUFA[3]	12.6	13.2	6.9	7.0	15.2	15.8	25.2	25.2	35.2	35.6	43.3	45.1
Total n-6 PUFA[4]	6.86	6.51	48.8	49.5	41.4	41.9	33.0	33.3	24.7	24.7	19.6	16.4
Total n-3/n-6 PUFA[5]	1.84	2.03	0.14	0.14	0.37	0.38	0.76	0.76	1.43	1.44	2.22	2.75

[1] Sum of saturated fatty acids, includes C10:0, C11:0, C12:0 and C13:0.

[2] Sum of monounsaturated fatty acids, includes C14:1 and C20:1n-9.

[3] Sum of omega-3 polyunsaturated fatty acids

[4] Sum of omega-6 polyunsaturated fatty acids

[5] Ratio of total omega-3 polyunsaturated fatty acids to total omega-6 polyunsaturated fatty acids.

Table 3. Sterol concentration of the experimental diets

Phytosterols (mg/kg)	**Diets**											
	FM/FO	PM/FO	SO100	SO100 +C	SO75 LO25	SO75 LO25 +C	SO50 LO50	SO50 LO50 +C	SO25 LO75	SO25 LO75 +C	L100	L100 +C
Brassicasterol	20	20	0	0	0	10	0	10	10	10	10	10
Campesterol	190	200	320	370	350	370	370	400	380	410	420	420
Campestanol	70	80	80	80	80	80	80	80	80	80	90	90
Stigmasterol	40	60	150	160	150	140	140	130	130	120	120	110
Unidentified sterols	30	30	40	50	40	50	50	50	50	50	50	50
Sitosterol	500	660	960	1000	1030	980	1070	1030	1080	1070	1130	1080
Sitostanol+ delta-5-avenasterol	210	270	290	290	320	190	350	320	370	350	430	380
Delta-5,24-stigmastadienol	10	10	20	20	20	20	30	20	30	30	20	20
Delta-7-stigmastenol	10	20	40	50	40	40	40	40	40	40	40	30
delta-7-Avenasterol	10	30	40	50	40	40	40	40	40	40	40	40
Total plant sterols + plant stanols	1090	1380	1940	2070	2070	1920	2170	2120	2210	2200	2350	2230
Cholesterol	2040	1430	55	1290	89	1230	93	1220	106	1270	92	1150

Table 4. Growth performance and feed utilization of rainbow trout juveniles fed all 12 experimental diets for 12 weeks[1,2]

	Diets											
	FM/FO	PM/FO	SO100	SO100 +C	SO75 LO25	SO75 LO25 +C	SO50 LO50	SO50 LO50 +C	SO25 LO75	SO25 LO75 +C	L100	L100 +C
Initial weight (g)	18.6±0.10	18.8±0.30	18.9±0.29	18.8±0.30	18.8±0.23	18.8±0.23	18.8±0.20	18.9±0.25	18.9±0.20	18.8±0.18	18.8±0.22	18.7±0.13
Final weight (g)	220±0.30^{ab}	222±1.30^{a}	211±2.44^{c}	212±0.69^{c}	209±1.26^{c}	211±1.73^{c}	211±2.70^{c}	210±2.84^{c}	210±1.40^{c}	210±2.59^{c}	213±2.00^{bc}	220±2.45^{ab}
Weight gain (g/fish)	201±0.26^{a}	203±1.26^{a}	192±2.15^{b}	194±0.81^{b}	190±1.21^{b}	193±1.50^{b}	192±2.57^{b}	191±2.60^{b}	191±1.30^{b}	191±2.48^{b}	195±1.86^{b}	201±2.42^{a}
SGR (%/day)	2.97±0.01^{a}	2.97±0.02^{a}	2.91±0.01^{c}	2.92±0.02^{c}	2.90±0.01^{c}	2.91±0.01^{c}	2.91±0.01^{c}	2.90±0.00^{c}	2.90±0.01^{c}	2.91±0.01^{c}	2.93±0.01^{bc}	2.97±0.01^{ab}
Feed intake (g/fish)	190±2.83^{a}	181±1.65^{ab}	174±3.55^{b}	174±3.48^{b}	171±1.92^{b}	172±4.66^{b}	177±0.68^{b}	175±8.22^{b}	172±3.10^{b}	173±2.73^{b}	177±5.05^{b}	192±4.13^{a}
FCR	0.94±0.01	0.89±0.01	0.90±0.01	0.90±0.01	0.90±0.00	0.89±0.02	0.92±0.01	0.91±0.04	0.90±0.02	0.91±0.02	0.91±0.01	0.96±0.02
Survival rate (%)	98.7±1.89	96.0±0.00	97.3±1.89	94.7±4.99	100±0.00	98.7±1.89	97.3±3.77	97.3±1.89	100±0.00	93.3±4.99	97.3±1.89	97.3±1.89

[1]Mean±SE (n=3) in the same row that share the same superscript are not statistically different (*P*> .05; Completely Randomized Design, One-way ANOVA; Tukey's HSD test).

[2]All calculations were performed on an average fish weight basis.

Table 5. Whole-body proximate composition (%, wet basis) of rainbow trout juveniles fed experimental diets for 12 weeks[1]

	Diets											
	FM/FO	PM/FO	SO100	SO100 +C	SO75 LO25	SO75 LO25 +C	SO50 LO50	SO50 LO50 +C	SO25 LO75	SO25 LO75 +C	L100	L100 +C
Proximate composition												
Dry matter (%)	32.1±0.96	33.0±0.75	32.1±0.51	32.8±0.29	32.1±0.22	32.3±0.14	32.1±0.73	31.8±0.99	32.2±0.85	32.8±0.84	32.7±0.45	33.3±0.31
Crude protein (%)	15.3±0.30	15.9±0.28	15.8±0.15	15.9±0.64	15.8±0.34	15.9±0.17	15.6±0.21	15.5±0.45	16.0±0.25	15.6±0.51	16.0±0.59	15.7±0.33
Crude fat (%)	14.1±0.89	14.4±0.70	13.9±0.64	14.3±0.51	13.8±0.21	14.0±0.39	13.9±0.47	13.7±1.37	13.9±0.81	14.6±0.85	14.6±0.78	15.2±0.10
Ash (%)	1.97±0.19	2.20±0.08	2.06±0.04	1.90±0.07	1.91±0.09	1.95±0.05	1.94±0.08	2.00±0.11	1.93±0.08	1.94±0.09	1.91±0.06	1.97±0.08
Gross energy (MJ/kg)	28.7±0.22	28.4±0.19	27.2±2.08	28.4±0.40	28.5±0.20	28.5±0.20	28.4±0.21	28.5±0.61	28.2±0.20	27.9±1.16	28.7±0.26	28.9±0.14

[1]Mean±SE (n=3); three fish from each tank were used for whole-body analysis. Proximate composition was not significantly different (*P*> .05; Completely Randomized Design, One-way ANOVA; Tukey's HSD test).

Table 6. Two-way ANOVA of growth performance and feed utilization of rainbow trout juveniles fed 10 experimental diets (w/o FM/FO, PM/FO) for 12 weeks[1].

Diets	Initial weight (g/fish)	FBW (g/fish)	WG (g/fish)	SGR (%/day)	Survival (%)	FI (g,DM/fish)	FCR
Means of main effects							
Cholesterol (mg/kg)							
0	18.8	211[b]	192[b]	2.91[b]	98.4	201	0.91
1430	18.8	213[a]	194[a]	2.92[a]	96.3	202	0.91
Soy oil / Linseed oil (%)							
100 / 0	18.8	211[b]	193[b]	2.91[b]	96.0	199[b]	0.90
75 /15	18.8	210[b]	191[b]	2.91[b]	99.3	196[b]	0.90
50 /50	18.8	210[b]	192[b]	2.91[b]	97.3	200[b]	0.92
25 / 75	18.6	210[b]	191[b]	2.91[b]	96.7	197[b]	0.90
0 / 100	18.8	217[a]	198[a]	2.95[a]	97.3	213[a]	0.93
Multi factors ANOVA (P Value)							
Cholesterol	0.794	**0.046**	**0.033**	**0.024**	0.111	0.573	0.367
Soy oil / Linseed oil	0.976	**<.001**	**<.001**	**<.001**	0.563	**<.001**	0.064
Cholesterol x Soy oil / Linseed oil	0.976	0.178	0.121	0.090	0.464	**0.042**	0.227

[1] Main effect means followed by a different letter are significantly different at $P < .05$, emphasized by bold *P values* in the ANOVA table.

Table 7. Chemical parameters of plasma of rainbow trout juvenile fed experimental diets for 12 weeks[1,2]

	Diets											
	FM/FO	PM/FO	SO100	SO100 +C	SO75 LO25	SO75 LO25 +C	SO50 LO50	SO50 LO50 +C	SO25 LO75	SO25 LO75 +C	L100	L100 +C
Total Choelsterol												
Plasma (mmol / L)	8.28 ± 0.27^{ab}	7.72 ± 0.29^{abc}	5.79 ± 0.58^{bc}	7.67 ± 0.47^{abc}	5.47 ± 0.11^{c}	7.45 ± 0.37^{abc}	6.06 ± 0.11^{abc}	8.73 ± 1.18^{a}	6.24 ± 0.24^{abc}	7.59 ± 0.61^{abc}	6.11 ± 0.52^{abc}	8.73 ± 2.04^{a}

[1]Mean±SE (n=9 fish per treatment) in the same row that share the same superscript are not statistically different (*P*> .05; Completely Randomized Design, One-factor ANOVA; Tukey's HSD test).

[2]Three fish from each tank were used for chemical analysis.

Table 8. Fillet fatty acid composition (% of total fatty acids) of rainbow trout juvenile fed experimental diets for 12 weeks[1]

Fatty acids (%)	**Diets**											
	FM/FO	PM/FO	SO100	SO100 +C	SO75 LO25	SO75 LO25 +C	SO50 LO50	SO50 LO50 +C	SO25 LO75	SO25 LO75 +C	L100	L100 +C
C14:0	3.27 ± 0.12	2.94 ± 0.31	0.61 ± 0.13	0.48 ± 0.03	0.55 ± 0.07	0.43 ± 0.04	0.48 ± 0.10	0.42 ± 0.05	0.43 ± 0.05	0.34 ± 0.06	0.55 ± 0.21	0.54 ± 0.06
C16:0	15.8 ± 0.31	15.5 ± 0.67	14.2 ± 0.74	13.6 ± 0.26	13.1 ± 0.34	12.3 ± 0.02	11.9 ± 1.04	12.1 ± 0.52	10.7 ± 0.20	10.7 ± 0.24	10.6 ± 0.65	11.0 ± 0.28
C18:0	3.93 ± 0.03	3.23 ± 0.11	4.78 ± 0.13	4.91 ± 0.01	4.29 ± 0.17	4.81 ± 0.24	4.44 ± 0.15	4.46 ± 0.18	4.46 ± 0.09	4.03 ± 0.06	3.27 ± 0.12	4.06 ± 0.10
C16:1n-7	4.37 ± 0.07	3.61 ± 0.22	0.94 ± 0.22	0.74 ± 0.08	0.94 ± 0.01	0.97 ± 0.17	0.67 ± 0.11	0.91 ± 0.17	0.79 ± 0.07	0.70 ± 0.28	0.92 ± 0.12	0.79 ± 0.12
C18:1n-7	2.84 ± 0.06	2.75 ± 0.20	1.20 ± 0.00	0.99 ± 0.06	1.02 ± 0.02	1.00 ± 0.03	0.85 ± 0.04	0.86 ± 0.05	0.83 ± 0.02	0.76 ± 0.03	0.76 ± 0.03	0.72 ± 0.02
C18:1n-9	14.6 ± 0.42	13.6 ± 0.67	15.2 ± 1.30	16.2 ± 0.79	16.5 ± 0.17	17.3 ± 0.48	14.6 ± 1.44	15.5 ± 1.18	16.1 ± 0.39	14.4 ± 2.29	16.2 ± 0.22	14.5 ± 1.00
C18:2n-6	4.66 ± 0.10^{g}	4.65 ± 0.09^{g}	29.3 ± 1.24^{ab}	33.0 ± 2.18^{a}	27.4 ± 0.06^{b}	29.6 ± 0.59^{ab}	21.2 ± 2.74^{c}	21.6 ± 1.59^{c}	17.5 ± 0.46^{cd}	16.4 ± 1.81^{de}	12.0 ± 0.35^{ef}	10.8 ± 0.83^{f}
C18:3n-6	n.d.	n.d.	1.02 ± 0.09	1.01 ± 0.13	0.78 ± 0.03	0.70 ± 0.08	0.50 ± 0.02	0.49 ± 0.00	0.39 ± 0.01	0.33 ± 0.02	0.25 ± 0.03	0.24 ± 0.03
C18:3n-3	0.64 ± 0.04^{f}	0.74 ± 0.02^{f}	3.25 ± 0.17^{e}	3.66 ± 0.16^{e}	8.33 ± 0.18^{d}	8.78 ± 0.50^{d}	13.1 ± 1.62^{c}	13.1 ± 1.13^{c}	19.4 ± 0.78^{b}	18.3 ± 1.52^{b}	24.9 ± 0.52^{a}	22.5 ± 1.06^{a}
C20:5n-3	5.34 ± 0.21^{a}	5.11 ± 0.24^{a}	0.80 ± 0.10^{e}	0.80 ± 0.13^{e}	1.21 ± 0.06^{de}	1.17 ± 0.07^{de}	1.70 ± 0.18^{cd}	1.74 ± 0.16^{cd}	1.90 ± 0.19^{c}	2.14 ± 0.19^{c}	2.23 ± 0.12^{bc}	2.79 ± 0.19^{b}
C22:6n-3	12.8 ± 1.13^{a}	13.3 ± 1.54^{a}	4.35 ± 0.34^{bcd}	3.68 ± 0.36^{d}	5.18 ± 0.31^{bcd}	4.05 ± 0.10^{cd}	5.50 ± 0.59^{bcd}	5.41 ± 0.62^{bcd}	5.69 ± 0.39^{bcd}	6.27 ± 0.75^{bc}	5.40 ± 0.69^{bcd}	6.72 ± 0.51^{b}
Total SFA[2]	26.0 ± 0.24^{a}	25.2 ± 1.04^{ab}	23.3 ± 0.59^{abc}	22.3 ± 0.32^{bcd}	21.1 ± 0.43^{cde}	20.4 ±0.28^{cdef}	20.5 ± 1.50^{cdef}	20.2 ± 1.05^{def}	18.7 ± 0.23^{ef}	19.0 ± 1.55^{ef}	18.1 ± 0.31^{f}	19.8 ± 0.43^{ef}
Total MUFA[3]	21.9 ± 0.55^{a}	20.1 ± 1.07^{ab}	18.2 ± 0.66^{b}	18.0 ± 0.69^{ab}	18.2 ± 0.53^{ab}	19.3 ± 0.68^{ab}	16.2 ± 1.46^{b}	17.3 ± 1.24^{b}	17.7 ± 0.47^{ab}	15.9 ± 2.58^{b}	17.9 ± 0.34^{ab}	16.1 ± 1.10^{b}
Total n-3 PUFA[4]	18.7 ± 1.32^{c}	19.2 ± 1.77^{c}	8.40 ± 0.46^{e}	8.13 ± 0.34^{e}	14.7 ± 0.30^{d}	14.0 ± 0.55^{d}	20.3 ± 1.10^{c}	20.3 ± 0.97^{c}	27.0 ± 0.80^{b}	26.7 ± 0.88^{b}	32.6 ± 0.64^{a}	32.0 ± 0.37^{b}
Total n-6 PUFA[5]	5.09 ± 0.20^{g}	5.91 ± 0.10^{g}	36.1 ± 1.02^{ab}	39.6 ± 2.36^{a}	32.3 ± 0.32^{b}	34.1 ± 0.67^{b}	24.8 ± 2.42^{cd}	25.3 ± 1.57^{c}	20.2 ± 0.46^{de}	17.8 ± 2.59^{de}	12.7 ± 0.58^{f}	11.6 ± 1.29^{f}
Total n-3/n-6 PUFA[6]	3.68 ± 0.18^{a}	3.24 ± 0.24^{ab}	0.23 ± 0.01^{f}	0.21 ± 0.02^{f}	0.46 ± 0.01^{ef}	0.41 ± 0.01^{ef}	0.82 ± 0.04^{e}	0.80 ± 0.05^{e}	1.34 ± 0.03^{d}	1.53 ± 0.20^{d}	2.58 ± 0.08^{c}	2.79 ± 0.30^{bc}

[1]Mean±SE (n=9 fish per treatment) in the same row that share the same superscript are not statistically different (*P*> .05; Completely Randomized Design, One-factor ANOVA; Tukey's HSD test).

[2] Sum of saturated fatty acids, includes C10:0, C11:0, C12:0 and C13:0.

[3] Sum of monounsaturated fatty acids, includes C14:1 and C20:1n-9.

[4] Sum of omega-3 polyunsaturated fatty acids

[5] Sum of omega-6 polyunsaturated fatty acids

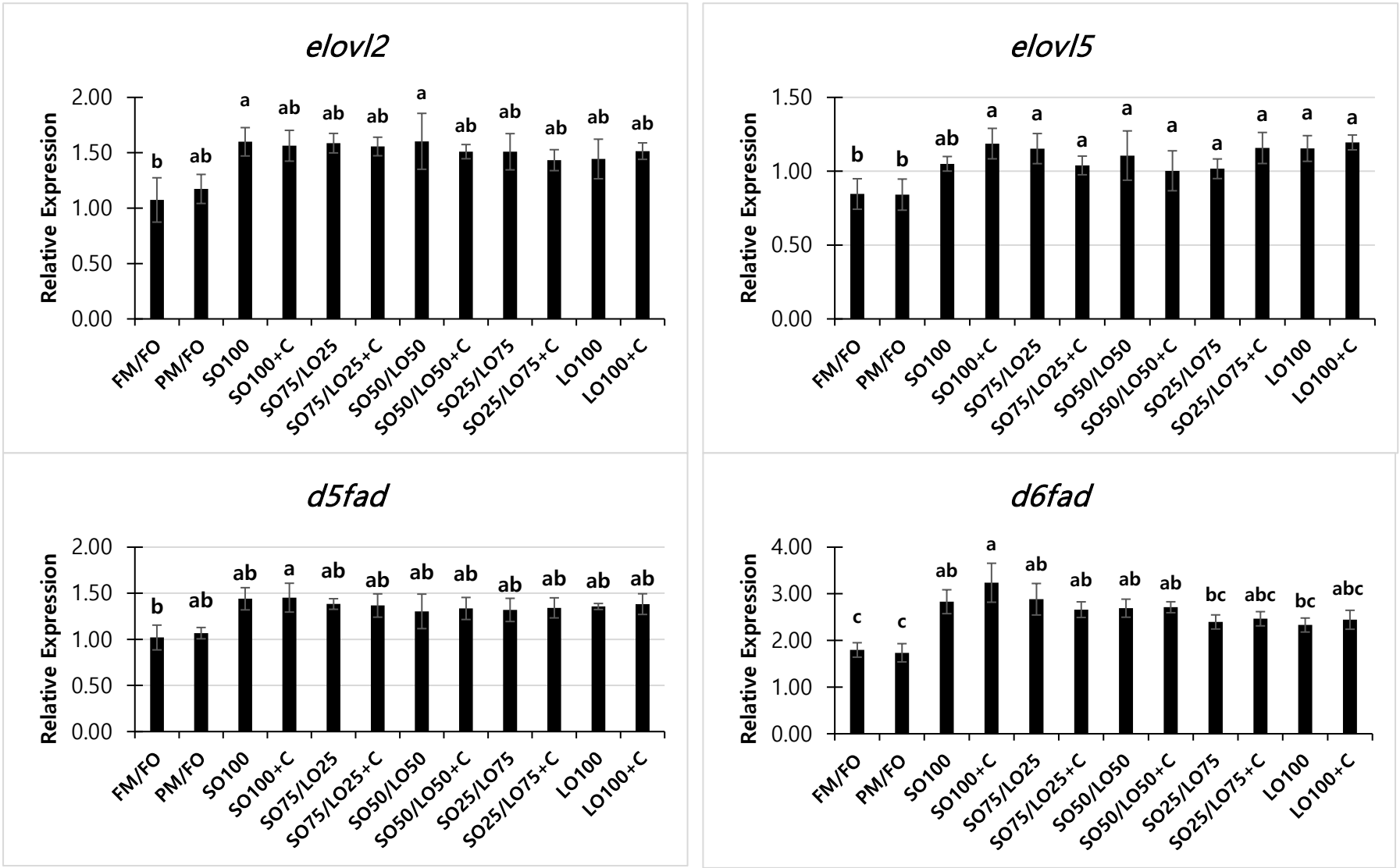
[6] Ratio of total omega-3 polyunsaturated fatty acids to total omega-6 polyunsaturated fatty acids.

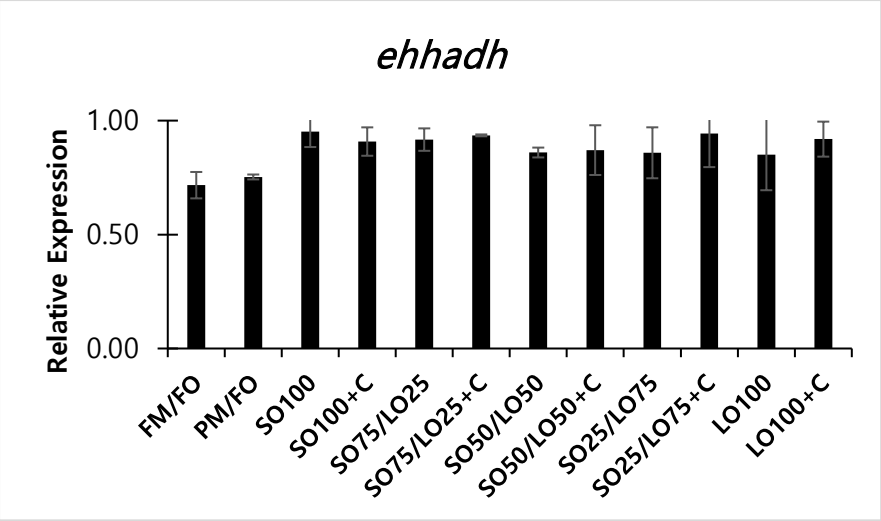
Table 9. Two-way ANOVA of fillet fatty acid composition of rainbow trout juveniles fed 10 experimental diets (w/o FM/FO, PM/FO) for 12 weeks[1].

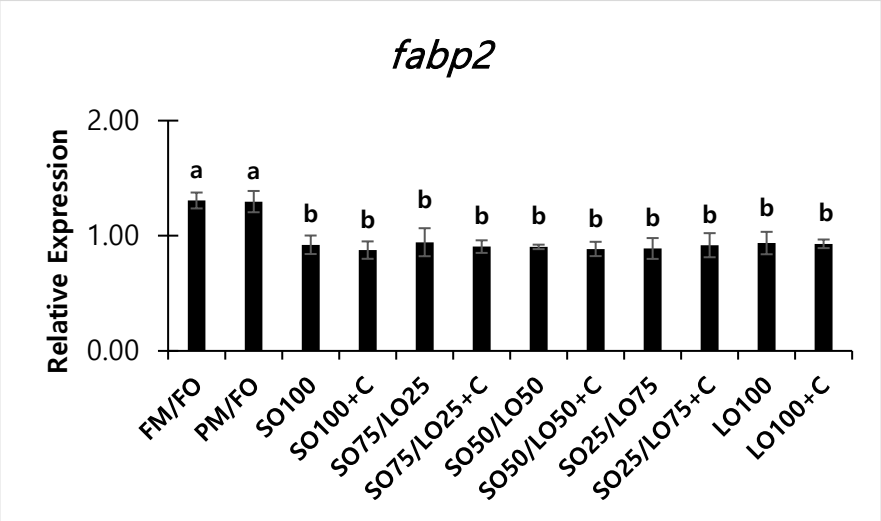
Diets	C18:2n-6	C18:3n-3	C20:5n-3	C22:6n-3	Total n-3 PUFA	Total n-6 PUFA
Means of main effects						
Cholesterol (mg/kg)						
0	21.5	13.8	1.57^{b}	5.22	20.6	25.2
1430	22.3	13.3	1.73^{a}	5.22	20.2	25.7
Soy oil / Linseed oil (%)						
100 / 0	31.1^{a}	3.45^{e}	0.80^{d}	4.01^{c}	8.28^{e}	38.0^{e}
75 /15	28.5^{a}	8.55^{d}	1.19^{c}	4.61^{bc}	14.4^{d}	33.2^{d}
50 /50	21.4^{b}	13.1^{c}	1.72^{b}	5.46^{ab}	20.3^{c}	25.0^{c}
25 / 75	17.0^{c}	18.9^{b}	2.02^{b}	5.98^{a}	26.9^{b}	19.0^{b}
0 / 100	11.4^{d}	23.7^{a}	2.51^{a}	6.06^{a}	32.3^{a}	12.1^{a}
Multi factors ANOVA (P Value)						
Cholesterol	0.245	0.205	**0.025**	1.000	0.229	0.430
Soy oil / Linseed oil	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
Cholesterol x Soy oil / Linseed oil	0.109	0.174	0.051	**0.019**	0.972	0.070

[1] Main effect means followed by a different letter are significantly different at $P < .05$, emphasized by bold *P values* in the ANOVA table.

Figure1. Realtive mRNA expression of genes (normalized against elf1α) involved in elongase (*elovl2* and *elovl5*), desaturase (*d5fad* and *d6fad*), β-oxidation (*ehhadh*) and fatty acid transport (*fabp2*) of liver of rainbow trout juveniles fed experimental diets for 12 weeks [1,2]





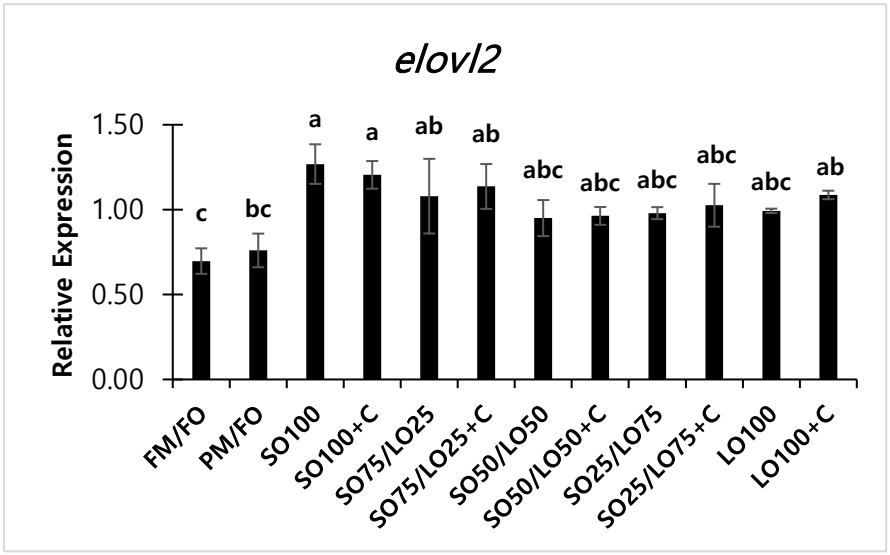


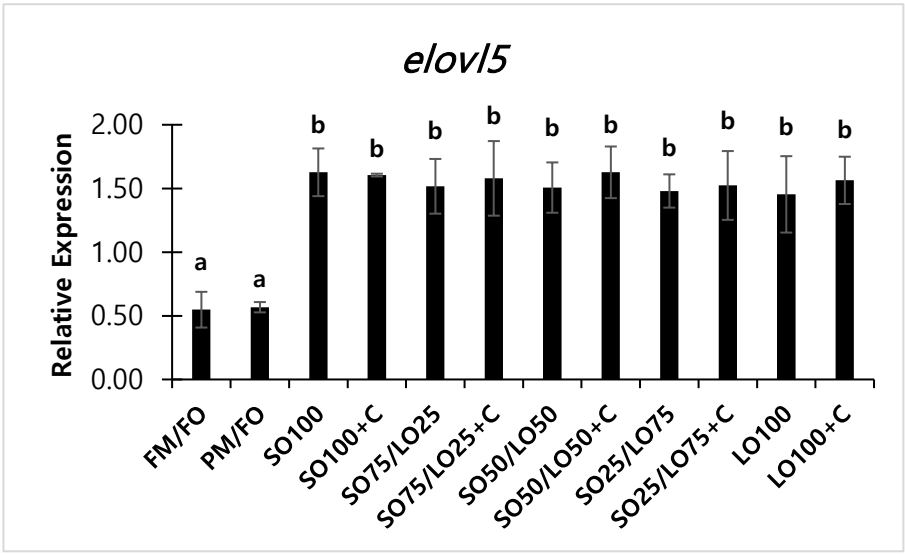
[1]Mean±SE (n=9 fish per treatment) in the same row that share the same superscript are not statistically different (*P*> .05; Completely Randomized Design, One-factor ANOVA; Tukey's HSD test).

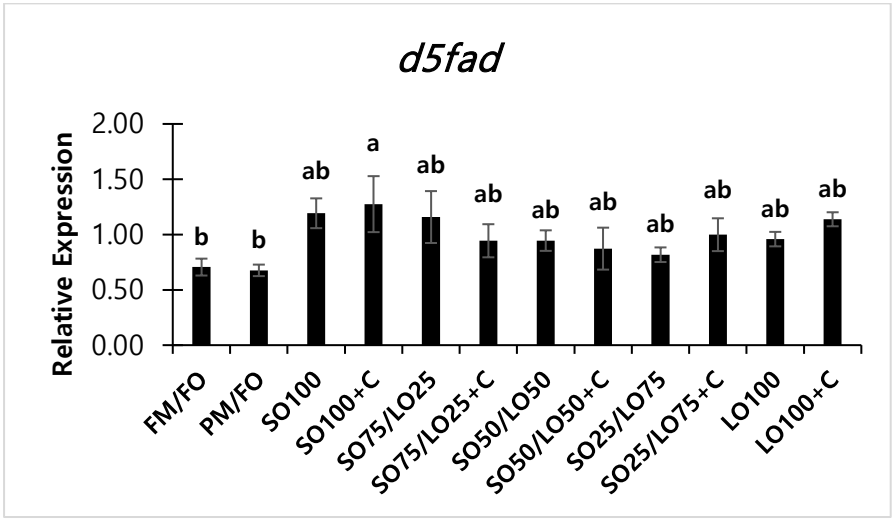
[2]Four fish from each tank were used for gene expression.

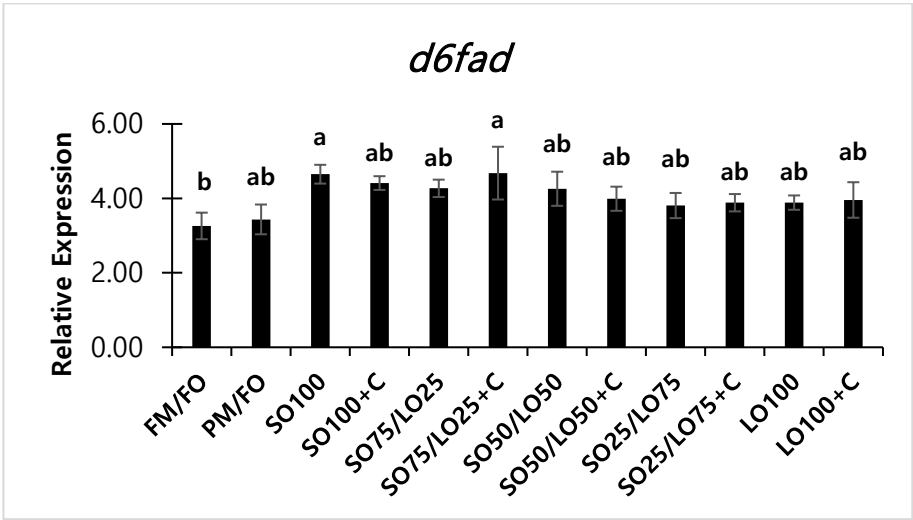
*elovl2: Elongation of very long chain fatty acids-like 2
elovl5: Elongation of very long chain fatty acids-like 5
d5fad: Delta-5 fatty acid desaturase
d6fad: Delta-6 fatty acid desaturase
ehhadh: Enoyl-coa hydratase and 3-hydroxyacyl coa dehydrogenase)
fabp2: Fatty acid binding protein-2

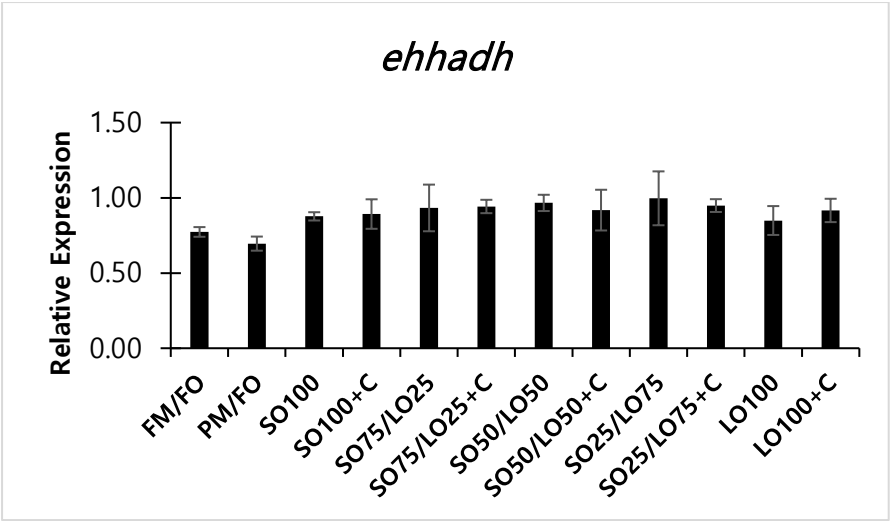
Figure 2. Realtive mRNA expression of genes (normalized against *arp*) involved in elongase (*elovl2* and *elovl5*), desaturase (*d5fad* and *d6fad*), β-oxidation (*ehhadh*) and fatty acid transport (*fabp2*) of muscle of rainbow trout juveniles fed experimental diets for 12 weeks [1,2]

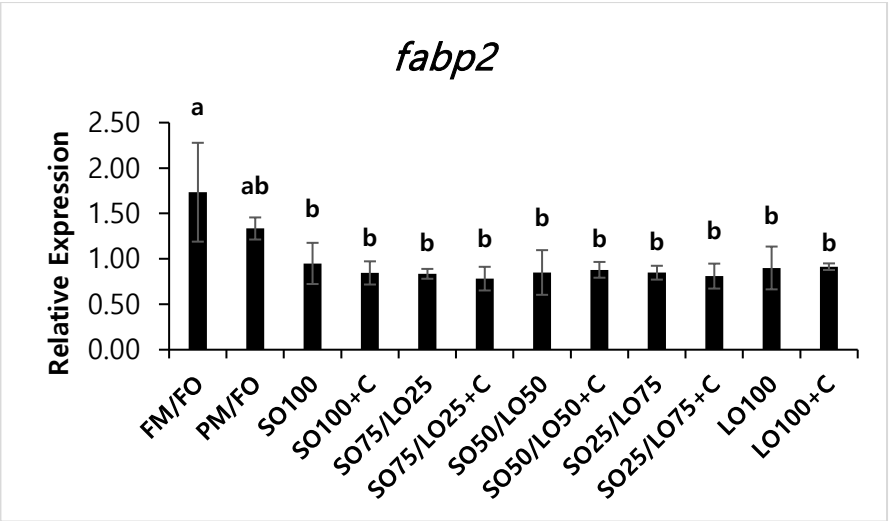









ehhadh
Relative Expression
1.50
1.00
0.50
0.00
FM/FO
PM/FO
SO100
SO100+C
SO75/LO25
SO75/LO25+C
SO50/LO50
SO50/LO50+C
SO25/LO75
SO25/LO75+C
LO100
LO100+C


fabp2
Relative Expression
2.50
2.00
1.50
1.00
0.50
0.00
a
ab
b
b
b
b
b
b
b
b
b
b
FM/FO
PM/FO
SO100
SO100+C
SO75/LO25
SO75/LO25+C
SO50/LO50
SO50/LO50+C
SO25/LO75
SO25/LO75+C
LO100
LO100+C