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Effects of dietary replacement of fish oil with different lipid sources on growth, fatty acid composition, mineral content and expression levels desaturase and elongase genes in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

In this study, the effects of alternative lipid sources on growth, mineral content, proximate composition, fatty acid profile and gene expression of rainbow trout were investigated. Four different diets were prepared with isonitrogenous and isolipid casein-gelatin based formulations, consisting of cod liver oil (CO), goose fat (GF), soybean oil (SO) and mixture of cod liver oil, goose fat and soybean oil (BLEND). Rainbow trout were randomly distributed in three replicate research tanks with 25 fish per tank for each diet group, and the feeding trial lasted for 56 days. Dietary lipid sources did not significantly affect growth performance, feed conversion ratio, survival rate and whole-body mineral composition. The crude lipid and neutral lipid contents were found to be highest in the fish fed GF diet and lowest in the CO group. MUFA content was increased by GF, while it was decreased by SO. Diets generally increased the amount of n-6 PUFA while it decreased the amount of n-3 PUFA and the ratio of n-3/n-6 PUFAs compared to control. The GF diet decreased whole body α-linolenic acid (ALA) and linolenic acid (LA) levels, while SO and BLEND increased these levels. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels were decreased generally in three diets compared to the control group. mRNA expression levels of delta-6-desaturase enzyme were increased in SO and BLEND groups with highest values in SO in both muscle and liver tissues. Elongase expression levels were increased in GF and BLEND groups with highest values in GF in both muscle and liver tissues. It was concluded that synthesis of EPA and DHA from LA and ALA is limited in fish fed all diets. Goose fat can be promising dietary source due to its potential of increasing the n-6 content, decreasing the moisture holding capacity and positive effect on flesh quality.

1. Introduction

Fishmeal production has increased in the recent years due to the remarkable developments in aquaculture (FAO, 2022). The demand for fish oil (FO) has been increasing every year globally (De Silva et al., 2011). However, the increase in the cost of fishmeal and FO, which are the most important feed ingredients, has led feed manufacturers and researchers to seek alternative raw materials in fishmeal production. Various alternative fish diets should be explored, especially with orientation to dietary lipids. For this reason, our aim is to increase the sources of fatty acids in the feed content by keeping the feed consumption at the same level (Hang et al., 2022; Örnek et al., 2021). The

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use of vegetable oil instead of FO in Atlantic salmon diets resulted in a reduction in n-3 highly unsaturated fatty acids (HUFAs) (Bell et al., 2003). It is clear that several vegetable oil sources cause different results in terms of fish nutrition (Menoyo et al., 2005; Regost et al., 2003; Torstensen et al., 2000). Thus, different effects can be seen on fish as the variety of oils used in fish feeding changes.

Fatty acids are substantial ingredients of human health and nutrition. In particular, LC-PUFA, (including arachidonic acid (ARA), EPA and DHA) has been found to play a significant physiological role in nutrition (Lee and Park, 2014). These fatty acids in vertebrates are derived in two ways, which include taking directly by diet, or by metabolism of the precursor fatty acids, LA and ALA, with the help of fatty acid desaturases and elongase (Nakamura et al., 2001; Wall et al., 2010). Although this transformation is limited in humans and even in marine fish, freshwater fish perform this transformation more effectively (Riediger et al., 2009; Sargent et al., 2003). The addition of double bonds to selected positions of fatty acyl chains is catalyzed by fatty acid desaturases (FADs). To be more specific, delta-6 fatty acid desaturase (Δ6 FAD), elongase and Δ5 FAD are involved in the synthesis of EPA and DHA from ALA and LA, respectively. In the synthesis of DHA, it occurs in two stages thanks to Δ6 FAD and peroxisomal chain shortening reactions (Cook and McMaster, 2002; Sprecher, 2000). Δ6 FAD is encoded by the *fads2* gene in mammals and teleost fish (Castro et al., 2012). However, this regulatory mechanism has not been fully elucidated. Changes in gene expression accompanying physiological and biochemical reactions of specialized cellular function are mainly controlled at the transcriptional level. Therefore, observing gene expression profiles can provide important insights for this process. It is known that dietary compositions affect fatty acids in fish. In particular, it was emphasized that fatty acid profiles may vary depending on the expression of desaturases and elongase. This variation may even be based on the fish species (Li et al., 2014; Seiliez et al., 2001; Zuo et al., 2016a,b).

It is acknowledged that the fatty acid and lipid compositions vary among fish tissues. The main parts of fish used in human nourishment are usually muscle tissues. Therefore, analysis of fatty acid profiles of muscle and liver tissues of fish living in their natural ecosystems can provide valuable data (Rodríguez et al., 2004). Elucidating the mechanism underlying enzyme activity and gene expression under different dietary conditions will offer a better understanding of the mechanistic basis of fish nutrition (Ostaszewska et al., 2010). Although alternative lipid sources have been investigated in aquaculture for the last 20 years (Alhazzaa et al., 2019), studies on goose fat and various oil blends are not sufficient. The aim of this study is to evaluate the effects of alternative lipid sources on the growth performances, mineral content, fatty acid profiles and the effects on expression profiles of Δ6-FAD and elongase in rainbow trout. For this purpose, 4 different diet groups containing cod liver oil (CO) as control diet, soybean oil (SO), goose fat (GF) and diet BLEND (CO+SO+GF) were formulated. The effect of diets on the growth parameters, as well as the mineral content and the effects of fatty acid profiles in the whole body were determined. In addition, the alteration of Δ6-FAD and elongase gene expressions in the muscle and liver tissue were investigated. This study will present a different perspective to the literature to evaluate the effects of SO, notably GF and BLEND compared to CO on fatty acid profiles and gene expressions of the mentioned enzymes in teleost fish.

2. Materials and methods

2.1. Experimental diets

This research is a continuation of our previously published feeding trial that examined the influences of different dietary lipids in rainbow trout diets on different rates of activities of antioxidant enzymes. As shown in Table 1, the feeding trial has been described in detail (Mercan et al., 2013)

The isonitrogenous and isocaloric diets used were CO, SO, GF, and a combination of all three. The first diet contained CO, which was rich in HUFAs. The second diet, GF, was not rich in HUFAs. The third diet, SO, was rich in linoleic acid, and a mixture of CO, GF, and SO (BLEND) were used as the fourth diet (Table 1). All of the feed ingredients were properly combined and pelleted to obtain a homogeneous mixture using a meat chopper machine with a 0.7–2 mm diameter. The diets were kept at − 20 °C until used. The pelletized feeds were lyophilized for 24 h.

Table 1
Ingredient and proximate composition of the experimental diets (% dry matter).

	Lipid Sources			
	Cod Liver Oil (CO)	Goose Fat (GF)	Soybean Oil (SO)	BLEND
Ingredients (%)				
Casein (vitamin free)	43.3	43.3	43.3	43.3
Gelatin	7.53	7.53	7.53	7.53
Dextrin (80% water soluble)	6.72	6.72	6.72	6.72
Wheat Meal	16.1	16.1	16.1	16.1
Fish protein concentrate[a]	5.26	5.26	5.26	5.26
Cod liver oil	15.00	0	0	5.00
Soybean oil	0	0	15.00	5.00
Goose fat	0	15.00	0	5.00
Vitamin Mixture[b]	4.08	4.08	4.08	4.08
Mineral Mixture[c]	3.06	3.06	3.06	3.06
Carboxymethylcellulose sodium salt (CMC)	2.04	2.04	2.04	2.04
L-arginine	0.50	0.50	0.50	0.50
L-methionine	0.38	0.38	0.38	0.38
L-lysine	0.60	0.60	0.60	0.60
Choline chloride (99%)	0.97	0.97	0.97	0.97
Proximate composition (%)				
Crude protein	61.6	61.0	60.7	61.5
Crude lipid	15.5	15.1	15.4	15.3
Ash	3.41	3.64	3.39	3.39
Moisture	7.91	8.11	9.38	6.63
Fatty acid composition (%)				
16:0	13.3	23.6	10.4	15.6
18:0	3.00	6.40	4.23	4.55
∑ SFA	25.8	31.3	15.7	23.9
16:1 n-7	5.75	3.92	0.35	3.18
18:1 n-9	21.2	51.2	23.2	13.8
18:1 n-7	3.10	1.72	1.58	2.11
∑ MUFA	35.8	57.7	25.7	39.4
18:3 n-3	2.65	0.88	7.78	3.78
20:5 n-3	9.82	0.33	0.70	3.34
22:5 n-3	2.37	0.07	0.13	0.72
22:6 n-3	10.8	0.45	0.50	3.66
∑ n-3	28.5	1.89	9.25	12.4
18:2n-6	6.56	8.71	48.9	23.2
20:2 n-6	0.60	0.09	0.06	0.22
20:4 n-6	1.00	0.02	-	0.31
∑ n-6	9.38	8.88	49.2	24.1
∑ PUFA	39.0	11.0	58.6	36.9
∑ n-3/∑ n-6	3.04	0.21	0.19	0.52

[a] Concentrate of fish-soluble protein (CPSP 90: crude protein, 82–84% wet weight [WW]; crude lipid, 9–13% WW); Sopropéche S.A., Boulogne-sur-mer, France.

[b] Roche Performance Premix (Hoffman-La Roche, Inc., Nutley, NJ, USA), composition in per gram of the vitamin mixture: vitamin A, 2645.50 IU; vitamin D3, 220.46 IU; vitamin E, 44.09 IU; vitamin B12, 13 mg; riboflavin, 13.23 mg; niacin, 61.73 mg; D-pantothenic acid, 22.05 mg; menadione, 1.32 mg; folic acid, 1.76 mg; pyridoxine, 4.42 mg; thiamin, 7.95 mg; and D-biotin, 0.31 mg.

[c] Bernhart Tomarelli salt mixture (ICN Pharmaceuticals, Costa Mesa, CA, USA), composition in g/100 g: calcium carbonate, 2.1; calcium phosphate dibasic, 73.5; citric acid, 0.227; cupric citrate, 0.046; ferric citrate (16–17 % Fe), 0.558; magnesium oxide, 2.5; manganese citrate, 0.835; potassium iodide, 0.001; potassium phosphate dibasic, 8.1; potassium oxide, 6.8; sodium chloride, 3.06; sodium phosphate, 2.14; and zinc citrate, 0.133. Five milligrams of Se in the form of sodium selenite was added per kilogram of the salt mixture.

2.2. Fish rearing and experimental design

A total of 300 three-month-old juvenile rainbow trout (*Oncorhynchus mykiss*) (initial weight, 1.18 ± 0.12 g) were utilized in the study. The fish were randomly distributed in three replicate research tanks (volume 50 L) with 25 fish per tank for each diet group and the feeding trial lasted for 56 days in a semi-recirculation system. During the trials, the dissolved oxygen, temperature, and pH levels of the water were maintained within the ideal limits for juvenile trout survival and feeding. The fish were fed the experimental diets four times a day (9:30, 12:30, 15:30 and 18:30) by hand until they were satiated. When the experimental treatment was terminated, fish were fasted for 24 h before they were slaughtered. Surgical procedures were started after the fish were exposed to hypothermia (ice slurry, 1:1). For further analysis, the samples were placed in liquid nitrogen and stored at − 80 °C. The treatments on the fish were carried out in accordance with the Applied Research Ethics National Association.

2.3. Proximate analysis

A methodology was used to establish the proximate components of the experimental diets and whole fish samples (AOAC, 1985). Samples were dehydrated in an oven at 105 °C for 6 h to achieve a consistent weight and to estimate the dry matter content. A semi-automatic Kjeldahl (Gerhardt Vapodest, 45 s) analysis was used to determine how much crude protein (N x 6.25) was in the sample. Ash content was obtained by burning the samples in a muffle furnace at 600 °C for 12 h.

2.4. Lipid and fatty acid analysis

Total lipid was extracted from the whole-body of rainbow trout using the method of Folch et al. (1957). Samples of rainbow trout (about 1 g) were homogenized for 1 min in chloroform/methanol (2:1 v/v) containing 0.01 % (w/v) of butylated hydroxytoluene [Sigma, 99.0 % (GC)]. At room temperature (20–22 °C), homogenization was done with ice and other materials. The lipid concentration was calculated gravimetrically after the organic solvent evaporated under a stream of nitrogen. Using Sep Pak silica cartridges (Waters, Milford, MA, USA), whole-body crude lipids were divided into polar lipids (PL) (phospholipids) and neutral lipids (NL). Cartridges' physical and chemical properties are as follows; Surface pH: 7; Activity grade: High; Pore size: 125 Å; Particle size: 80 μm; flow rate: 2 ml/min. The neutral lipids were eluted with 30 ml chloroform (Sigma, ⩾99–99.4 % (GC), product number: 24216) after the residual lipid (about 0.15 g) was applied to a silica filter. Then, the polar fraction was eluted with 30 ml methanol (Sigma, ⩾99.9 %, Product number: 34885). The quantities of polar and neutral lipids were then measured gravimetrically subsequent to the evaporation of solvent from both fractions under nitrogen (Czesny and Dabrowski, 1998). To synthesize fatty acid methyl esters (FAMEs), the technique developed by Metcalfe and Schmitz, 1961 was practiced. The FAMEs were analysed by means of gas chromatography (GC, Hewlett Packard, 6890 N) on a DB-23 capillary column. Detector temperature program was set to 190 °C for 35 min and subsequently to 220 °C, with an increase of 30 °C per minute; and this temperature was maintained for 5 min. A carrier gas flow rate of 2 ml/min of hydrogen was supplied. The individual fatty acids were identified by comparing the retention period of each fatty acid to that of a reference fatty acid combination (Supelco 37-component FAME mix). The quantity of various fatty acids was calculated according to the peak area of the C19:0 internal standard (Sigma, USA) and reported as a percentage of the total FAME.

2.5. Mineral analysis

The whole body of the fish was analysed for microelements (Fe, Mn, Zn, and Cu) and macroelements (P, K, Ca, Mg, S and Na). Samples were taken and mineralized in a mixture of 70% HNO_3 (Sigma-Aldrich) and of 30% H_2O_2 at 85 °C in a microwave oven (Bergof Speedwave Microwave Digestion Equipment MWS-2). The levels of P, K, Ca, Mg, Fe, and Cu in whole-body were measured using an inductively couple plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484–4794, USA).

2.6. RNA isolation and cDNA synthesis

RNA was isolated using TRIzol® Reagent (Life Technologies, Carlsbad, CA, USA) from liver and muscle tissues sampled from the different experimental groups. DNase was used on the RNA to prevent the possibility of genomic contamination. 1 % agarose gel electrophoresis and Nanodrop spectrophotometer (Thermo Scientific) were used to determine the quality and quantity of the isolated RNA. Each 2 μg RNA was reverse-transcripted using SuperScript™ III to synthesize first-strand cDNA and stored at − 20 °C until used for RT-qPCR.

2.7. Primer and probe design

The rainbow trout primers and TaqMan probes were designed using Primer3 software (v.0.4.0) (http://frodo.wi.mit.edu/). Δ6 FAD (GenBank accession no AF301910.1) and elongase (GenBank accession no AY605100.1) sequences BLASTed to correct mRNA sequences. *β*-actin (GenBank accession no AF254414) was used as a housekeeping gene since it was not affected by any of the target genes. The TaqMan probes for the reference gene and the target gene were attached with Cy5/Blackhole Quencher 2 and FAM/TAMRA, respectively (Table 2).

2.8. Real-time quantitative PCR

A thermal cycler was used to quantify gene expression by real-time PCR analysis (RotorQ-Gene, Qiagen, Germany). Template DNA, 900 nM of forward and reverse primers (target and reference), 250 nM TaqMan probes, 25 μl of FastStart TaqMan® Probe Master Mix (Applied Biosystems) were used to perform real-time PCR. This mix contained AmpliTaq Gold DNA Polymerase, AmpErase uracil N-glycosylase (UNG), dNTP with dUTP, and an optimized buffer component in a total volume of 50 μl. The following thermal cycling settings were used for the amplification and detection of the samples and the standards: AmpliTaq Gold DNA polymerase was activated at 95 °C for 10 min as a hot start, then AmpErase UNG optical enzyme was activated at 50 °C for 2 min. Afterwards 45 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 1 min, and extension at 72 °C for 15 s. The efficiency $(e)^{(\Delta Ct)}$ method used to compare mRNA levels in gene expression with the control group and β-Actin, was employed to assess real-time PCR data. The analytical sensitivity was confirmed by slopes of standard curves and the formula e = 10(1/slope) (Pfaffl, 2001), where amplification efficiency (e) was calculated using Rotor-Gene software.

2.9. Calculation of growth performance

Initial body weight (g/fish) (IBW), survival ratio (SR), final body weight (g/fish) (FBW), weight gain (WG), specific growth rates (SGR), and feed conversion ratio (FCR) were calculated according to the following formulas:

SR= (final fish number/initial number of fish) x100

WG= (final weight–initial weight) ×100/initial weight

SGR= 100 × (ln final weight – ln initial weight)/days

FCR = dry feed intake (g)/weight gain

Table 2
Sequence, amplification length, genbank accession number of primers and probes used for real-time PCR.

Gene	Sequence (5′→3′)	Amplification length(bp)	GenBank accession no
Δ6 Desaturase Forward	ACCTAAAGGGTGCCTCTGCT		
Δ6 Desaturase Reverse	TTGTCTCCCAGGACGAAGAC	120	AF301910.1
Δ6 Desaturase Prob	FAM- TCGTCACTTCCAGCACCACGC -TAMRA		
Elongase Forward	TCTTACTATGGGCTCTCTGCTGT		
Elongase Reverse	AGAAAAGGGCAATAAGTGTGATG	195	AY605100.1
Elongase Prob	FAM- TGATTTCCCCAGAGGGTGGCTG -TAMRA		
β-Actin Forward	TGGCCGTACCACCGGTAT		
β-Actin Reverse	GCAGAGCGTAGTCCTCGTAGATG	79	AF254414
β-Actin Prob	Cy5- CTCCGGTGACGGCGTGACCC -BQ2		

2.10. Statistical analysis

The data was represented as mean ± SD (n = 5). The analysis was conducted using SPSS statistics 21.0 software. The growth, and the effect of dietary lipid sources on expression levels of the rainbow trout were analyzed using one-way analysis of variance (ANOVA) to determine the significant differences ($p < 0.05$). Statistical differences among dietary groups were indicated by different lowercase letters ($p < 0.05$).

3. Results

3.1. Growth performance

The growth performance and survival rate of rainbow trout are shown in Table 3. Dietary treatments had no significant effect on FBW (g/fish), WG (% g/100 g) and SGR (%/day) although fish fed with the GF diet showed very slight retardation in growth performance.

3.2. Proximate composition

The GF group showed the highest lipid content of 11.4 ± 1.11%, whereas the CO group had the lowest value of 9.52 ± 0.56 % (Table 4). In line with this observation, neutral lipid levels were significantly higher in the GF and BLEND group than those of other two groups. No difference in phospholipid levels was observed among groups. Crude protein and ash amounts did not statistically differ among groups.

3.3. Fatty acid composition

The fatty acid composition of whole-body lipids of rainbow trout fed different lipid sources (CO, GF, SO, and BLEND) are summarized in Table 5.

The amounts of total SFA in total lipid showed important differences between the groups. The highest SFA level was observed in the CO group, with the ratio of 26.2 ± 0.17 %, whereas the lowest value was observed in the SO group (19.9 ± 1.53 %). In contrast, SFA levels in the neutral lipids were detected to be lowest in the CO group and highest in the GF group (22.0 ± 0.63 % and 25.9 ± 0.26 %, respectively). No difference was found among groups in SFA levels of phospholipids. Palmitic acid (16:0) was the most common SFA found both in neutral lipids and phospholipids. Most of the differences in SFA levels between groups were due to differences in the levels of fatty acids in the diets.

The variations in the percentage of MUFA were significant between groups that were fed different dietary lipids (Table 5). It was highest with respective to total, neutral and phospholipids in the fish fed the GF. 18:1 n-9 (oleic acid-OA) was the predominant MUFA among all experimental groups.

The CO group had the maximum levels of n-3 PUFA in total, neutral, and phospholipid fractions, which was followed by the BLEND, SO and GF groups. EPA and DHA constitute the main components of the n-3 PUFA in the fish fed CO and BLEND diets.

Consequently, it is likely that the n-3 PUFA levels of rainbow trout reflected those levels in the diets. In addition, LA was the main n-6 PUFA in fish fed the SO diet. The highest value of EPA+DHA was determined in CO group in total, neutral and phospholipids obtained from whole body of fish. This results from the abundance of EPA and DHA in CO. The BLEND group had the second highest rate of EPA+DHA, while the lowest values were seen in GF group.

3.4. Mineral content

Ten mineral elements determined in whole-body of fish are shown in Table 6. In general, GF and SO groups contained similar amounts of minerals. Ca levels were found to be highest in GF groups compared to the control group. Also, it was found that SO group had the highest levels of P compared to the others ($p < 0.05$). It is interesting that both GF and SO groups increased the whole-body Ca and P content and decreased the Cu, Fe, K, Mg, S and Zn content compared to CO ($p < 0.05$). The Blend group showed an intermediate pattern, suggesting that the changes in mineral concentration are primarily attributed to the difference in dietary lipids.

3.5. Expression of genes related to lipid metabolism

The expression of the Δ6 FAD and elongase in rainbow trout muscle and liver tissue after 8 weeks of feeding on the various lipid sources is

Table 3
Growth parameters and survival in rainbow trout fed the experimental diets for 8 weeks.

Diets	IBW[1]	FBW[2]	WG[3]	SGR[4]	FCR[5]	SR[6]
CO	1.18 ± 0.04	6.51 ± 0.13	453.93 ± 8.14	3.11 ± 0.03	1.04 ± 0.01	96.00 ± 4,00
GF	1.18 ± 0.02	6.36 ± 0.24	439.42 ± 20.16	3.06 ± 0.07	1.02 ± 0.04	94.66 ± 6.14
SO	1.18 ± 0.01	6.49 ± 0.24	450.23 ± 37.57	3.09 ± 0.12	1.16 ± 0.20	93.33 ± 6.11
BL	1.18 ± 0.03	6.51 ± 0.21	463.67 ± 23.51	3.14 ± 0.07	1.05 ± 0.04	94.66 ± 6,13

Data are presented as means ± SD (n = 5); 1 IBW, Initial body weight; 2 FBW, Final body weight; 3WG, Weight gain; 4SGR, Specific growth; 5FCR, Food conversion ratio; 6SR, Survival rate

Table 4
Whole body proximate composition in rainbow trout fed the experimental diets for 8 weeks (%)[a].

Diets	Crude lipid	PL	NL	Crude protein	Ash	Moisture
CO	9.52 ± 0.56[b]	5.30 ± 0.20	4.19 ± 0.39[b]	20.6 ± 0.76	1.76 ± 0.07	66.5 ± 0.23[a]
GF	11.4 ± 1.11[a]	5.35 ± 0.33	6.26 ± 0.96[a]	21.2 ± 0.58	1.80 ± 0.17	64.5 ± 1.41[b]
SO	9.61 ± 0.38[b]	5.50 ± 0.51	4.11 ± 0.87[b]	20.8 ± 1.09	1.84 ± 0.10	65.9 ± 0.43[ab]
BLEND	10.8 ± 0.42[ab]	5.29 ± 0.31	5.48 ± 0.11[a]	20.6 ± 0.56	1.73 ± 0.17	65.2 ± 0.36[ab]

[a] Means with different superscript letters in a column are significantly different ($p < 0.05$).

Table 5
Fatty acid content of whole body total lipids in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different lipid sources[a].

	TL				NL				PL			
Fatty Acid	CO	GF	SO	BLEND	CO	GF	SO	BLEND	CO	GF	SO	BLEND
14:0	3.58 ± 0.16^{a}	1.21 ± 1.05^{b}	0.47 ± 0.60^{b}	1.56 ± 0.50^{b}	1.56 ± 0.34^{b}	1.96 ± 0.15^{ab}	2.25 ± 1.90^{a}	1.74 ± 0.08^{b}	1,99 ± 0.04^{a}	1.58 ± 0.6^{ab}	1.29 ± 0.28^{b}	1.59 ± 0.39^{ab}
15:0	0.29 ± 0.01^{a}	0.12 ± 0.01^{b}	0.07 ± 0.05^{b}	0.12 ± 0.05^{b}	0.18 ± 0.04^{a}	0.12 ± 0.03^{bc}	0.16 ± 0.02^{ab}	0.10 ± 0.03^{c}	0.75 ± 0.07	0.72 ± 0.08	0.67 ± 0.09	0.73 ± 0.05
16:0	15.4 ± 0.03b^{c}	17.4 ± 0.23^{a}	14.0 ± 1.51^{c}	15.8 ± 0.22^{b}	14.9 ± 0.33^{b}	16.4 ± 0.34^{a}	15.3 ± 0.59^{b}	15.7 ± 0.56^{ab}	15.9 ± 0.08	15.1 ± 0.10	14.9 ± 0.76	15.1 ± 0.51
17:0	0.27 ± 0.01^{a}	0.12 ± 0.02^{b}	0.10 ± 0.04^{b}	0.11 ± 0.04^{b}	0.07 ± 0.04^{b}	0.11 ± 0.01^{ab}	0.14 ± 0.02^{a}	0.08 ± 0.01^{b}	0.77 ± 0.07	0.73 ± 0.08	0.69 ± 0.06	0.74 ± 0.07
18:0	4.01 ± 0.27^{b}	4.63 ± 0.24^{a}	4.58 ± 0.41^{a}	4.98 ± 0.03^{a}	4.15 ± 0.07^{c}	6.65 ± 0.16^{a}	4.86 ± 0.28^{b}	5.13 ± 0.01^{b}	4.99 ± 0.14^{b}	4.85 ± 0.02^{b}	5.72 ± 0.38^{a}	5.33 ± 0.27^{ab}
20:0	2.21 ± 0.08^{a}	0.37 ± 0.06^{b}	0.51 ± 0.21^{b}	0.59 ± 0.41^{b}	0.48 ± 0.07^{a}	0.17 ± 0.06^{b}	0.53 ± 0.08^{a}	0.27 ± 0.03^{b}	0.65 ± 0.05^{b}	0.77 ± 0.12^{ab}	0.85 ± 0.25^{ab}	1.01 ± 0.04^{a}
22:0	0.37 ± 0.04^{a}	0.08 ± 0.01^{b}	0.10 ± 0.06^{b}	0.10 ± 0.06^{b}	0.68 ± 0.04^{bc}	0.54 ± 0.13^{c}	1.07 ± 0.04^{a}	0.81 ± 0.07^{b}	0.89 ± 0.04	0.78 ± 0.03	0.88 ± 0.032	1.04 ± 0.13
∑ SFA	**26.2 ± 0.17^{a}**	**23.9 ± 1.43^{ab}**	**19.9 ± 1.53^{c}**	**23.3 ± 1.31^{b}**	**22.0 ± 0.63^{c}**	**25.9 ± 0.26^{a}**	**24.3 ± 0.04^{b}**	**23.8 ± 0.43^{b}**	**25.9 ± 0.40**	**24.6 ± 0.31**	**25.1 ± 1.47**	**25.5 ± 0.61**
14:1	0.20 ± 0.02	0.09 ± 0.01	0.17 ± 0.23	0.07 ± 0.03	0.26 ± 0.16^{a}	0.09 ± 0.01^{b}	0.11 ± 0.01^{b}	0.10 ± 0.01^{b}	0.62 ± 0.05	0.68 ± .11	0.58 ± 0.08	0.66 ± 0.07
15:1	0.32 ± 0.11	0.37 ± 0.39	0.28 ± 0.25	0.35 ± 0.24	0.06 ± 0.03^{a}	0.02 ± 0.01^{b}	0.05 ± 0.02^{a}	0.03 ± 0.01^{b}	1.03 ± 0.09	1.118 ± 0.23	1.04 ± 0.13	1.10 ± 0.13
16:1 *n-7*	6.09 ± 0.19^{a}	6.12 ± 0.37^{a}	1.71 ± 0.38^{c}	4.11 ± 0.05^{b}	0.12 ± 0.03^{a}	0.02 ± 0.01^{b}	0.06 ± 0.04^{b}	0.13 ± 0.02^{a}	3.15 ± 0.32^{a}	3.48 ± 0.08^{a}	1.80 ± 0.59^{b}	2.21 ± 0.68^{b}
17:1	0.19 ± 0.09	0.19 ± 0.07	0.43 ± 0.42	0.14 ± 0.03	6.08 ± 0.54^{b}	5.80 ± 0.05^{b}	4.99 ± 0.11^{a}	6.00 ± 0.11^{b}	0.85 ± 0.09	0.89 ± 0.14	0.78 ± 0.09	0.85 ± 0.13
18:1 *n-7*	3.19 ± 0.14^{a}	2.77 ± 0.12^{a}	2.08 ± 0.41^{b}	2.14 ± 0.38^{a}	3.18 ± 0.04^{a}	2.74 ± 0.05^{b}	2.25 ± 0.36^{b}	2.52 ± 0.06^{bc}	2.68 ± 0.07^{a}	2.65 ± 0.05^{a}	2.00 ± 0.20^{b}	2.11 ± 0.27^{b}
18:1 *n-9*	24.0 ± 0.86^{c}	45.3 ± 0.64^{a}	22.5 ± 0.56^{d}	31.5 ± 0.21^{b}	31.7 ± 0.39^{d}	46.9 ± 1.85^{a}	34.3 ± 1.85^{c}	39.5 ± 0.14^{b}	14.9 ± 3.43^{b}	21.4 ± 1.54^{a}	12.8 ± 2.09^{b}	13.2 ± 2.47^{b}
20:1 *n-11*	0.74 ± 0.36	0.77 ± 0.98	0.62 ± 0.91	0.14 ± 0.08	0.20 ± 0.02	0.25 ± 0.03	0.28 ± 0.04	0.24 ± 0.07	0.63 ± 0.01	0.69 ± 0.06	0.58 ± 0.09	0.69 ± 0.05
20:1 *n-9*	1.68 ± 1.23	1.40 ± 1.03	0.68 ± 0.46	1.44 ± 0.63	2.40 ± 0.29^{a}	2.38 ± 0.25^{a}	1.93 ± 0.05^{b}	1.50 ± 0.06^{c}	1.64 ± 0.15^{ab}	1.81 ± 0.68^{a}	1.17 ± 0.19^{b}	1.54 ± 0.38^{ab}
22:1 *n-11*	0.12 ± 0.03^{b}	0.03 ± 0.05^{c}	0.22 ± 0.03^{a}	0.16 ± 0.03^{ab}	0.17 ± 0.01^{a}	0.10 ± 0.07^{b}	0.04 ± 0.01^{b}	0.02 ± 0.01^{b}	0.57 ± 0.06^{b}	0.67 ± 0.08^{ab}	0.86 ± 0.12^{a}	0.67 ± 0.15^{ab}
22:1 *n-9*	0.30 ± 0.01	0.55 ± 0.11	0.66 ± 0.34	0.74 ± 0.49	0.05 ± 0.05^{c}	0.15 ± 0.01^{b}	0.17 ± 0.01^{a}	0.04 ± 0.01^{c}	1.06 ± 0.18	1.96 ± 0.27	2.19 ± 0.74	2.16 ± 1.10
24:1 *n-9*	0.61 ± 0.03a	0.38 ± 0.06^{b}	0.27 ± 0.07^{b}	0.30 ± 0.07^{b}	0.48 ± 0.07^{a}	0.32 ± 0.01^{b}	0.10 ± 0.08^{c}	0.27 ± 0.05^{b}	1.02 ± 0.07	0.96 ± 0.11	0.85 ± 0.17	0.86 ± 0.07
∑ MUFA	**37.5 ± 0.07^{c}**	**57.9 ± 0.79^{a}**	**29.6 ± 1.32^{d}**	**41.1 ± 0.62^{b}**	**44.7 ± 1.91^{c}**	**58.8 ± 0.91^{a}**	**44.2 ± 2.19^{c}**	**50.3 ± 1.00^{b}**	**28.2 ± 3.42^{b}**	**36.4 ± 0.91^{a}**	**24.8 ± 1.91^{b}**	**26.0 ± 2.09^{b}**
18:3 *n-3*	1.77 ± 0.12^{b}	0.45 ± 0.01^{c}	3.17 ± 0.75^{a}	2.47 ± 0.38^{ab}	1.62 ± 0.05^{b}	0.50 ± 0.07^{c}	2.36 ± 0.25^{a}	1.80 ± 0.04^{b}	1.15 ± 0.07^{b}	1.09 ± 0.11^{b}	1.84 ± 0.35^{a}	1.73 ± 0.12^{a}
18:4 n-3	1.22 ± 0.09^{a}	0.34 ± 0.03^{c}	0.75 ± 0.17^{b}	0.72 ± 0.18^{b}	0.22 ± 0.07	0.24 ± 0.12	0.17 ± 0.05	0.25 ± 0.08	0.91 ± 0.02	0.74 ± 0.02	0.86 ± 0.21	0.73 ± 0.06
20:3 *n-3*	0.26 ± 0.02	0.23 ± 0.20	0.16 ± 0.01	0.15 ± 0.04	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.73 ± 0.08	0.71 ± 0.08	0.73 ± 0.09	0.74 ± 0.85
20:4 *n-3*	1.03 ± 0.08^{a}	0.17 ± 0.04^{c}	0.36 ± 0.07^{b}	0.35 ± 0.11^{b}	0.24 ± 0.01^{ab}	0.19 ± 0.05^{bc}	0.29 ± 0.03^{a}	0.14 ± 0.01^{c}	1.07 ± 0.07^{a}	0.84 ± 0.07^{b}	0.89 ± 0.10^{ab}	0.87 ± 0.14^{b}
20:5 *n-3*	5.21 ± 0.17^{a}	1.62 ± 0.14^{b}	1.82 ± 0.11^{b}	1.76 ± 0.44^{b}	5.09 ± 0.21^{a}	1.36 ± 0.03^{c}	1.58 ± 0.23^{c}	2.17 ± 0.04^{b}	5.37 ± 0.35^{a}	2.99 ± 0.15^{b}	3.15 ± 0.46^{b}	3.44 ± 0.69^{b}
22:5 *n-3*	2.16 ± 0.11^{a}	0.59 ± 0.12^{b}	0.75 ± 0.03^{b}	0.74 ± 0.20^{b}	0.83 ± 0.03^{a}	0.36 ± 0.30^{b}	0.64 ± 0.13^{ab}	0.52 ± 0.03^{ab}	2,24 ± 0.08^{a}	1.48 ± 0.10^{b}	1.61 ± 0.18^{b}	1.72 ± 0.14^{b}
22:6 *n-3*	15.4 ± 0.23^{a}	4.76 ± 0.81^{d}	6.49 ± 0.93^{c}	8.26 ± 0.16^{d}	12.5 ± 0.35^{a}	5.03 ± 0.49^{c}	5.60 ± 0.39^{c}	8.20 ± 0.24^{b}	23.0 ± 2.48^{a}	13.6 ± 0.91^{b}	16.8 ± 3.62^{b}	18.2 ± 1.43^{b}
∑ *n-3*	**27.0 ± 0.14^{a}**	**8.17 ± 1.32^{c}**	**13.5 ± 0.76^{b}**	**14.4 ± 0.07^{b}**	**20.5 ± 0.51^{a}**	**7.70 ± 1.33^{d}**	**10.7 ± 0.29^{c}**	**13.4 ± 0.29^{b}**	**34.5 ± 3.17^{a}**	**21.4 ± 1.10^{c}**	**25.9 ± 3.87^{ab}**	**27.4 ± 2.16^{b}**
18:2 *n-6*	6.60 ± 0.22^{c}	6.54 ± 0.22^{c}	31.3 ± 1.49^{a}	15.9 ± 0.03^{b}	8.59 ± 0.26^{c}	6.24 ± 0.11^{d}	18.1 ± 1.98^{a}	10.71 ± 1.12^{b}	3.08 ± 0.52^{c}	5.68 ± 1.33^{bc}	11.73 ± 4.77^{a}	9.14 ± 1.73^{ab}
18:3 *n-6*	0.19 ± 0.02	0.46 ± 0.07	0.94 ± 0.44	0.95 ± 0.63	0.68 ± 0.04^{a}	0.35 ± 0.07^{b}	0.43 ± 0.02^{b}	0.19 ± 0.03^{c}	0.69 ± 0.01^{b}	0.95 ± 0.11^{ab}	1.25 ± 0.36^{a}	0.99 ± 0.27^{ab}
20:2 *n-6*	0.71 ± 0.05^{b}	0.89 ± 0.09^{b}	1.44 ± 0.31^{a}	1.35 ± 0.16^{a}	0.64 ± 0.03^{b}	0.69 ± 0.04^{b}	0.81 ± 0.10^{a}	0.52 ± 0.03^{c}	1.02 ± 0.02^{c}	2.10 ± 0.09^{a}	1.61 ± 0.06^{b}	1.67 ± 0.27^{b}
20:3 *n-6*	0.37 ± 0.01^{b}	0.74 ± 0.19^{ab}	1.39 ± 0.61^{a}	1.37 ± 0.52^{a}	0.38 ± 0.16^{a}	0.39 ± 0.03^{a}	0.48 ± 0.03^{a}	0.16 ± 0.01^{b}	1.03 ± 0.02^{b}	2.33 ± 0.14^{a}	2.43 ± 0.78^{a}	2.29 ± 0.70^{a}
20:4 *n-6*	0.65 ± 0.01	1.01 ± 0.06	1.31 ± 0.44	1.34 ± 0.66	1.51 ± 0.01^{a}	0.39 ± 0.06^{d}	0.50 ± 0.05^{c}	0.78 ± 0.06^{b}	1.92 ± 0.10^{b}	3.16 ± 0.32^{ab}	3.80 ± 0.80^{a}	3.24 ± 1.35^{ab}
22:2 *n-6*	0.42 ± 0.02^{a}	0.21 ± 0.05^{b}	0.16 ± 0.05^{bc}	0.09 ± 0.09^{c}	0.27 ± 0.05^{a}	0.12 ± 0.04^{b}	0.15 ± 0.03^{b}	0.12 ± 0.01^{b}	0.83 ± 0.07	0.77 ± 0.06	0.67 ± 0.15	0.82 ± 0.07
22:4 *n-6*	0.17 ± 0.08	0.22 ± 0.09	0.45 ± 0.50	0.09 ± 0.09	0.19 ± 0.02	0.16 ± 0.16	0.11 ± 0.01	0.08 ± 0.01	0.80 ± 0.10	0.81 ± 0.12	1.05 ± 0.35	0.94 ± 0.35
∑ *n-6*	**9.11 ± 0.12^{c}**	**10.1 ± 0.44^{c}**	**37.0 ± 2.39^{a}**	**21.2 ± 1.93^{b}**	**12.3 ± 0.22^{b}**	**8.33 ± 0.16^{c}**	**20.5 ± 1.82^{a}**	**12.5 ± 0.20^{b}**	**9.39 ± 0.25^{b}**	**15.8 ± 0.55^{ab}**	**22.5 ± 5.85^{a}**	**19.3 ± 4.73^{a}**
∑ HUFA												

(*continued on next page*)

Table 5 (*continued*)

	TL				NL				PL			
Fatty Acid	CO	GF	SO	BLEND	CO	GF	SO	BLEND	CO	GF	SO	BLEND
	26.4 ± 0.05[a]	10.5 ± 1.37[c]	14.3 ± 0.44[b]	15.5 ± 0.70[b]	21.7 ± 0.61[a]	8.74 ± 0.94[d]	10.2 ± 0.15[c]	12.7 ± 0.35[b]	28.1 ± 3.36[a]	28.8 ± 1.61[b]	32.7 ± 4.20[ab]	34.1 ± 0.42[ab]
EPA+DHA	20.6 ± 0.06[a]	6.38 ± 0.95[d]	8.31 ± 1.03[c]	10.0 ± 0.28[b]	17.6 ± 0.55[a]	6.39 ± 0.79[c]	7.18 ± 0.16[c]	10.4 ± 0.20[b]	28.4 ± 2.84[a]	16.6 ± 1.05[b]	19.9 ± 4.06[b]	21.6 ± 2.13[b]
∑ *n*-3/ *n*-6	2.97 ± 0.05[a]	0.81 ± 0.15[b]	0.37 ± 0.03[c]	0.69 ± 0.07[b]	1.68 ± 0.07[a]	0.93 ± 0.17[d]	0.52 ± 0.03[b]	1.04 ± 0.04[c]	3.68 ± 0.43[a]	1.35 ± 0.11[b]	1.24 ± 0.55[b]	1.50 ± 0.49[b]

[a] Means with different superscript letters in a row are significantly different ($p < 0.05$).

Table 6
Mineral concentration in whole body of rainbow trout (%)[a].

Diets	CO	GF	SO	BLEND
Ca	27.2 ± 1.86[d]	38.8 ± 1.69[a]	35.4 ± 0.12[b]	31.5 ± 0.34[c]
Cu	0.12 ± 0.02[a]	0.06 ± 0.01[b]	0.05 ± 0.00[b]	0.05 ± 0.01[b]
Fe	0.20 ± 0.00[a]	0.14 ± 0.03[b]	0.17 ± 0.02[a]	0.12 ± 0.02[b]
K	22.1 ± 0.27[a]	15.8 ± 2.12[b]	15.4 ± 0.05[b]	17.2 ± 0.32[b]
Mg	4.17 ± 0.12[a]	3.48 ± 0.23[b]	3.33 ± 0.26[b]	3.23 ± 0.49[b]
Mn	0.03 ± 0.01[a]	0.02 ± 0.00[a]	0.01 ± 0.01[a]	0.01 ± 0.00[b]
Na	6.31 ± 0.36[a]	6.40 ± 0.12[a]	6.42 ± 0.15[a]	5.75 ± 0.35[b]
P	30.1 ± 0.09[b]	31.8 ± 0.34[b]	36.4 ± 0.61[a]	36.1 ± 2.84[a]
S	9.07 ± 1.49[a]	3.08 ± 0.06[bc]	2.44 ± 0.29[c]	5.72 ± 2.71[b]
Zn	0.63 ± 0.05[a]	0.36 ± 0.06[b]	0.39 ± 0.03[b]	0.31 ± 0.03[b]

[a] Means with different superscript letters in a row are significantly different ($p < 0.05$)

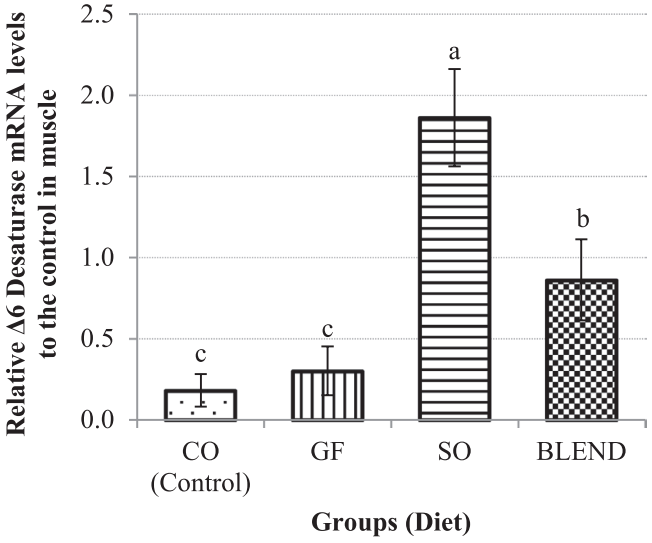


Fig. 1. Relative mRNA expression levels of Δ6 Desaturase gene in muscle of rainbow trout fed diets containing different lipid sources for 8 weeks. Values are means±SE, n = 3 per group; different lowercase letters above the bars show that there are significant differences among different treatments (P < 0.05).

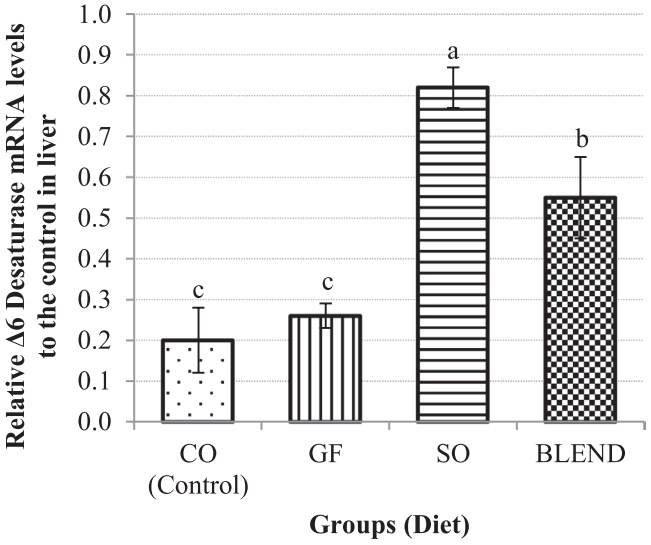


Fig. 2. Relative mRNA expression levels of Δ6 Desaturase gene in liver of rainbow trout fed diets containing different lipid sources for 8 weeks. Values are means±SE, n = 3 per group; different lowercase letters above the bars show that there are significant differences among different treatments (P < 0.05).

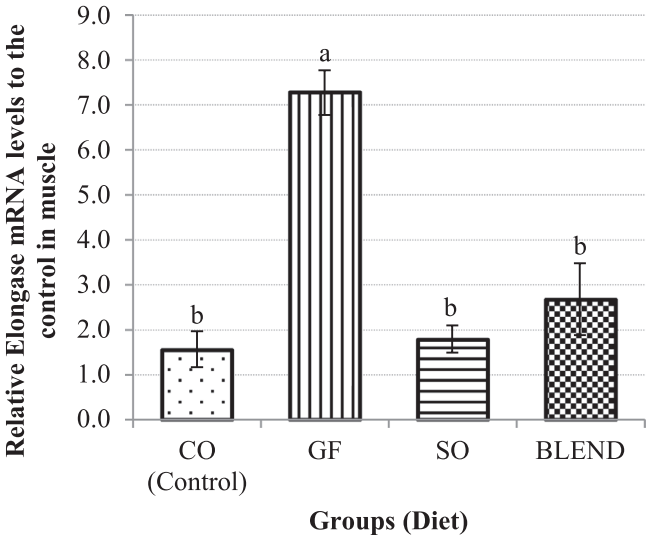


Fig. 3. Relative mRNA expression levels of Elongase gene in muscle of rainbow trout fed diets containing different lipid sources for 8 weeks. Values are means ±SE, n = 3 per group; different lowercase letters above the bars show that there are significant differences among different treatments (P < 0.05).

shown Figs. 1 and 2.

Muscle and liver tissues showed the lowest Δ6 FAD expression level in CO group, followed by the GF, BLEND and SO groups. In addition, SO group was significantly up-regulated in both tissues compared to the other groups ($p < 0.05$).(Fig. 3, 4).

Elongase gene expressions were significantly up-regulated in the GF group compared to the others in both muscle and liver tissues ($p < 0.05$). There was no statistical difference between the other groups in muscle.

4. Discussion

Over the last two decades, higher use of protein and oil components as alternatives to fish meal and FO has been a valuable strategy to boost the sustainability of aquaculture (Naylor et al., 2021). Within this context, the current investigation attempted to establish a relationship

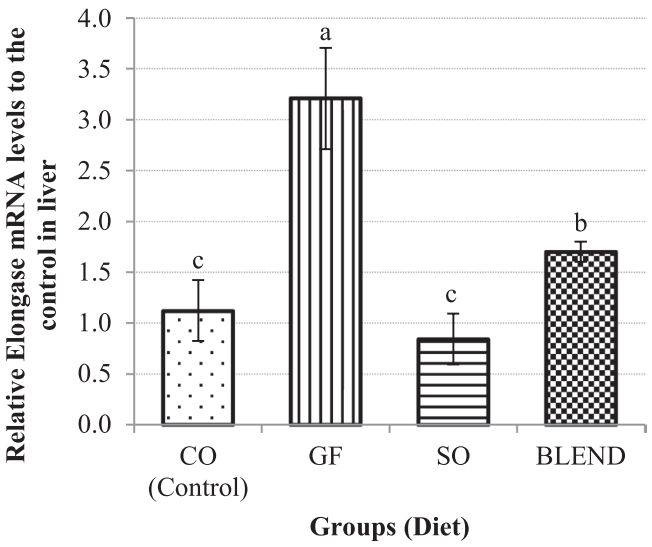


Fig. 4. Relative mRNA expression levels of Elongase gene in liver of rainbow trout fed diets containing different lipid sources for 8 weeks. Values are means ±SE, n = 3 per group; different lowercase letters above the bars show that there are significant differences among different treatments ($P < 0.05$).

between the differing effects of GF, SO and BLEND diets on growth, fatty acid composition, mineral content and mRNA levels of Δ6 FAD and elongase genes in rainbow trout.

4.1. Effects of different lipid sources on the growth performance

The growth performance of rainbow trout in the SO, GF, and BLEND groups exhibited no significant difference. Previous studies have highlighted that vegetable oils could replace FO in the diet without influencing fish growth parameters (Du et al., 2017; Mu et al., 2018; Piedecausa et al., 2007). Liu et al., 2022 and He et al. (2021) reported that replacing dietary FO with SO did not affect the growth of grass carp (*Ctenopharyngodon idella*) and groupers *Epinephelus coioides*, respectively. Xu et al., 2021 reported that substituting high levels of SO with FO in the diet significantly reduced the growth performance of yellow croakers (*Larimichthys crocea*). In contrast, some studies have shown that the effects of dietary replacement of fish oil by vegetable oil can result in good growth performance in marine and freshwater fish (Ghosi Mobaraki et al., 2020; Wu et al., 2019). The fact that the growth performances of fish fed SO and GF were not positively affected in this study may be due to the profile of fatty acids in these diets. Moreover, the effects of diets on growth performances may be due to different fatty acid utilization by different fish species.

4.2. Effects of different lipid sources on the proximate composition

It was observed that the whole-body crude lipid level of the fish was significantly affected by replacing CO diet with GF and BLEND. Fish fed the GF and BLEND diets had the highest crude lipid and crude protein levels, but the lowest moisture content, relative to the other diet groups. In the present study, an inverse relationship was observed between moisture and lipid percentage in the whole-body. Another study in which a high amount of crude lipid accompanied low levels of moisture overlaps with our study (Chaiyapechara et al., 2003). The obtained moisture contents were found to be between 64.5 % and 66.5 %. Especially fish fed GF diet have lower moisture content in the whole-body composition. High humidity can cause degradation of polyunsaturated fatty acids, increase the vulnerability of fish to degradation by microorganisms, and ultimately reduce flesh quality for longer storage (Olagunju et al., 2012). Although the protein content of the fish may vary according to the species, size, season and gender of the fish (Erkan and Özden, 2007). Ash content refers to the total mineral content in the tissue (Nair and Mathew, 2000). In this study, no significant difference was found between the diet groups in terms of ash content. The Ca and P levels in the GF and SO groups increased notably in comparison to other minerals, possibly because the trout is an active swimmer and low levels of ash and protein would suggest high physical activity. Also, Ca and P levels are directly relevant to bone density and hardness (Nikiforidou et al., 2020; Toppe et al., 2007). Therefore, we think that GF and SO diets positively affected bone development of fish relative to the control group.

4.3. Effects of different lipid sources on the fatty acid profile

In teleost fish, body fat is mainly affected by fatty acid concentrations in the diet (Tocher et al., 2008). We determined that MUFA content was increased by GF, while SO diet decreased it. The high MUFA contents probably reflected fatty acid composition of diet since GF contained the highest amount of MUFA among diets tested. MUFAs (especially 18:1 n-9) are used for growth and energy supply in fish (Tocher, 2009). It is always essential for fish to expend additional energy to adapt to stressful conditions and achieve lipid homeostasis. Thus, just like other fish species, the levels of MUFAs and SFAs affects how well rainbow trout digest FA (Xu et al., 2020). Dietary phospholipids can increase the transference of lipids and dietary fatty acids from the intestine to the whole-body probably via enhanced lipoprotein synthesis and are essential for the early development of larval and juvenile fish (Tocher et al., 2008).

The CO group showed the highest n-3 PUFA content in total, neutral and phospholipids, followed by the BLEND, SO and GF groups. Similar findings are observed in largemouth bass (*Micropterus salmoides*) (Chen et al., 2020) and *Onychostoma macrolepis* (Gou et al., 2021). In this study, it was determined that levels of n-3 PUFA and total HUFA in phospholipids are higher than those of neutral lipid fractions. Dietary phospholipids can increase the transport of dietary fatty acids and lipids from the gut to the whole-body, probably via enhanced lipoprotein synthesis, and are essential for the early development of larval and juvenile fish (Tocher et al., 2008). Similar to our study, Mu et al. (2021) determined the maximum n-6 PUFA content and the minimum n-3/n-6 ratio in the fillet due to the high LA level provided by SO in fish fed with SO. They emphasized that the fatty acid composition of vegetable oils will alter this ratio compared to FO. Besides, we predict that the decrease in the n-3/n-6 PUFA ratio in fatty acid profiles in GF, SO and BLEND diet groups compared to control had no impact on the growth performance of the fish. A study on *Megalobrama amblycephala* demonstrated that the ratio of n-3/n-6 PUFAs was negatively associated with liver fat deposition (Li et al., 2015).

It has been stated that fatty acid retention in fish is directly related to the fatty acid content of the diets taken (Rosenlund et al., 2010; Turchini et al., 2009; Vyas et al., 2012). In this respect, our study is consistent with previous research that shows the fatty acid contents in the diets and the whole-body fatty acid contents are related to each other. The retention and synthesis of lipids in fish is closely associated with the uptake of dietary lipids, which are converted and synthesized as fatty acids by the liver and then transported by the blood to other tissues by lipoproteins. Therefore, the fatty acid composition in fish tissues often represents dietary fatty acid compositions (Liu et al., 2022).

4.4. Effects of different lipid sources on Δ6 desaturase and elongase gene expressions

In this study, the SO supplementation significantly increased the expression of Δ6-FAD in liver and muscle tissue. It is likely that the mRNA level of Δ6 FAD was negatively correlated with dietary PUFA levels. Elongase levels were at control levels in fish fed SO diet. Elongase gene expressions were highest in the GF group compared to other groups in both muscle and liver tissues. Δ6 FAD mRNA level in fish fed GF diet

was at control levels. The low expression of desaturase and elongase may be due to the intake of diets rich in EPA and DHA that might reduce the need for n-3 PUFA synthesis. Namely, when fish are fed diets containing PUFA (mainly EPA, DHA and ARA) that are required for their normal growth and development, fish have less requirement to synthesize these fatty acids (Tocher, 2010). These results overlap with data of previous studies on rainbow trout and other fish species fed vegetable sources (Du et al., 2017; Li et al., 2015; Örnek et al., 2021). Our results would contribute to the identification of metabolic pathway(s) that regulates expression of elongase genes. Also, the BLEND diet group showed a regulatory pattern of lipid metabolism in both genes in liver and muscle tissues.

5. Conclusion

Here we evaluated the effect of replacement of fish oil with alternative lipid sources. Dietary lipids we used in this study did not significantly affect growth performance, feed conversion ratio, survival rate and whole-body mineral composition compared to the control. The crude lipid and neutral lipid contents were found to be highest in the fish fed GF diet. MUFA content was also increased by GF. Elongase expression level was the highest in GF diet whereas Δ6 FAD expression was at the maximum level in SO group. According to the findings of our study, GF can be a promising dietary source due to increasing elongation of n-6 PUFAs, decreasing moisture holding capacity and its positive effect on flesh quality. Moreover, further research should be carried out under different stress conditions on the partial replacement of fish oil with goose fat and other lipid based alternatives in fish feeding.

Authorship Contribution Statement

EA and MK conceived the idea, designed the Project, analyzed the data and wrote the manuscript. OV and AT performed the experiments. DE corrected the language/grammar inconsistencies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

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