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ORIGINAL PAPER

Chronic hypoxia and hyperoxia alter tissue-specific fatty acid profile and FD6D and elongase gene expression levels in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Commercially important trout species, especially rainbow trout, are under great threat due to several negative factors affecting oxygen levels in water such as global warming and eutrophication. In our study, rainbow trout (*Oncorhynchus mykiss*) was exposed to chronic (for 28 days) hypoxia (4.0 ± 0.5 mg/L) and hyperoxia (12 ± 1.2 mg/L) in order to evaluate the alteration of fatty acid profiles in muscle, liver and gill tissues. In addition, delta-6-desaturase and elongase gene expression profiles were measured in liver, kidney and gill tissues. The amount of saturated fatty acids increased by oxygen applications in the liver, while it decreased in the muscle and gill tissues compared to normoxia ($p < 0.05$). Monounsaturated fatty acids levels increased in muscle and gill ($p < 0.05$). Although n-3 polyunsaturated fatty acid (PUFA) decreased in muscle tissue, n-6 PUFA increased ($p < 0.05$). The n-3/n-6 ratio decreased in muscle tissue in response to the both exposures ($p < 0.05$) as well as eicosapentaenoic acid/docosahexaenoic acid ratio ($p < 0.05$). Hypoxia exposure generally increased delta-6-desaturase and elongase mRNA levels in all tissues ($p < 0.05$). However, gene expression profiles were variable in fish exposed to hyperoxia. As a result of oxygen exposures, the lipid profile of muscle tissue, which stores dense fat, was negatively affected more than that of liver and gill tissues. We determined that the change in expression levels was tissue specific.

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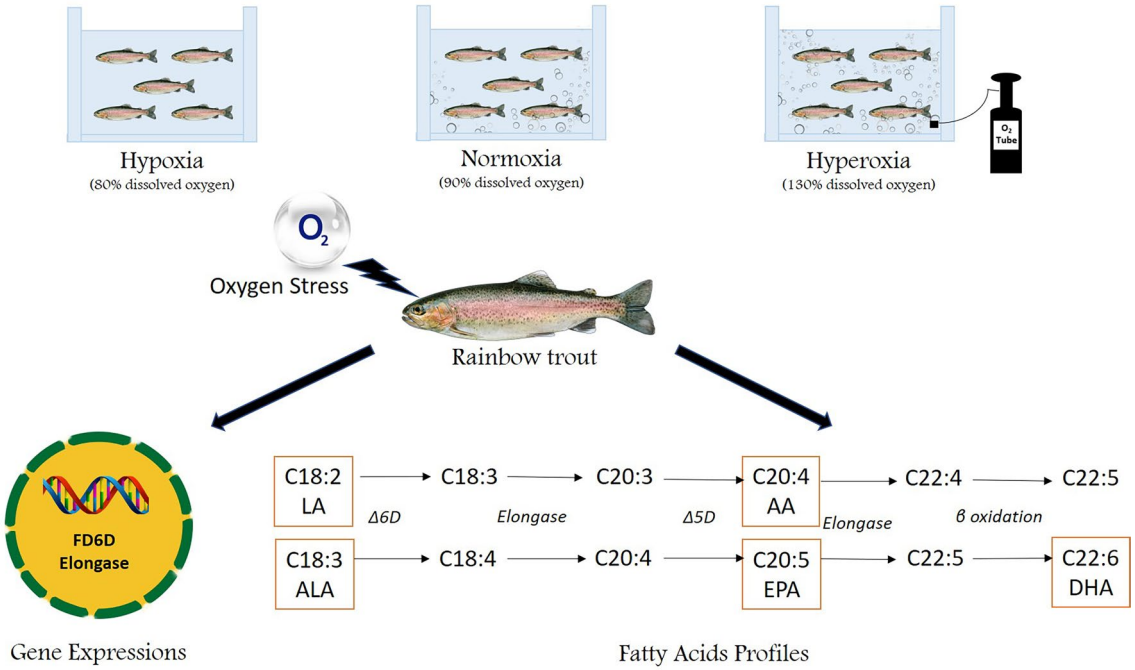
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Graphical abstract



Keywords Hypoxia · Hyperoxia · Fatty acid profile · Delta-6-desaturase · Elongase · Rainbow trout

Introduction

Fish meat, especially trout, is an important food source to meet the animal protein needs of humans due to its rich nutritional components (Justi et al. 2003). However, depending on various environmental factors, such as temperature, eutrophication and global warming, dissolved oxygen concentration in fresh waters is unstable, which has serious negative effects on the organism. Hypoxia, a naturally and frequently occurring condition in multiple ecosystems, has increased in frequency, and severity as a function of nutrient loading and global warming (Verberk and Atkinson 2013; Nudds et al. 2020).

Polyunsaturated fatty acid (PUFA) biosynthesis in animals generally occurs by desaturases via aerobic pathways. Vertebrates lack delta-12 (Δ12) desaturase and delta-15 (Δ15) desaturase. Unlike vertebrates, these desaturases are commonly found in many invertebrate classes (Kabeya et al. 2018). Δ12 desaturase is involved in the synthesis of linolenic acid (LA; 18:2 n-6) from precursor oleic acid (18:1 n-9), and Δ15 desaturase synthesizes α-linolenic acid (ALA; 18:3 n-3) from LA (Nakamura and Nara 2004). ALA and LA are the precursors of PUFA (Arachidonic Acid (20:4 n-6), eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3)) which play important roles in growth, development and reproduction of multicellular organisms in animals including humans. Thus, LA should be supplied by diet (Riediger et al. 2009). In addition to being important energy sources, these long-chain fatty acids are structural components of cell membranes, and these fatty acids can regulate enzyme activity, transcription of genes and membrane fluidity (Castillero et al. 2009). Depending on species, HUFAs (highly unsaturated fatty acids) can be synthesized from n-3 and n-6 unsaturated fatty acids, namely LA and ALA through a series of biochemical pathways involving alternating steps of elongation and desaturation. PUFAs that play important physiological roles in fish are synthesized by fatty acid desaturases (FADS) and elongases, which are regulated by PUFA consumption after metabolization of LA and ALA (Leonard 2004; Venegas-Calerón et al. 2010). FADS catalyze the addition of double bonds at selected positions of their acyl chains. More specifically, delta-6-desaturase (Δ6D) and delta-5-desaturase (Δ5D) catalyze desaturation at the Δ6 and Δ5 positions on the carbon backbone during the synthesis of AA from LA and EPA and DHA from ALA, respectively (Sprecher 1981). On the other hand, they have key roles in growth and survival (Ruyter et al. 2000), pigmentation, and resistance to stress and disease (Anderson and Fritsche 2002). Δ6D is encoded by putative delta-6-desaturase (FD6D) gene (Genebank accession number: AF301910.1) in *Oncorhynchus mykiss*. Changes

in the activity of the Δ6D alter fatty acid profiles and are associated with metabolic and inflammatory diseases (Ruxton et al. 2007). However, the regulatory mechanism underlying FD6D is not fully understood. Changes in gene expression accompanying physiological and biochemical reactions of specialized cellular functions are mainly controlled at the transcriptional level. Therefore, observing gene expression profiles can provide important insights into this process.

Fish tissues are rich in PUFA (Ruxton et al. 2005). However, hypoxia and hyperoxia can be negative factors especially for lipid-rich tissues due to the risk of peroxidation (Bal et al. 2022). PUFAs are susceptible to oxidation because oxygen affects double bonds to form lipoperoxides that produce reactive oxygen species (ROS) and finally induce oxidative stress (Jin et al. 2017). The sustained presence of low oxidative stress state is essential for optimum lipid homeostasis. Since PUFAs tend to react with free radicals, they lead to further degradation with the loss of unsaturated lipids (Sevanian and Hochstein 1985; Sies 1986). The presence of PUFA-containing lipid peroxidation due to oxidative stress has been reported especially in patients with human nonalcoholic fatty liver disease (Videla et al. 2004). In addition, PUFAs can be oxidized and cause changes in oxidative stress conditions caused by many factors such as herbicide (Mercan et al. 2013; Ali et al. 2015; Gonçalves et al. 2016) and metal (Gabryelak et al. 2000) exposure. Therefore, changes in FA profiles are sensitive indicators of stress (Sánchez-Muros et al. 2013).

Fluctuations in dissolved oxygen concentration in water are known to have negative indirect effects on growth parameters and immune system in aquatic organisms (Verghese et al. 2007; Aksakal and Ekinci 2021). In light of these data, we predict that fluctuations in dissolved oxygen concentration in water may also change the fatty acid composition directly related to nutrition in the economically important rainbow trout. In addition, determining the expression behavior of the genes of fatty acid desaturases and elongation enzymes, which play an active role in the conversion of fatty acids, will help in interpreting the results. There are many papers in the literature regarding the effects of various factors (Özyurt and Polat 2006; Gill and Weatherley 1984; Kaçar et al. 2021; Sun et al. 2022) on the fatty acids of rainbow trout, but our study will be unique in terms of investigating the effects of oxygen stress on fatty acid profiles.

Therefore, we aimed in this study to investigate fatty acid profile and molecular responses of rainbow trout against hypoxia and hyperoxia. Accordingly, we analyzed the effects of long-term hypoxia and hyperoxia on fatty acid composition in white muscle, liver and gill tissues in addition to how FD6D and elongase gene expression profiles in liver, kidney and gill tissues are affected in this process.

Materials and methods

Experimental materials and design

Current research is a follow-up study of a previously published experiment on the biological and molecular responses of rainbow trout under hypoxia and hyperoxia conditions. The experimental design has been described in detail by Aksakal and Ekinci (2021).

Three-month-old juvenile rainbow trout, *Oncorhynchus mykiss*, with an initial weight of 8.79 ± 0.12 g, were acquired from a local fish farm. Fish were acclimated in the recirculating aquaculture system's 60 L fiberglass tanks for 2 weeks. Following this, healthy fish were randomly distributed with 20 fish in each fiberglass tank. The experiment had three groups, a total of 18 tanks. During the experiment, the oxygen levels in the hypoxia group (6 tanks), the normoxia group (as control group) (6 tanks), and the hyperoxia group (6 tanks) were maintained at 4.0 ± 0.5, 7.5 ± 0.5, and 12 ± 1.2 ppm, respectively. To induce hypoxic stress in fish, the dissolved oxygen concentration was decreased in 6 tanks by reducing water flow. Hyperoxia was produced by an aeration stone emitting pure oxygen gas bubbles (McArley et al. 2021). A Smart Oxy Oximeter Probe was used to measure oxygen saturation, pH, temperature, and NH_3 and data were recorded on a computer. During the 28-day experiment, temperature was 13.0 ± 0.2 °C and the fish were fed commercial trout diets (~ 1.5% body weight/day). Fish fasted for 24 h before the start of the experiment. One fish was randomly taken from each tank. Three of them were used for fatty acid experiments and the other three were used for gene expression studies. Following the experiment, liver, muscle, gill and kidney samples were taken from each fish group on day 28. Experimental approach with summary of the research is shown in Fig. 1.

This experiment and treatments were approved by the Chair of the Local Ethics Committee of Animal Experiments, Atatürk University, Turkey (Number: 36643897-119).

RNA extraction and complementary DNA (cDNA) synthesis

Three fish (1 fish/tank) were taken from each group and liver, gill and kidney tissues were surgically removed immediately after hypothermia with ice slurry. In addition, fish samples were collected tissues at the initial steps of experiments. 500 mg of each tissue was homogenized in a TissueLyser (Qiagen, Hilden, Germany) prior to RNA isolation. Total RNA was successfully extracted from 500 mg

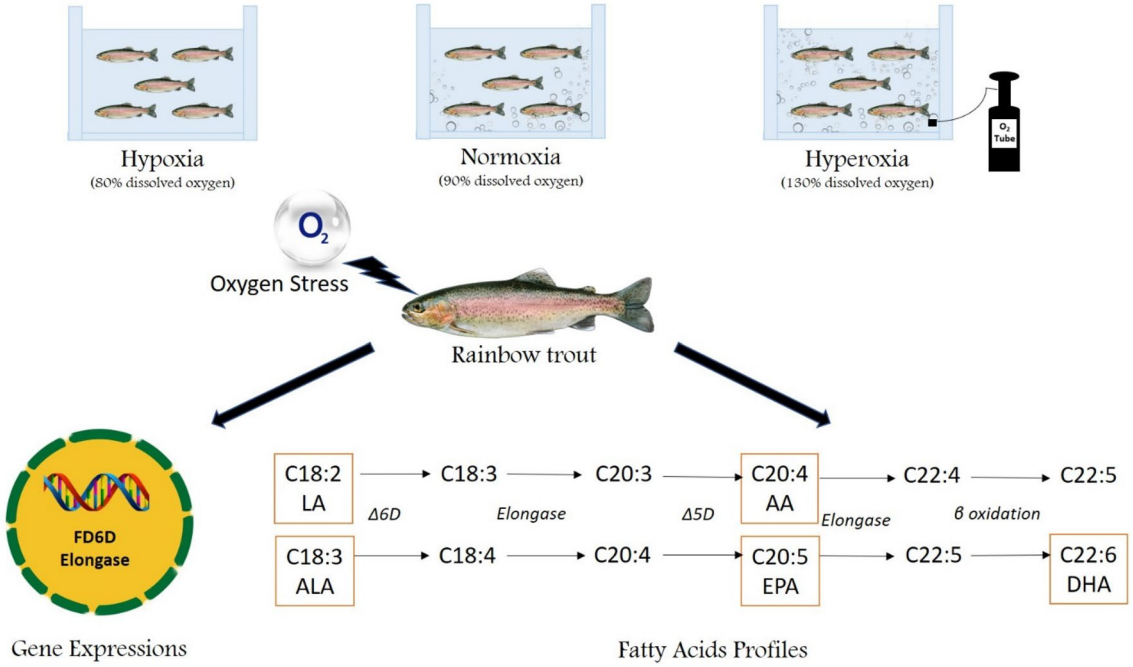


Fig. 1 The experimental design of the study demonstrates the effects of hypoxia and hyperoxia on fatty acid composition and their desaturase and elongase gene expressions on rainbow trout

of each tissue, using the Qiacube robot (Qiagen, Hilden, Germany) and an RNeasy Lipid Tissue Mini Kit (Qiagen, catalog no. 74104). DNase was used to prevent the possibility of genomic contamination. A spectrophotometer (Nanodrop) and agarose gel electrophoresis (1%) were utilized to analyze the quantity and purity of the RNA. cDNA was synthesized using the ThermoScriptTM RT-PCR System for a First-Strand cDNA Synthesis Kit (Invitrogen no. 11146-016). The procedure was carried out by the guidelines provided by the manufacturer. All the cDNA was stored at − 20 °C until use.

Primer and probe design

The primer and TaqMan probe sequences used in the study are shown in Table 1. Two genes involved in fatty acids, including delta-6-desaturase (AF301910.1) and elongase (AF254414), were selected as potential targets. The β-actin gene (AF254414) was used as a housekeeping gene. Primers and TaqMan probes were designed using Primer 3 (version 0.4.0) software (http://frodo.wi.mit.edu/). Correct mRNA sequences were verified by BLAST analysis. The TaqMan probes for the reference gene and the target gene were attached with Cy5/Blackhole Quencher 2 and FAM/TAMRA, respectively (Table 1).

Table 1 Sequence, amplification length, and GenBank accession number of primers and probes used for real-time PCR

Gene	Sequence (listed 5′→3′)	Amplification length (bp)	GenBank accession no
FD6D forward	ACCTAAAGGGTGCCTCTGCT		
FD6D reverse	TTGTCTCCCAGGACGAAGAC	120	AF301910.1
FD6D 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 TaqMan Prob	^{Cy5}-CTCCGGTGACGGCGTGACCC-^{BQ2}		

Quantitative real-time PCR (qRT-PCR)

qRT-PCR were carried out using a Rotor-Gene Q 5-channel instrument (Qiagen, Germany). The reaction was performed with 50 µL of amplification mix containing 5 µL of cDNA template, 5 µL of PCR forward/reverse primers (20 pmol), 1 µL of target and reference TaqMan probes (10 pmol), and 25 µL of 2 × TaqMan® FAST Advanced Master Mix (Thermo Fisher Scientific) with the following thermal pattern: 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 15 s. Each sample was run with three technical replicates. A single peak at the predicted Tm on the melting curve verified the reaction's specificity. To determine relative expression levels, previously reported $2^{-\Delta\Delta Ct}$ technique was used (Pfaffl 2001).

Lipid and fatty acid analysis

At the end of the 28-day experiment, three fish were randomly selected from each group and their white muscles, livers, and gills were collected to determine the fatty acid composition. Total lipid was extracted from samples according to the method of Folch et al. (1957). Samples of rainbow trout (about 1 g) were homogenized by means of Ultra-Turrax for 1 mine in chloroform/methanol (2:1 v/v) containing 0.01% (w/v) of butylated hydroxytoluene [Sigma, 99.0% (GC)]. To synthesize fatty acid methyl esters, the technique developed by Metcalfe and Schmitz (1961) was practiced (FAMEs). The FAMEs were injected onto a DB-23 column (60 m, 0.25 mm, and 0.25 m) using an Agilent 6890 GC. Peaks were identified using standards from Supelco (37 FAME mix, Sigma-Aldrich). According to the peak area of the C19:0 internal standard (Sigma, USA), different fatty acids were reported as a percentage of total FAME.

Statistical analysis

All data were analyzed using SPSS 22.0 (Chicago, IL, USA). Both in gene expression analysis and in fatty acid analysis, differences of each experimental groups were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at a significance level of $p \leq 0.05$. All results were expressed as mean ± SEM (standard error mean). Initial data were obtained before exposures and excluded from the statistics.

Results

The FA compositions of muscle, liver, and gill phospholipids of rainbow trout (*Oncorhynchus mykiss*) after 28 days under hypoxia, normoxia, and hyperoxia stress are shown in Table 2.

Saturated fatty acids (SFAs)

The proportion of SFA in muscle and gill tissues of fish decreased in the hypoxic and hyperoxia treatments relative to the normoxia group ($p < 0.05$). In addition, the liver tissues of hyperoxia-exposed fish have the highest SFA level at 27.08 ± 1.03%. The dominant fatty acid of the SFAs is 16:0 (PA: Palmitic acid) fatty acid in all groups.

Monounsaturated fatty acids (MUFAs)

The levels of MUFA fatty acids in the tissues of fish exposed to hypoxia and hyperoxia were higher than in normoxic groups. The most significant of these increases, from 24.34% to 28.66%, was observed in the muscle tissues of fish treated to hyperoxia ($p < 0.05$). 18:1 n-9 (Oleik acid-OA) was the most predominant MUFA among all experimental groups.

Polyunsaturated fatty acids (PUFAs)

Omega-3 (n-3) PUFAs in the muscle tissues of fish exposed to oxygen stress decreased significantly compared to the normoxia group ($p < 0.05$). In addition, n-3 PUFA ratios in the liver and gill tissues of fish were not affected by 28-day hypoxia and hyperoxia. There was no statistically significant difference between treatments in terms of liver and gill tissues ($p > 0.05$).

Among the 7 PUFAs detected, the predominant fatty acids were 22:6 n-3 (DHA: docosahexaenoic acid), 20:5 n-3 (EPA: eicosapentaenoic acid) and 18:3 n-3 (ALA: α-linolenic acid), respectively. DHA, EPA, and ALA levels were significantly affected only in fish muscle tissues under hypoxic and hyperoxic stress compared to the normoxia group ($p < 0.05$). The EPA + DHA levels of all tissues analyzed in this study were decreased compared to the normoxia group ($p < 0.05$). However, EPA/DHA levels increased in the muscle and gill tissues of fish exposed to hyperoxia and the liver tissues of fish exposed to hypoxia ($p < 0.05$).

Omega-6 (n-6) PUFAs were not affected by oxygen stress. But it significantly increased the n-6 fatty acid content in muscle ($p < 0.05$). In addition, 18:2 n-6 (LA: Linoleic acid) was the major n-6 PUFA in fish. In addition, although the liver n-3/n-6 ratio was the highest in hypoxia treatment, it was not statistically significant ($p > 0.05$).

In general, hypoxia and hyperoxia treatments significantly affect the fatty acid profile of fish muscle.

mRNA levels of fatty acid Δ6-desaturase and elongase

Expression profiles of FD6D and elongase in the liver, kidney, and gill tissue after 28-day oxygen treatment are shown in Figs. 2, 3, 4, 5, 6 and 7.

Table 2 The fatty acid profiles of the muscle, liver, and gills of rainbow trout (*Oncorhynchus mykiss*) in hypoxia, normoxia and hyperoxia

Fatty acid	Muscle				Liver				Gill			
	Initial	Hypoxia	Normoxia	Hyperoxia	Initial	Hypoxia	Normoxia	Hyperoxia	Initial	Hypoxia	Normoxia	Hyperoxia
14:0	1.56	1.86 ± 0.01^{b}	1.77 ± 0.03^{c}	1.96 ± 0.0^{a}	0.82	3.38 ± 0.77^{ab}	2.83 ± 0.71^{b}	5.69 ± 0.67^{a}	2.39	2.59 ± 0.03^{a}	2.68 ± 0.01^{a}	2.41 ± 0.05^{b}
15:0	0.12	0.21 ± 0.03^{a}	0.12 ± 0.03^{ab}	0.07 ± 0.02^{b}	0.00	0.05 ± 0.0^{a}	0.06 ± 0.0^{a}	0.06 ± 0.01^{a}	0.04	0.07 ± 0.0^{a}	0.03 ± 0.0^{c}	0.05 ± 0.0^{b}
16:0	14.85	15.26 ± 0.01^{a}	15.81 ± 0.10^{a}	14.26 ± 0.27^{b}	19.11	16.15 ± 0.71^{a}	15.05 ± 0.32^{a}	14.8 ± 0.24^{a}	15.08	15.51 ± 0.03^{b}	15.73 ± 0.02^{a}	14.55 ± 0.10^{c}
17:0	0.48	0.49 ± 0.0^{a}	0.46 ± 0.0^{b}	0.50 ± 0.01^{a}	0.43	0.41 ± 0.03^{a}	0.54 ± 0.01^{a}	0.46 ± 0.02^{ab}	0.58	0.26 ± 0.01^{b}	0.63 ± 0.0^{a}	0.43 ± 0.11^{ab}
18:0	4.56	4.38 ± 0.11^{a}	4.36 ± 0.07^{a}	3.87 ± 0.05^{b}	6.37	4.67 ± 0.08^{a}	4.74 ± 0.24^{a}	4.59 ± 0.23^{a}	4.63	4.90 ± 0.10^{a}	5.49 ± 0.19^{a}	5.06 ± 0.40^{a}
20:0	0.61	0.57 ± 0.0^{b}	0.54 ± 0.0^{c}	0.73 ± 0.01^{a}	0.30	0.35 ± 0.06^{a}	0.32 ± 0.03^{a}	0.44 ± 0.05^{a}	0.26	0.50 ± 0.15^{a}	0.74 ± 0.0^{a}	0.80 ± 0.01^{a}
22:0	0.78	0.81 ± 0.05^{a}	0.81 ± 0.0^{a}	0.97 ± 0.10^{a}	0.43	0.62 ± 0.13^{a}	0.52 ± 0.01^{a}	0.50 ± 0.08^{a}	0.80	0.82 ± 0.01^{a}	0.86 ± 0.01^{a}	0.90 ± 0.05^{a}
24:0	0.47	0.30 ± 0.11^{a}	0.60 ± 0.03^{a}	0.74 ± 0.23^{a}	0.65	0.48 ± 0.04^{a}	0.52 ± 0.10^{a}	0.56 ± 0.05^{a}	0.21	0.16 ± 0.0^{a}	0.19 ± 0.01^{a}	0.25 ± 0.06^{a}
∑ SFA	23.42	23.89 ± 0.03^{b}	24.47 ± 0.06^{a}	23.09 ± 0.04^{c}	28.11	26.09 ± 0.26^{ab}	24.58 ± 0.09^{b}	27.08 ± 1.03^{a}	23.99	24.81 ± 0.24^{b}	26.34 ± 0.21^{a}	24.46 ± 0.56^{b}
14:1	0.05	0.05 ± 0.01^{b}	0.07 ± 0.0^{ab}	0.08 ± 0.01^{a}	0.00	1.36 ± 0.10^{a}	1.59 ± 0.07^{a}	1.51 ± 0.24^{a}	0.09	0.13 ± 0.0^{a}	0.11 ± 0.0^{c}	0.12 ± 0.0^{b}
15:1	0.20	0.26 ± 0.0^{a}	0.27 ± 0.0^{a}	0.27 ± 0.01^{a}	0.21	0.29 ± 0.02^{b}	0.35 ± 0.0^{a}	0.28 ± 0.01^{b}	0.36	0.44 ± 0.0^{a}	0.37 ± 0.0^{b}	0.43 ± 0.0^{a}
16:1 *n*-7	2.63	2.50 ± 0.02^{a}	2.60 ± 0.10^{a}	2.70 ± 0.02^{a}	1.24	1.89 ± 0.12^{a}	1.88 ± 0.11^{a}	2.32 ± 0.26^{a}	3.92	4.14 ± 0.09^{a}	4.14 ± 0.0^{a}	4.00 ± 0.06^{a}
17:1	0.33	0.32 ± 0.0^{b}	0.31 ± 0.01^{b}	0.37 ± 0.01^{a}	0.22	0.28 ± 0.01^{a}	0.31 ± 0.0^{a}	0.28 ± 0.03^{a}	0.36	0.36 ± 0.01^{b}	0.39 ± 0.0^{a}	0.36 ± 0.01^{b}
18:1 *n-9*	24.41	22.91 ± 0.25^{b}	19.90 ± 0.16^{c}	23.88 ± 0.18^{a}	15.19	17.23 ± 2.91^{a}	16.34 ± 0.69^{a}	17.98 ± 1.69^{a}	33.68	31.23 ± 0.27^{a}	29.42 ± 0.13^{b}	31.40 ± 0.03^{a}
20:1 *n-9*	0.18	0.18 ± 0.0^{b}	0.17 ± 0.01^{b}	0.25 ± 0.02^{a}	0.15	0.13 ± 0.02^{a}	0.11 ± 0.01^{a}	0.16 ± 0.03^{a}	0.23	$0.23 \pm 0.0a^{a}$	0.22 ± 0.0^{a}	0.23 ± 0.01^{a}
22:1 n-9	0.40	0.38 ± 0.0^{a}	0.39 ± 0.0^{a}	0.46 ± 0.10^{a}	0.31	0.40 ± 0.06^{a}	0.44 ± 0.02^{a}	0.36 ± 0.0^{a}	0.06	0.10 ± 0.04^{a}	0.04 ± 0.01^{a}	0.10 ± 0.04^{a}
22:1 *n-11*	0.04	0.07 ± 0.0^{a}	0.06 ± 0.0^{a}	0.04 ± 0.0^{b}	0.25	0.26 ± 0.07^{a}	0.21 ± 0.02^{a}	0.19 ± 0.05^{a}	0.09	0.07 ± 0.0^{a}	0.08 ± 0.0^{a}	0.09 ± 0.01^{a}
24:1 *n-9*	0.48	0.45 ± 0.02^{a}	0.56 ± 0.1^{a}	0.62 ± 0.12^{a}	0.58	0.51 ± 0.10^{a}	0.48 ± 0.06^{a}	0.64 ± 0.06^{a}	0.16	0.11 ± 0.0^{a}	0.12 ± 0.0^{a}	0.21 ± 0.07^{a}
∑ MUFA	28.71	27.11 ± 0.25^{b}	24.34 ± 0.24^{c}	28.66 ± 0.06^{a}	18.15	22.33 ± 2.90^{a}	21.71 ± 0.77^{a}	23.69 ± 2.15^{a}	38.95	36.81 ± 0.33^{a}	34.89 ± 0.14^{b}	36.95 ± 0.09^{a}
18:3 *n*-3	2.65	2.53 ± 0.01^{a}	1.16 ± 0.66^{b}	2.83 ± 0.01^{a}	1.37	1.61 ± 0.29^{a}	1.52 ± 0.12^{a}	1.70 ± 0.26^{a}	0.65	1.98 ± 0.73^{a}	3.09 ± 0.0^{a}	3.25 ± 0.01^{a}
18:4 n-3	0.25	0.21 ± 0.01^{a}	1.24 ± 0.60^{a}	0.29 ± 0.03^{a}	0.15	0.17 ± 0.02^{a}	0.17 ± 0.0^{a}	0.18 ± 0.02^{a}	3.37	1.72 ± 0.83^{a}	0.26 ± 0.0^{a}	0.27 ± 0.0^{a}
20:3 *n*-3	0.69	0.61 ± 0.01^{ab}	0.57 ± 0.0^{b}	0.71 ± 0.05^{a}	0.83	0.79 ± 0.09^{a}	0.80 ± 0.03^{a}	0.81 ± 0.02^{a}	0.50	0.50 ± 0.01^{a}	0.44 ± 0.0^{c}	0.46 ± 0.01^{b}
20:4 *n-3*	0.28	0.27 ± 0.0^{a}	0.25 ± 0.0^{a}	0.36 ± 0.01^{a}	0.22	0.35 ± 0.07^{a}	0.20 ± 0.0^{b}	0.21 ± 0.01^{b}	0.59	0.40 ± 0.08^{a}	0.52 ± 0.0^{a}	0.52 ± 0.01^{a}
20:5 *n*-3	3.84	4.18 ± 0.10^{b}	4.70 ± 0.04^{a}	4.39 ± 0.02^{b}	4.42	4.42 ± 0.50^{a}	4.16 ± 0.09^{a}	3.55 ± 0.37^{a}	2.33	2.58 ± 0.01^{a}	2.63 ± 0.0^{a}	2.54 ± 0.08^{a}
22:5 *n*-3	1.58	1.23 ± 0.0^{a}	1.24 ± 0.0^{a}	1.33 ± 0.08^{a}	1.16	1.11 ± 0.09^{a}	0.91 ± 0.01^{a}	0.93 ± 0.05^{a}	0.62	0.73 ± 0.01^{a}	0.66 ± 0.01^{a}	0.74 ± 0.04^{a}
22:6 *n*-3	20.17	20.82 ± 0.31^{b}	24.27 ± 0.10^{a}	17.65 ± 0.40^{c}	31.70	25.86 ± 3.90^{a}	27.69 ± 1.15^{a}	24.35 ± 2.21^{a}	6.38	7.42 ± 0.0^{a}	7.61 ± 0.05^{a}	6.85 ± 0.22^{b}
∑ *n*-3	29.46	29.86 ± 0.46^{b}	33.43 ± 0.20^{a}	27.57 ± 0.20^{c}	39.86	34.31 ± 4.34^{a}	35.44 ± 1.13^{a}	31.72 ± 2.36^{a}	14.44	15.31 ± 0.17^{a}	15.21 ± 0.06^{a}	14.62 ± 0.36^{a}
18:2 *n*-6	13.93	14.92 ± 0.15^{b}	13.69 ± 0.06^{c}	16.15 ± 0.10^{a}	7.90	11.17 ± 1.41^{a}	11.27 ± 0.59^{a}	11.58 ± 1.37^{a}	17.67	18.33 ± 0.32^{b}	18.84 ± 0.01^{ab}	19.16 ± 0.08^{a}
18:3 *n-6*	0.28	0.23 ± 0.0^{b}	0.20 ± 0.0^{c}	0.33 ± 0.01^{a}	0.22	0.19 ± 0.02^{ab}	0.16 ± 0.01^{b}	0.23 ± 0.02^{a}	0.28	0.34 ± 0.0^{b}	0.29 ± 0.0^{c}	0.38 ± 0.01^{a}
20:2 *n*-6	1.68	1.59 ± 0.05^{ab}	1.46 ± 0.06^{b}	1.64 ± 0.03^{a}	0.74	1.21 ± 0.24^{a}	1.28 ± 0.01^{a}	1.22 ± 0.19^{a}	2.31	2.15 ± 0.01^{a}	2.18 ± 0.01^{a}	2.17 ± 0.02^{a}
20:3 *n-6*	0.92	0.96 ± 0.01^{a}	0.91 ± 0.01^{a}	0.99 ± 0.04^{a}	0.95	1.09 ± 0.02^{a}	1.29 ± 0.04^{a}	1.15 ± 0.10^{a}	1.20	1.03 ± 0.0^{b}	1.07 ± 0.0^{a}	1.01 ± 0.0^{c}
20:4 *n*-6	1.29	1.17 ± 0.04^{ab}	1.23 ± 0.0^{a}	1.10 ± 0.02^{b}	3.43	2.60 ± 0.37^{a}	2.97 ± 0.16^{a}	2.69 ± 0.32^{a}	0.83	0.91 ± 0.02^{a}	0.84 ± 0.0^{b}	0.85 ± 0.01^{b}
22:2 *n*-6	0.08	0.07 ± 0.0^{a}	0.07 ± 0.0^{a}	0.19 ± 0.07^{a}	0.04	0.11 ± 0.02^{a}	0.05 ± 0.0^{b}	0.05 ± 0.0^{b}	0.21	0.19 ± 0.0^{ab}	0.18 ± 0.0^{b}	0.22 ± 0.02^{a}
22:4 *n*-6	0.23	0.20 ± 0.0^{ab}	0.19 ± 0.0^{b}	0.29 ± 0.04^{a}	0.59	0.91 ± 0.08^{ab}	1.24 ± 0.12^{a}	0.60 ± 0.10^{b}	0.13	0.11 ± 0.03^{a}	0.17 ± 0.0^{a}	0.18 ± 0.03^{a}

Table 2 (continued)

Fatty acid	Muscle				Liver				Gill			
	Initial	Hypoxia	Normoxia	Hyperoxia	Initial	Hypoxia	Normoxia	Hyperoxia	Initial	Hypoxia	Normoxia	Hyperoxia
$\sum n$-6	18.11	18.88 ± 0.17^{b}	17.49 ± 0.02^{c}	20.20 ± 0.0^{a}	13.25	16.21 ± 1.28^{a}	16.98 ± 0.40^{a}	16.86 ± 1.36^{a}	22.29	22.76 ± 0.29^{b}	23.21 ± 0.01^{ab}	23.57 ± 0.06^{a}
$\sum$ HUFA	30.76	31.12 ± 0.41^{b}	34.90 ± 0.06^{a}	28.66 ± 0.04^{c}	44.09	38.44 ± 4.90^{a}	40.59 ± 1.57^{a}	35.54 ± 2.77^{a}	15.10	16.01 ± 0.13^{a}	16.29 ± 0.08^{a}	15.55 ± 0.40^{a}
EPA + DHA	24.01	25.00 ± 0.42^{b}	28.97 ± 0.14^{a}	22.04 ± 0.42^{c}	36.12	30.28 ± 4.40^{a}	31.85 ± 1.23^{a}	27.90 ± 2.57^{a}	8.71	10.0 ± 0.02^{ab}	10.23 ± 0.05^{a}	9.39 ± 0.30^{b}
EPA/DHA	0.19	0.20 ± 0.0^{b}	0.19 ± 0.0^{b}	0.25 ± 0.0^{a}	0.14	0.17 ± 0.01^{a}	0.15 ± 0.0^{b}	0.15 ± 0.0^{b}	0.37	0.35 ± 0.0^{b}	0.35 ± 0.0^{b}	0.37 ± 0.0^{a}
$\sum n$-3/ n-6	1.63	1.58 ± 0.04^{b}	1.91 ± 0.01^{a}	1.36 ± 0.01^{c}	3.01	2.18 ± 0.44^{a}	2.09 ± 0.12^{a}	1.93 ± 0.30^{a}	0.65	0.67 ± 0.02^{a}	0.66 ± 0.0^{ab}	0.62 ± 0.01^{b}

Different superscript letters indicate the significant difference between hypoxia, normoxia and hyperoxia within each tissue ($p < 0.05$). It was not included in the initial statistical calculation. Results represent means ± S.D. ($n = 3$). SFA

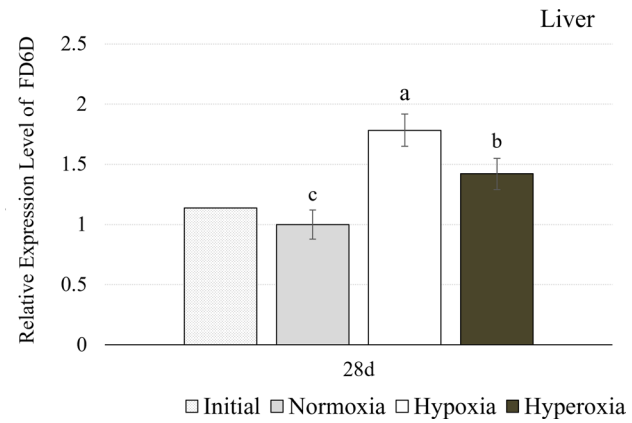


Fig. 2 Relative expression of FD6D in liver of *Oncorhynchus mykiss* exposed to hypoxia, normoxia and hyperoxia at 28 days. Different letters indicate statistically significant difference determined by Duncan's multiple range test ($p < 0.05$). Initial data were obtained before exposures and it was excluded from the statistics. Data are mean ± SEM ($n = 3$)

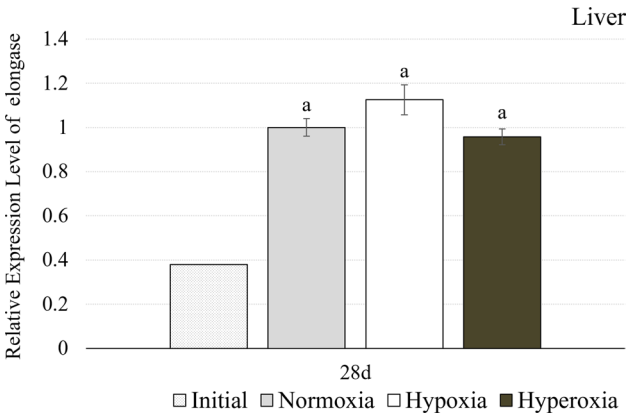


Fig. 3 Relative expression of elongase in liver of *Oncorhynchus mykiss* exposed to hypoxia, normoxia and hyperoxia at 28 days. Different letters indicate statistically significant difference determined by Duncan's multiple range test ($p < 0.05$). Initial data were obtained before exposures and it was excluded from the statistics. Data are mean ± SEM ($n = 3$)

In liver tissue, FD6D mRNA levels were up-regulated under hypoxia stress relative to normoxia and hyperoxia groups ($p < 0.05$) (Fig. 2). In addition, the elongase mRNA levels in the liver of rainbow trout were dramatically up-regulated 2.5-fold in hypoxia, normoxia and hyperoxia treatment compared to the initial time of fish. But no statistical difference was found between the treatments ($p > 0.05$) (Fig. 3). In kidney tissue, FD6D and elongase mRNA levels were down-regulated relative to the initial time. The hypoxia group was statistically different from the other groups in both genes ($p < 0.05$) (Figs. 4 and 5). It was determined that the gill tissues differed significantly in terms of FD6D expressions. Accordingly, we found

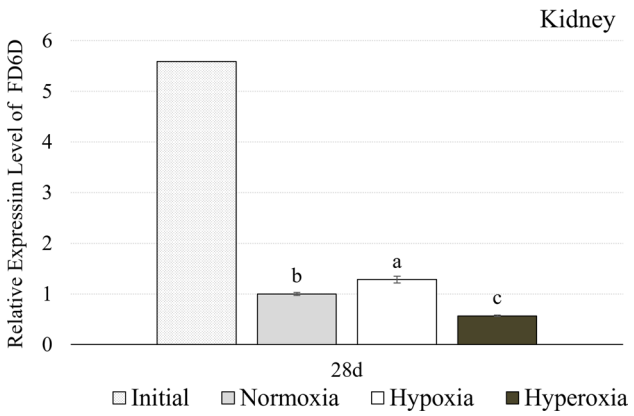


Fig. 4 Relative expression of FD6D in kidneys of *Oncorhynchus mykiss* exposed to hypoxia, normoxia and hyperoxia at 28 days. Different letters indicate statistically significant difference determined by Duncan's multiple range test ($p < 0.05$). Initial data were obtained before exposures and it was excluded from the statistics. Data are mean ± SEM ($n = 3$)

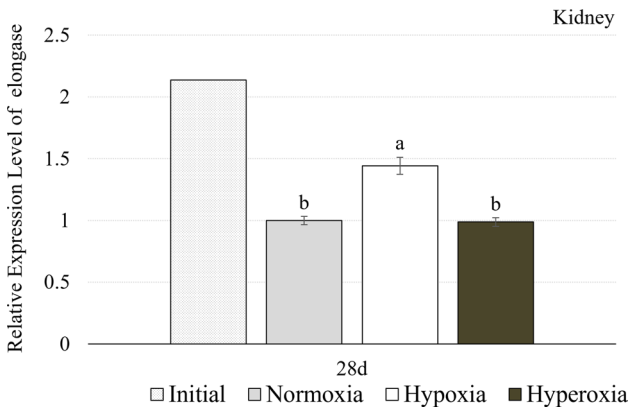


Fig. 5 Relative expression of elongase in kidneys of *Oncorhynchus mykiss* exposed to hypoxia, normoxia and hyperoxia at 28 days. Different letters indicate statistically significant difference determined by Duncan's multiple range test ($p < 0.05$). Initial data was obtained before exposures and it was excluded from the statistics. Data are mean ± SEM ($n = 3$)

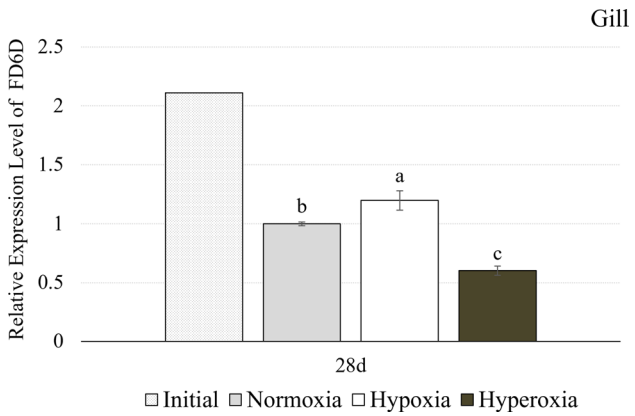


Fig. 6 Relative expression of FD6D in gills of *Oncorhynchus mykiss* exposed to hypoxia, normoxia and hyperoxia at 28 days. Different letters indicate statistically significant difference determined by Duncan's multiple range test ($p < 0.05$). Initial data was obtained before exposures and it was excluded from the statistics. Data are mean ± SEM ($n = 3$)

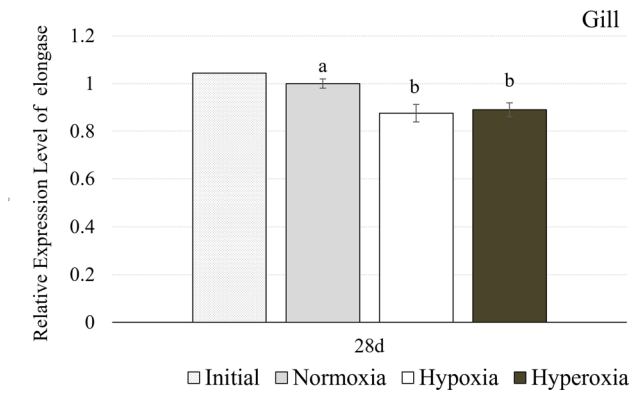


Fig. 7 Relative expression of elongase in gills of *Oncorhynchus mykiss* exposed to hypoxia, normoxia and hyperoxia at 28 days. Different letters indicate statistically significant difference determined by Duncan's multiple range test ($p < 0.05$). Initial data were obtained before exposures and it was excluded from the statistics. Data are mean ± SEM ($n = 3$)

that the hypoxia group was up-regulated compared to the other groups ($p < 0.05$) (Fig. 6). In addition, we found that elongase expression levels in the gill tissues of fish under long-term stress were different compared to the normoxia group ($p < 0.05$) (Fig. 7).

According to our real-time PCR data, FD6D and elongase expression levels in muscle, kidney and gill tissues of rainbow trout exposed to oxygen stress were affected. Expression levels showed marked variability in tissues, especially in response to exposure to hypoxia stress.

Discussion

This study is a follow-up of a previous one (Aksakal and Ekinci 2021), in which it was shown that both growth parameters and transcription levels of growth, immune system and stress-related genes were decreased when rainbow trout were exposed to hypoxia and hyperoxia. However, to the best of our knowledge, no study has been found on how hypoxia and hyperoxia affect the FA profile of rainbow trout and their relationship with the responsible genes.

Changes in fish lipid metabolism can occur under different stress conditions (Li et al. 2022; Gonçalves et al. 2016; Verberk and Atkinson 2013). In the current study, total MUFA level in white muscle tissue was found to be highest in the hyperoxia groups compared to the control group. MUFAs (especially 18:1 n-9) are used for growth and energy supply in fish. It is always essential for fish to expend additional energy to adapt to stressful conditions and achieve lipid homeostasis. Therefore, just like other fish species, the order of MUFAs and SFAs affects how well rainbow trout digest FA (Xu et al. 2020).

It is well known that n-3/n-6 PUFA, EPA, and DHA play crucial roles in regulating lipid metabolism, meat quality, growth performance, and immunological function in fish muscle (Wang et al. 2021). Because of the significant decrease in n-3/n-6 PUFA, DHA, EPA, and DHA + EPA in the muscle fatty acid profiles of the fish compared to the normoxia group, we assume that the quality of the fillets was negatively impacted. In this study, the levels of n-6 PUFA increased in exposure to oxygen stress, whereas n-3 PUFA values decreased in muscle tissue. Bao and collaborators (2018) also reported an inverse relationship between n-3 PUFA and n-6 PUFA in rainbow trout exposed to heat stress (6 h). This is probably due to the metabolic challenges that fish experience with feeding under both hypoxia and hyperoxia stress, as the fatty acid composition of organisms is often a reflection of their dietary fatty acid profile. In this study, it is of importance to evaluate the possibility that hypoxia and hyperoxia are chronic stress factors in trout, causing changes in feeding behavior and decreasing feed intake. Our results, together with evidence from previous research, show that low oxygen is directly related to feed intake and reduces digestible protein and lipid retention, energy intake, and storage in fish (Glencross 2009; Remen et al. 2014; Magnoni et al. 2019). As a consequence, it has been shown that the food taken in hypoxia conditions are used directly as energy since it is costly to store (Vikeså et al. 2017). The decrease in the n-3 PUFA level is largely due to the decrease in EPA and DHA levels. Similar to hyperoxia, we determined that these parameters decreased in muscle tissue. Moya-Falcon et al. (2006) in their study found that heat stress causes a decrease in 22:6 n-3 DHA levels in hepatocytes. It has been emphasized that this decrease may be related to meeting the increased energy demand from fatty acids due to stress conditions in muscle tissue (Dahlhoff 2004). It is also noteworthy that the most affected tissue among the three tissues is muscle tissue. The fact that muscle tissue is rich in lipids, especially in fish, may be the reason for this effect (Huss 1988).

Liver fatty acid profiles were not significantly altered in response to experimental conditions in trout. The absence of a significant change in n-6 PUFA content as well as n-3 PUFA such as 20:5 n-3 and 22:5 n-3 content in the liver may be related to the unchanged elongase gene expression. The increase in FD6D expression in the liver may be associated with increase of 18:3 n-6 fatty acid compared to the control in effect of hyperoxia. It can be suggested that the increase in hypoxia is due to other fatty acids in the fatty acid pathway of FD6D or may be due to post-transcriptional modifications because the 18:4 n-3 fatty acid was unchanged compared to control. As a matter of fact, elongase and FD6D are genes of important enzyme systems involved in the elongation of fatty acids (Henderson 1996). Therefore, it seems that rainbow trout can effectively adjust to the hypoxic and hyperoxia conditions. These results are consistent with findings of previous studies on rainbow trout and teleost fish (Uribe et al. 2011; Wischhusen et al. 2020). Similarly, it has been stated that the n-3 PUFA content stored in phospholipids can be improved for tolerance of ischemia damage in rat heart tissue that develops as a result of adaptation to hypoxia (Ježová et al. 2002). Fish increased cortisol levels under hypoxic conditions (Magnoni et al. 2019). In this state, it can be seen as an indicator that the liver and gill tissues adapt to oxygen stress.

The gills are the main organs for respiration, ion and water exchange, and nitrogenous waste excretion. Furthermore, when subjected to environmental stress, the gills are the first organs to be affected in fish (Evans et al. 2005). In the current study, MUFA (especially 18:1 n-9) levels of gill tissue fatty acid profile exposed to oxygen stress were significantly increased compared to the normoxia group. The reason for this is that metabolism requires a lot of energy to balance with the uneven osmotic pressure (Zhang et al. 2021). This was similar to the findings of research on the gills of *Neptunea cumingii* by Zhang et al. (2021) when the temperature is reduced below 16 °C. In our study, gene expression data revealed that exposure to hypoxia increased the expression level of the Δ6-desaturase gene compared to other treatments. These results suggest that rainbow trout gills improve energy supply during acute hypoxia and hyperoxia stress (Liu et al. 2022). In this study, the down-regulated elongase gene levels were noteworthy, but our investigation found no significant difference between long-term hypoxia and hyperoxia. In addition, it was shown that acute stress had no effect on the primary FA composition in rainbow trout gill phospholipids but decreased levels of DHA, an essential fatty acid. This is due to the fact that the gills are not metabolically intended to store lipids (Liu et al. 2018).

To better understand the regulation of HUFA biosynthetic pathways in commercially important cultured fish species, it is important to better understand the regulation of FD6D and elongase gene expression. The importance of highly unsaturated fatty acids (HUFA, n-6 and n-3 fatty acids) in fish nutrition is well known (Sargent et al. 2003). The status of fatty acids such as EPA and DHA are largely dependent on the conversion capacity of FD6D and elongase and C18 fatty

acids rather than dietary intake. Although a large number of studies have focused on the effect of various dietary sources or dietary stress on FD6D and elongase gene expression, there are no papers investigating the effects of hypoxia and hyperoxia on these genes. While PUFA levels such as ALA and LA are increased by the effect of hypoxia and hyperoxia in muscle tissue, the decrease in n-3 PUFA levels such as EPA and DHA indicate low Δ6D and elongase activity. As an indicator of this, the gene expression profile is expected to decrease. Similar results were seen in a diet study (Thomassen et al. 2012). But it should be noted that a decrease in activity may not always be the result of a decrease in gene expression. Changes in the mRNA degradation rate of these enzymes are significantly regulated at the post-transcriptional level (Clerch 2000). The increase of DHA can be regulated by elongation and desaturase enzymatic activity on ALA. Therefore, for the genes encoding these enzymes to be up-regulated, either an increase in the amount of ALA or a decrease in the n-3 PUFA content is required (Tocher 2015). In our study, we determined that FD6D expression level increased as a result of hypoxia exposure, but it increased in the liver and decreased in the kidney and gill as a result of hyperoxia exposure. This suggests that FD6D expression is tissue-specific. In addition, high FD6D expression levels may be caused by high ALA and LA levels. As a result of hypoxia and hyperoxia, levels of ALA and LA were found to be increase, as well as AA, while EPA and DHA levels decreased. Shi et al. (2015) reported that Δ6D converts ALA to stearidonic acid (SDA, 18:4) better than LA to ALA in *Mortierella alpina*. Therefore, it is possible that Δ6D might be more effective in the transformation of ALA in rainbow trout under oxygen stress. On the other hand, elongase was generally detected to be low, suggesting that Δ6D may be more sensitive than elongase under stress conditions.

Conclusions

This is the first study to show the effect of oxygen stress on fatty acids in muscle, liver and gill, also FD6D and elongase mRNA levels in muscle, liver and kidney in rainbow trout. We determined that this stressor did not cause any change in fatty acid profiles in the liver and gills, but had significant effects on muscle tissue. Following hypoxia and hyperoxia adaptation, fatty acid profiles were tissue-specifically altered or remained unaffected. The fact that this situation does not meet the expectations in terms of gene expression profiles can be explained by the change in oxidative stress and post-transcriptional modifications.

Despite all, metabolic pathways underlying the changes observed in the FA profile and the regulatory mechanism of FD6D expression in tissues by oxygen stress following exposure to long-term hypoxia or hyperoxia were not discussed in detail in this study and remain to be defined.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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