Ann. Anim. Sci., Vol. 17, No. 4 (2017) 1135–1153 DOI: 10.1515/aoas-2017-0010

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EFFECT OF DIETARY FISH OIL REPLACEMENT WITH PLANT OILS ON GROWTH PERFORMANCE AND GENE EXPRESSION IN JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Abstract

An eight-week feeding trial was conducted to evaluate the effects of total (100%) replacement of dietary fish oil with alternative lipid sources in juvenile rainbow trout. Six iso-nitrogenous and iso-lipidic experimental diets were formulated: CO (14%) – cod liver oil; SSO (14%) – safflower seed oil; SBO (14%) – soybean oil; LO (14%) – linseed oil; SBO (7%) + LO (7%) – a blend of soybean oil and linseed oil; and SSO (7%) + LO (7%) – a blend of safflower seed oil and linseed oil. Growth performance [specific growth rate (SGR), weight gain (WG), food conversion ratio (FCR) and survival rate (SR)], growth hormones [growth hormone (GH-I), insulin-like growth factor-I (IGF-I) and insulin-like growth factor-II (IGF-II)], immune transforming growth factor-β (TGF-β) and antioxidant [superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and glutathione S-transferase (GST)] response, and heat shock protein 70 (HSP70) mRNA levels were determined in muscle and liver. Our data indicated that final weight, weight gain, FCR and SGR showed significant difference among the six dietary treatments ($P<0.05$) while there were no significant differences in survival rate between the rainbow trout from supplement fed groups and control group. HSP70 mRNA level expression in muscle was higher in fish fed SSO ($P<0.05$) while highest level in liver was obtained from fish fed SBO compared to the other treatments ($P<0.05$). There were no significant differences among treatments for TGF-β mRNA expression level in muscle and liver. In conclusion, growth performance and expression levels of growth hormones, antioxidants, HSP70, except TGF-β were affected by five separate lipid sources. In addition, LO positively increased growth performance of juvenile rainbow trout by means of preventing oxidative stress and HSP70 and, enhanced expression of growth hormone related gene.

Key words: dietary lipid, gene expression, oxidative stress, HSP70

Plant oils (PO) are readily available, utilizable in feedstuffs, cost-effective, environmentally and economically sustainable (Turchini et al., 2003; Teoh et al., 2011). On the other hand, PO do not contain *n-3* long-chain polyunsaturated fatty acids (*n-3* LC-PUFA) (Teoh et al., 2011). As documented in the previous studies, single or a mixture of plant oils can be used as alternative lipid sources (Sargent and Tacon, 1999; Bell et al., 2003; Richard et al., 2006; Gatlin et al., 2007; Webster et al., 2007; Bell and Waagbo, 2008; Panserat et al., 2009; Teoh et al., 2011; Bogevik et al., 2014; Moldal et al., 2014; Carmona-Osalde et al., 2015; Geay et al., 2015; Han et al., 2015; Betiku et al., 2016; Carbone and Faggio, 2016; Teoh and Ng, 2016; Azeredo et al., 2017; Brum et al., 2017; Caballero-Solares et al., 2017; Nayak et al., 2017).

Oncorhynchus mykiss is the most important salmonid fish species owing to its aquaculture potential, economic value and wide consumer demand (Kocabas et al., 2011). Global production of rainbow trout is rapidly increased depending on increasing demand. Moreover, requirement of commercial pelleted feeds and importance of alternative feedstuffs is increased in global production (Turchini and Francis, 2009; Teoh et al., 2011). Concomitantly, nutritional status affects the endocrine system having a central role in the modulation of growth and nutrient utilization (Chen et al., 2012). In this framework, the aim of the study was to examine effect of supplementation of different dietary lipid sources (cod liver oil, CO; soybean oil, SO, rich in *n-6* PUFA; linseed oil, LO, rich in *n-3* PUFA; safflower seed oil, SSO, rich in 18:2*n-6*) on growth performance (SGR, WG, FCR, SR) of rainbow trout juveniles. The specific objectives were to: (1) assess expression of growth hormones (GH-I, IGF-I, IGF-II); (2) evaluate expression levels of antioxidant enzymes (SOD, CAT, GR, GPx, GST); (3) determine expression of heat shock protein 70 (HSP70); (4) assess expression of immune response genes (TGF-β).

Material and methods

Fish (n=600; 2.28±0.02 g, mean±SEM) were obtained from a local supplier (Erzurum, Turkey) and transferred to the laboratory. The fish were acclimatized to the laboratory conditions in a fiberglass tank for two weeks. Fish were then randomly stocked into tanks (50 L). The feeding trial was conducted for 8 weeks. The photoperiod was 14L:10D. Dissolved oxygen was measured with a Smartoxy Oxymeter (Technos Company, Chioggia, Italy). Temperature, pH and dissolved oxygen saturation in the treatments were 13.0±0.2^{o}C, 7.4 and 75–80%, respectively. The fish were fed to satiety four times at a day. At the end of the feeding trial, five fish per tank were sacrificed and were sampled and stored at –80^{o}C for molecular analysis.

Experimental diets

Six iso-nitrogenous and iso-lipidic casein-gelatin based experimental diets with varying dietary sources were formulated. The sources of dietary lipid were cod liver oil [CO (14%) diet, rich in highly unsaturated fatty acids]; safflower seed oil [SSO (14%) diet, rich in linoleic acid]; soybean oil [SBO (14%) diet, rich in linoleic acid];

linseed oil [LO (14%) diet, rich in linolenic acid]; a blend of soybean oil and linseed oil [SBO (7%) + LO (7%) diet]; and a blend of safflower seed oil and linseed oil [SSO (7%) + LO (7%) diet] (Table 1). Dry ingredients were mixed homogenously in a Hobart mixer. Then, the oil was thoroughly mixed with the ingredient mixture and distilled water was added. The moist dough was screw pressed through a 2-mm die and the feed pellets formed were fan-dried and stored frozen at −20°C until use.

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

	Lipid sources					
	CO	SSO	SBO	LO	SBO+LO	SSO+LO
Ingredients (%)						
casein (vitamin free)	43.19	43.19	43.19	43.19	43.19	43.19
gelatin	8.64	8.64	8.64	8.64	8.64	8.64
dextrin[1]	6.25	6.25	6.25	6.25	6.25	6.25
wheat meal	15.00	15.00	15.00	15.00	15.00	15.00
fish protein concentrate[2]	5.00	5.00	5.00	5.00	5.00	5.00
cod liver oil (CO)	14.00	-	-	-	-	-
safflower seed oil (SSO)	-	14.00	-	-	-	7.00
soybean oil (SO)	-	-	14.00	-	7.00	-
linseed oil (LO)	-	-	-	14.00	7.00	7.00
vitamin mixture[3]	2.00	2.00	2.00	2.00	2.00	2.00
mineral mixture[4]	3.00	3.00	3.00	3.00	3.00	3.00
CMC[5]	1.00	1.00	1.00	1.00	1.00	1.00
L-arginine	0.50	0.50	0.50	0.50	0.50	0.50
L-methionine	0.40	0.40	0.40	0.40	0.40	0.40
L-lysine	0.80	0.80	0.80	0.80	0.80	0.80
Stay-C 35[6]	0.06	0.06	0.06	0.06	0.06	0.06
choline chloride (99%)	0.17	0.17	0,17	0.17	0.17	0.17
Proximate composition (%)						
crude protein	64.18	64.10	64.17	64.41	64.27	64.01
crude lipid	15.27	15.10	15.40	15.18	15.28	15.31
ash	5.91	5.64	5.39	5.35	5.38	5.49
moisture	7.6	8.11	9.38	8.99	9.27	8.93

[1]Water soluble 80%.

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

[3]Trouw Nutrition Premix (Ankara, Turkey), composition per g of the vitamin mixture: vitamin A – 2645.50 IU; vitamin D_3 – 220.46 IU; vitamin E – 44.09 IU; Vitamin B_{12} – 13 g; 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; D-biotin – 0.31 mg.

[4]Bernhart Tomarelli salt mixture (ICN Pharmaceuticals, Costa Mesa, CA), composition (g/100 g): calcium carbonate – 2.1; calcium phosphate dibasic – 73.5; citric acid – 0.227; cupric citrate – 0.046; ferric citrate (16 to 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.

[5]Carboxymethylcellulose sodium salt.

[6]Phosphitan C (Mg-L-ascorbyl-2-phospahte), Sigma, Germany.

Growth measurements and sampling

Fish were weighed at the start (initial weight) and the end (final weight) of the experiment, and fish mortality was recorded daily. Each fish was anesthetized (Benzocaine, 30 mg/L), and body weight (BW: body weight) recorded. Specific growth rate (SGR) was calculated as $[(\ln W_2-\ln W_1)/(t_2-t_1)*100]$ (W_1: initial weight, W_2: final weight, t_1: initial time, t_2: final time). Percentage of weight gain was calculated as [WG=(final weight–initial weight)×100/initial weight]. Food conversion ratio was calculated as [FCR=feed consumed/weight gain].

Total RNA isolation and cDNA preparation

Triplicates of fish (n=15) from each feeding group were analyzed for gene expression of GH-I, IGF-1, IGF-II, TGF- β, SOD, CAT, GR, GPx, GST and HSP70 in muscle and liver. Total RNA was isolated from 50 mg frozen muscle and liver tissues with a RNeasy Lipid Tissue Mini Kit (Qiagen cat. no. 74804) using the Qiacube robot (Qiagen, Hilden, Germany). RNA was treated with DNase I in order to avoid genomic contamination. RNA concentrations and quality were verified by means of nanodrop spectrophotometer and RNA gel electrophoresis, respectively. Following isolation, cDNA synthesis was performed using the ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis Kit (Invitrogen) according to the manufacturer's protocol. All cDNA was stored at −20°C until use.

TaqMan probe and primer design

Primers and TaqMan probes were designed in Primer3 software (v. 0.4.0) (http://bioinfo.ut.ee/primer3/) using rainbow trout muscle and liver GH-I and TGF-β BLASTed to ensure correct mRNA sequences. IGF-I and IGF-II primers and TaqMan probes were used from Aksakal et al. (2010). *β*-actin was used as a suitable reference gene since it was not affected by any of the treatments (Johansen and Overturf, 2005). In order to perform real-time PCR, TaqMan probe of the target and reference gene was conjugated with FAM/TAMRA. The primer and probe sequences, amplification length and GenBank accession number for real-time PCR are provided in Table 2.

Real-time PCR and gene expression analysis

Quantification of gene expression by real-time PCR analysis was performed using a thermal cycler Qiagen Rotor-Gene. The real-time PCR was carried out in a reaction volume of 50 µl containing template DNA, 900 nM of both target and reference forward and reverse primers, 250 nM of both target and reference TaqMan probes, and 25 µl of Fast Start TaqMan Probe Master (Applied Biosystems) which consists of AmpliTaq Gold DNA Polymerase, Amp Erase uracil N-glycosylase (UNG), dNTP with dUTP, and optimized buffer component. Amplification and detection of the samples and the standards were performed using the following thermal cycling conditions: 50°C for 2 min for activation of optical Amp Erase UNG enzyme, 95°C for 10 min as hot start to activate Ampli Taq Gold DNA polymerase followed by 45 cycles of denaturation at 95°C for 15 s, and annealing and extension at 60°C for 1 min. Real-time PCR data were analysed using the efficiency (e)(−ΔCt) method, which is used to determine mRNA levels in gene expression against control group and reference gene

β-actin. Analytical sensitivity was confirmed by running standard curves. Amplification efficiency (E) was calculated based on the slopes of the curves (slope) using the formula $E=10^{(-1/slope)}$ (Pfaffl 2001), and the slope value via Rotor-Gene software. Also, we used the GeneGlobe Data Analysis Center (Qiagen) to construct hierarchical clustering analysis of the genes as up- or down-regulated.

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

Genes	Primers and Probes	Sequence (5'-3')	Amplification length (bp)	GenBank accession number
GH-I	forward	TCTGCTGATGCCAGTCTTACTG	118	NM_001124689.1
	reverse	CTGAGCCAATAGGTGGAGATGT		
	probe	FAM-ACGGCTCTTCAACATCGCGGTC-TAMRA		
IGF-I	forward	ATGTGCTGTGTCTCCTGTACCC	149	M95183.1
	reverse	TAAAAGCCTCTCTCTCCACACA		
	probe	FAM-TAACCCTGACTTCGGCGGCA-TAMRA		
IGF-II	forward	GAAGGTCAAGATGATGTCTTCG	108	M95184.1
	reverse	AGTTCTCCTCCACATAGCGTTT		
	probe	FAM-TCGAGTGCTGGTCATTGCGC-TAMRA		
TGF-*β*	forward	AGCTCTCGGAAGAAACGACA	134	X99303.1
	reverse	TAGCCAGTGGGTTCATGGA		
	probe	FAM-TCCGTAAGGACCTGGGCTGGAA-TAMRA		
SOD	forward	TGCTTATGGAGACAACACCAA	156	AF469663.1
	reverse	TGGATGTTGATCTTAGCCACA		
	probe	FAM-CCACTGATGCTGTTCGGCACGT-TAMRA		
CAT	forward	TCCTTTATCCACTCTCAGAAGCG	125	NM_001140302.1
	reverse	CCACGGTCACTGAACAGGAA		
	probe	FAM-TGGTGTGGGACTTCTGGAGCCTGC-TAMRA		
GR	forward	ATCACGCCATCACCACCAG	117	HF969248.1
	reverse	CTTGCAACATCTCATCACAGCC		
	probe	FAM-GGCAAGGAGGAGAAGGTGGTTG-GCC -TAMRA		
GPx	forward	GTGCCCTGCAACCAGTTT	134	AF281338.1
	reverse	TTCCCATTCACATCCATCTTC		
	probe	FAM- CCGTCCCGGAAATGGCTTTGA -TAMRA		
GST	forward	TGGCTGACGTTATTGTCTTCC	112	NM_001160559.1
	reverse	CTGGGTCTGTCCTTCACCATA		
	probe	FAM-CGGCGCGTTACCCCAAACTG -TAMRA		
HSP70	forward	GACATCAGCCAGAACAAGCG	136	NM_001124228.1
	reverse	TGGAGGTGTAGAAGTCGATGC		
	probe	FAM- TCCAGCTCCCAGGCCAGCATTGAG _TAMRA		
β-Actin	forward	TGGCCGTACCACCGGTAT	79	AF254414
	reverse	GCAGAGCGTAGTCCTCGTAGATG		
	probe	FAM-CTCCGGTGACGGCGTGACCC-TAMRA		

Statistical analysis

All experimental data analyses were performed by one-way analysis of variance (ANOVA) using the software package SPSS 14.0 for Windows. Duncan's multiple range test was applied if there were significant differences among the treatments means. All percentage data were arcsine transformed prior to being analysed statistically. Normality and homogeneity of variance were confirmed prior to analysis. Data was subjected to a Student–Newman–Keuls post-hoc test for homogenous subsets. Significance was set at $P<0.05$ following confirmation of normality and homogeneity of variance.

Results

The means of initial weight, final weight, weight gain, SGR, FCR and survival rate in the six dietary treatments are presented in Table 3. Final weight, weight gain, SGR and FCR were significantly affected by different lipid sources ($P<0.05$) while survival rate was not affected by dietary treatments.

Table 3. Growth and feed utilization of juvenile rainbow trout fed fish oil replaced diets

Growth performance	Experimental diets					
	CO	SBO	SSO	LO	SBO+LO	SSO+LO
Initial weight ±g	2.29±0.01	2.28±0.01	2.27±0.02	2.27±0.01	2.28±0.01	2.29±0.01
Final weight ±g	11.55±0.09 a	10.70±0.13 c	11.00±0.05 b	11.72±0.10 a	11.74±0.01 a	11.20±0.04 b
Weight gain ±%	402.66±4.90 ab	369.82±3.82 d	385.51±3.16 c	416.29±8.53 a	414.25±1.36 a	388.75±1.45 bc
SGR ±%	2.69±0.02 a	2.58±0.02 c	2.63±0.01 b	2.74±0.03 a	2.73±0.01 a	2.64±0.01 b
FCR	0.96±0.01 ab	0.95±0.03 ab	1.02±0.04 a	0.92±0.01 b	0.97±0.02 ab	0.97±0.04 ab
Survival ±%	90.00±1.44	90.83±0.83	90.83±0.83	91.67±0.83	90.83±0.83	91.66±1.66

a, b, c – values in rows with different letters differ significantly ($P\leq0.05$).

Growth hormone genes mRNA expression levels in rainbow trout muscle and liver are presented in Figure 1. GH-I mRNA expression level in muscle and liver was highest in fish fed LO and SBO+LO compared to other treatments ($P<0.05$). Fish fed diet SBO+LO had significantly higher IGF-I mRNA expression level in muscle and liver than the other experimental groups ($P<0.05$). IGF-II mRNA level expression in muscle was higher in fish fed SBO than other diets ($P<0.05$) while highest level in liver was obtained from fish fed SSO+LO ($P<0.05$).

There were no significant differences among treatments for TGF-β mRNA expression level in muscle and liver (Figure 2).

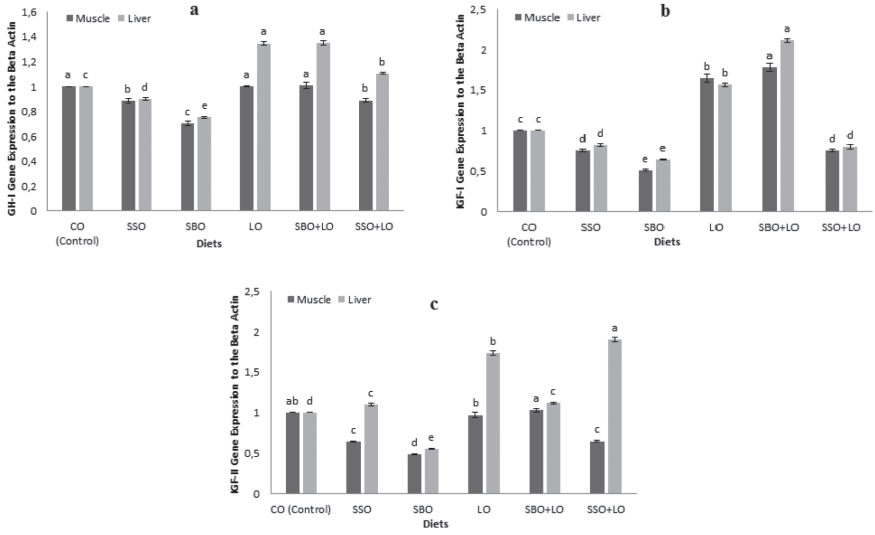

Figure 1. The mRNA expression levels of growth hormones in rainbow trout muscle and liver; a) GH-I b) IGF-I and c) IGF-II. The mRNA expression at 100% FO group was chosen as the calibrator and in other groups was represented as fold changes from the calibrator. Data are presented as means±SEM. Superscript letters indicate significant differences among treatments (P<0.05)

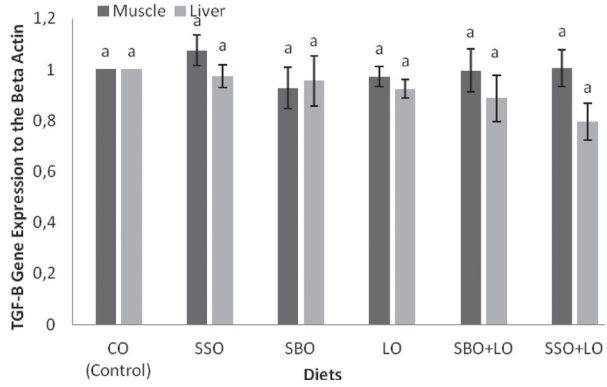

Figure 2. The mRNA expression levels of TGF-*β* in rainbow trout muscle and liver. TGF-*β* expression at 100% FO group was chosen as the calibrator and in other groups was represented as fold changes from the calibrator. Data are presented as means±SEM. Superscript letters indicate significant differences among treatments (P<0.05)

HSP70 mRNA expression level in muscle was higher in fish fed SSO (P<0.05) while the expression level of liver in SBO diets were significantly higher than the other groups (P<0.05) (Figure 3).

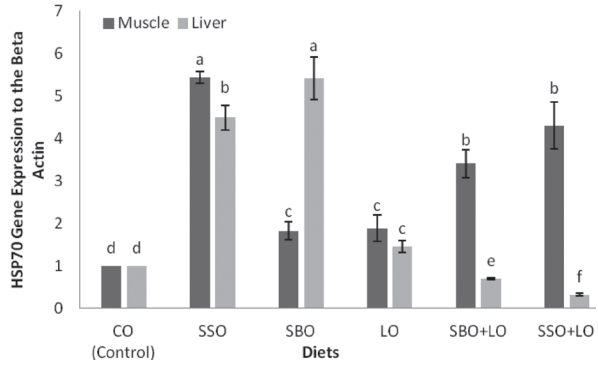

Figure 3. The mRNA expression levels of HSP70 in rainbow trout muscle and liver. HSP70 expression at 100% FO group was chosen as the calibrator and in other groups was represented as fold changes from the calibrator. Data are presented as means±SEM. Superscript letters indicate significant differences among treatments (P<0.05)

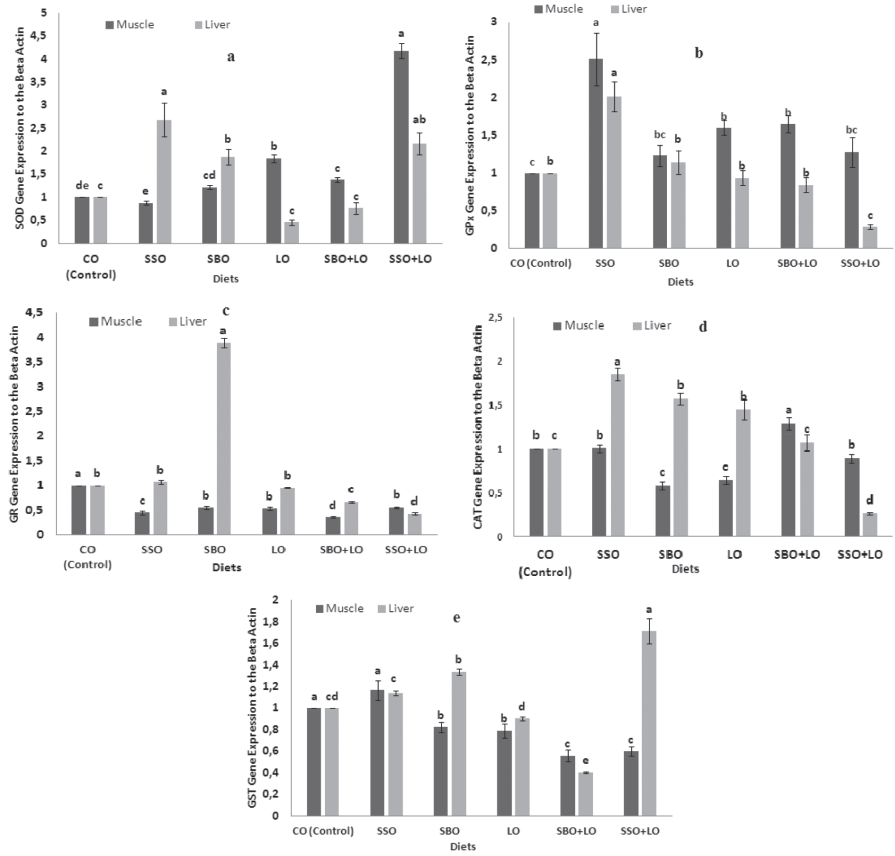

Figure 4. The mRNA expression levels of antioxidant enzymes in rainbow trout muscle and liver; a) SOD, b) GPx, c) GR, d) CAT and e) GST. The mRNA expression at 100% FO group was chosen as the calibrator and in other groups was represented as fold changes from the calibrator. Data are presented as means±SEM. Superscript letters indicate significant differences among treatments (P<0.05)

Antioxidant related genes mRNA expression levels in rainbow trout muscle and liver are given in Figure 4. SOD mRNA expression level in muscle was higher in fish fed SSO compared to the other treatments ($P<0.05$) while highest level in liver was obtained from fish fed SSO+LO ($P<0.05$). Fish fed diet SSO had significantly higher GPx mRNA expression level in muscle and liver compared to the other dietary treatments ($P<0.05$). GR mRNA expression level of muscle in group CO was higher compared to other groups ($P<0.05$) while highest level in liver was obtained from fish fed SBO ($P<0.05$). CAT mRNA expression level in muscle was higher in fish fed SBO+LO compared to the other treatments ($P<0.05$) while highest level in liver was obtained from fish fed SSO ($P<0.05$). GST mRNA expression level in muscle was higher in fish fed SSO compared to the other groups ($P<0.05$) while highest level in liver was obtained from fish fed SSO+LO ($P<0.05$).

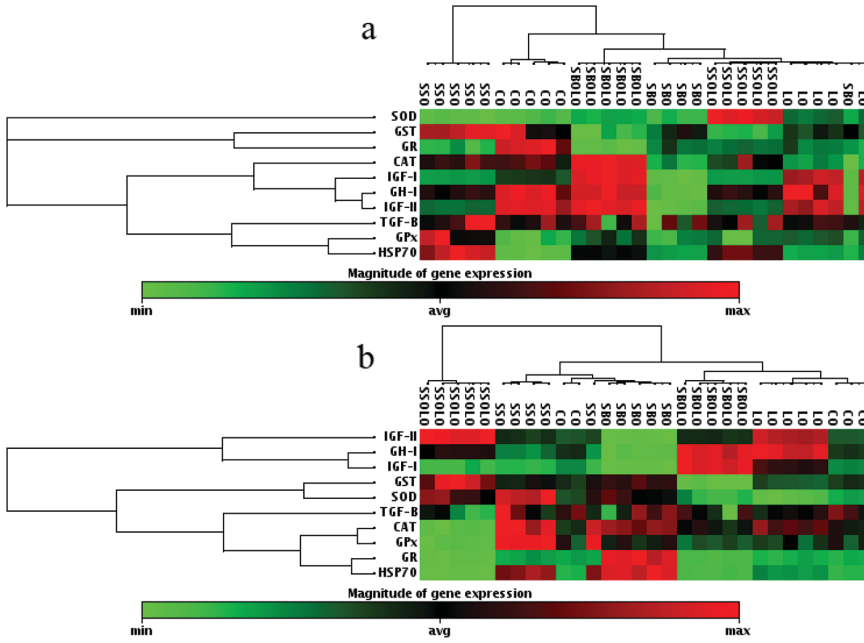

Figure 5. Unsupervised hierarchical clustering analysis of the genes in muscle (a) and liver (b) according to the similarity in their expression across different diets. Each row represents the expression pattern of a single gene across diets and each column corresponds to a single sample. Expression levels are represented by a colour tag, with red representing the highest levels and green the lowest levels of expression. Missing values are indicated by black squares

Hierarchical clustering of gene expression was used as a visualization tool to identify expression patterns among replicates. Genes were clustered vertically according to the similarity in their expression across different diets (Figure 5). In fast muscle, CAT was placed in a separate node relative to all of the antioxidant related genes, indicating a distinct expression pattern whereas the SOD, GST and GR were

clustered together. GH-I, IGF-I, IGF-II and CAT were clustered together. TGF-*ß*, GPx and HSP-70 were clustered together (Figure 4 a). In the liver, two main clusters were obtained: one with GH-I, IGF-I and IGF-II another containing with GST, SOD, TGF-ß, CAT, GPx, GR and HSP-70, in which TGF-*ß* occupied a superior hierarchical position (Figure 4 b).

Discussion

Fish oil (FO) has been used as the major source of lipid in aquaculture due to containing excessively high *n-3* essential fatty acids [the highly unsaturated fatty acids (HUFA) eicosapentaenoic (EPA, 20:5*n-3*) and docosahexaenoic (DHA, 22:6*n-3*) acids] thus far (Turchini and Francis, 2009; Tocher 2010, 2015; González-Félix et al., 2016). Interest has recently grown in the role and usage of new lipid resources due to increasing demand for global aquafeed production, the economic pressure, decreased production and increased prices of fish oil (FO) (Teoh et al., 2011; Han et al., 2015; González-Félix et al., 2016). Hence, trials using plant oils (PO) as alternative lipid sources have been conducted in aquatic animals on account of being cheap, abundant, and relatively stable (Sargent and Tacon, 1999; Bell et al., 2003; Richard et al., 2006; Gatlin et al., 2007; Webster et al., 2007; Bell and Waagbo, 2008; Panserat et al., 2009; Teoh et al., 2011; Bogevik et al., 2014; Moldal et al., 2014; Carmona-Osalde et al., 2015; Geay et al., 2015; Han et al., 2015; Betiku et al., 2016; Carbone and Faggio, 2016; Teoh and Ng, 2016; Azeredo et al., 2017; Brum et al., 2017; Caballero-Solares et al., 2017; Nayak et al., 2017). In addition, in recent years, researchers are interested to investigate hepatic gene expression variation related to either fish oil or fish meal replacement in salmonids due to being new molecular markers associated with the intake of the plant based feeds (Jordal et al., 2005; Panserat et al., 2008; Leaver et al., 2008; Panserat et al., 2009; Wacyk et al., 2012: Peng et al., 2014; Rolland et al., 2015; Mente et al., 2017). As far as the authors of this work are aware, there are no reports on the effect of different dietary plant oils on growth performance and expression of growth hormones, oxidative stress-related genes, heat shock protein 70 (HSP70) and immune response gene (TGF-ß) in liver and muscle of juvenile rainbow trout. In light of the above researches, we examined different dietary lipid sources on growth performance and expression levels of growth hormones, antioxidants, HSP70 and TGF-*ß* in juvenile rainbow trout. Overall, we fundamentally evaluated replacement of SBO with LO and SSO. In addition, diets were supplemented with LO for compensation of linoleic acid (LA;18:2n-6) deficiency. Briefly, the positive results were not obtained from SSO as alternative plant lipid source instead of SBO in terms of growth parameters unlike LO. Besides all these results, it was determined that increased growth rate likely correlated with enhanced growth hormone levels and reduced oxidative stress and HSP70.

The replacement of dietary FO with alternative plant oils has been studied in freshwater and marine organisms thus far (Sargent and Tacon, 1999; Bell et al., 2003; Turchini et al., 2003; Richard et al., 2006; Gatlin et al., 2007; Webster et al., 2007;

Bell and Waagbo, 2008; Panserat et al., 2009; Teoh et al., 2011; Emre et al., 2015; Han et al., 2015; González-Félix et al., 2016). In some studies, it was determined that growth performance in some fish species and invertebrates (sea bream *Sparus aurata*, rainbow trout *Oncorhynchus mykiss*, cobia *Rachycentron canadum*, meagre *Argyrosomus regius*, Senegalese sole *Solea senegalensis*, swimming crab *Portunus trituberculatus*) was not influenced by FO replacement with plant oils (Martinez-Llorens et al., 2007; Turchini et al., 2009; Trushenski et al., 2011; Sales and Glencross, 2011; Nasopoulou and Zabetakis, 2012; Benítez-Dorta et al., 2013; Emre et al., 2015). Consistent with previous studies, survival rate was not significantly affected by different lipid sources compared to the control diet. Contrary to findings by previous studies, our results showed that weight gain, SGR and survival rate were highest in diet containing LO compared to the control diet. This is because essentially fatty acids are synthesized from α-linolenic acid (ALA; 18:3*n-3*) in LO by fish.

The growth hormone (GH) is a pluripotent hormone secreted by the somatotrophs of the anterior pituitary gland in teleosts (Reinecke et al., 2005; Futawaka et al., 2016) and modulates growth by way of increased protein synthesis and decreased lipolysis in vertebrates (Biga and Meyer, 2009). In addition, GH plays a crucial role in physiological processes such as reproduction, skeletal and soft tissue, immune function, and the regulation of ionic and osmotic balance, metabolism of lipid, protein and carbohydrate in fish (Reinecke et al., 2005; Hoseinifar et al., 2017). The GH/IGF (growth hormone/insulin-like growth factor) system is organized by GH, GH receptor (GHR), IGF-I and IGF-II, IGF receptors (IGFRI and IGFRII), and IGF binding proteins (IGFBPs) (Gabillard et al., 2006). In particular, insulin-like growth factor-I (IGF-I) promotes growth in large part depending on nutrient availability (Duan, 1998; Moriyama et al., 2000; Leroith et al., 2001; Wood et al., 2005; Terova et al., 2007; Fox et al., 2010). The production of IGF-I and activation of the Janus kinase (JAK)/signal transduction and activator of transcription (STAT) pathway are induced by the binding of GH to GH receptors (GHR) on the hepatocyte membrane (Futawaka et al., 2016). Insulin-like growth factor-II (IGF-II) is indicated to show a high structural homology with IGF-I (Vong et al., 2003; Terova et al., 2007; Fox et al., 2010) and extensively expressed in juvenile and adult fish (Reinecke et al., 2005; Fox et al., 2010). IGFs stimulate growth, myocyte hypertrophy during muscle regeneration and, proliferation and differentiation of muscle precursor cells (myoblasts or satellite cells) (Florini et al., 1996; Musaro et al., 1999; Terova et al., 2007). There is a need to examine effect of dietary lipid sources on expression of the GH/IGF system because nutrient considerably affects the GH/IGF system being complex regulation network (Gabillard et al., 2006; Safari et al., 2016, 2017). Consistent with result of the growth parameters, GH-I, and IGF-I gene expression levels of muscle and liver in group SBO+LO were higher compared to other dietary treatments in present study.

The main responsible enzymes [catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione S-transferase (GST) and glutathione peroxidase (GPx)] protect the biological systems against ROS-mediated damage (Cabrita et al., 2014; Saddick et al., 2015). Feeding is important in terms of maintaining the pro-oxidant-antioxidant balance (Covey, 1986; Martinez-Alvarez et al., 2005; Alu-

wong et al., 2013). Therefore, studies about the role of dietary nutrients or additives on the antioxidant enzyme activities in animals have been increased recently. In addition, as mentioned previously, antioxidant enzyme mRNA expression levels have been increased by dietary fish oil while free radical-induced tissue damage decreased (Fernandes et al., 1996; De Pablo and De Cienfuegos, 2000). Furthermore, the oxidation is reduced by low levels of lipid in diets and the growth rate increase in fish (Rueda-Jasso et al., 2004). Hence, gene expression of the main enzymatic antioxidants was assessed in present study. Our results indicated that CAT, SOD, GST, GR and GPx gene expression levels varied in muscle and liver. Interestingly, SOD, GPx, CAT and GST gene expression levels were significantly increased by SSO and diets containing SSO. In contrast, CAT, SOD, GST, GR and GPx gene expression levels were highly reduced in fish fed LO or diets including LO. In parallel with these results, the best results were obtained from LO in terms of growth parameters. The polyunsaturated fatty acids (PUFA) are essential for fish development and classified as *n-3* long chain polyunsaturated fatty acids (*n-3* LC-PUFA) and *n-6* long chain polyunsaturated fatty acids (*n-6* LC-PUFA). 18:*n-6* (ALA) and 20:4*n-6* (ARA) are most important fatty acids of *n-6* LC-PUFA. SSO and SBO used in this study are rich in 18:2*n-6*. However, these fatty acids are not the substrate for synthesis of EPA and DHA, which are incorporated into membrane structure. Moreover, *n-6* fatty acids are more susceptible to lipid peroxidation compared to *n-3* fatty acids and oxidized rapidly (Lands et al., 1973; Culp et al., 1979; Venkatraman et al., 1994). 18:3*n-3* (ALA) is the most important fatty acid of *n-3* LC-PUFA. The fatty acid is the substrate for synthesis of EPA and DHA for fish. In present study, LO has lipid source containing *n-3* long chain polyunsaturated fatty acids. For these reasons, SSO or SBO were supplied with LO for providing *n-3* long chain polyunsaturated fatty acids.

Heat shock proteins (Hsps) are important due to involvement in the folding and translocation of newly synthesized proteins and repair of damaged proteins denatured after stress (Kalmar and Greensmith, 2009; Tovar-Ramírez et al., 2010; Wang et al., 2016). HSP70 is an environmentally inducible heat shock protein (HSP), and moves as a chaperone in modulation of normal protein function (Santacruz et al., 1997). HSP70 is one of biomarkers for cellular stress and revealing the effect of dietary treatments and, provides maintenance of cellular homeostasis (Iwama et al., 1998; Ravagnan et al., 2001; Kim et al., 2005; Tovar-Ramírez et al., 2010; Sankar Giri et al., 2016). The expression of stress proteins (HSP70) is stimulated by environmental and physiological stresses and synthesised in response to stresses (Pelham, 1986; Voznesensky et al., 2004; Rajeshkumar et al., 2013). In this study, the variations were observed in HSP70 mRNA expression level at different tissues. HSP70 mRNA expression level in muscle was lower in fish fed CO while lower levels in liver were obtained from fish fed LO or diets including LO. The reduction of HSP70 gene expression in the rainbow trout following administration of SSO+LO and SBO+LO is possibly due to containing 18-3*n-3* (ALA) of LO and resistance to common unwanted stresses increased during rearing period (Yarahmadi et al., 2014). The results were similar with antioxidants mRNA expression levels in present study.

Immune system is most important for fish in terms of protection against pathogens (Whyte, 2007) and influenced by nutrition and feed additives (Yarahmadi et al.,

2014; Hoseinifar et al., 2015, 2016; Carbone and Faggio, 2016; Wang et al., 2016). Growth rate is influenced by immune system and disease resistance (Tocher et al., 2001, 2006; Fonseca-Madrigal et al., 2005; Jordal et al., 2005; Zheng et al., 2005; Li et al., 2007; Jaya-Ram et al., 2008; Zheng et al., 2009; Geay et al., 2010; Navarro-Guillen et al., 2014; Geay et al., 2015; Chen et al., 2015). As documented in previous studies, it has been suggested that gene expression can be altered by dietary components (Fonseca-Madrigal et al., 2005; Jordal et al., 2005; Zheng et al., 2005; Tocher et al., 2001, 2006; Jaya-Ram et al., 2008; Zdunczyk and Pareek, 2009; Geay et al., 2010; Navarro-Guillen et al., 2014; Geay et al., 2015). TGF-*β* is one of anti-inflammatory cytokines and important for improvement of cellular defences in terms of enhancement of disease resistance (Whyte, 2007; Chen et al., 2015). In addition to all these growth factors, the transforming growth factor-β (TGF-*β*) family is comprised of dimeric proteins modulating growth, differentiation and metabolism of many cell types (Funkenstein et al., 2010). TGF-*β*s are crucial regulators of vertebrate muscle growth (Funkenstein et al., 2010). Furthermore, TGF-β has a key role in the control of liver structure and liver disease progression is inhibited by the TGF-*β* signalling pathway (Fabregat et al., 2014, 2016). Therefore, TGF-β gene expression level was measured in muscle and liver. Our results indicated that TGF-*β* mRNA expression fluctuated with different lipid sources. In contrast to results of growth parameters, expression of oxidative stress-related genes and growth hormone related genes, dietary SSO supplementation insignificantly increased expression of TGF-*β* mRNA.

Conclusions

In conclusion, the current study reveals that LO has an inhibitory effect on oxidative stress-related genes. Highest growth rate was obtained from LO. Results of growth parameters comply with oxidative stress-related genes, growth hormone-related genes and HSP70. Our results support the results of previous studies about using a transcriptomic approach for explaining reasons of lower or higher growth. Additionally, more investigations in these areas would validate the significance of using different varieties of alternative oil resources ingredients with respect to immune function gene expression in fish.

Acknowledgment

Research was supported by a grant from Centre of Scientific Research Project of Atatürk University (Project Numbers: BAP-2012-247 and BAP-2015-160). Therefore, the authors would like to acknowledge Atatürk University for supporting this project.

References

Aksakal E., Ceyhun S.B., Erdoğan O., Ekinci D. (2010). Acute and long term genotoxicity of deltamethrin to insulin-like growth factors and growth hormone in rainbow trout. Comp. Biochem. Phys. C, 152: 451–455.

Aluwong T., Kawu M., Raji M., Dzenda T., Govwang F., Sinkalu V., Ayo J. (2013). Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens. Antioxidants, 2: 326–339.

Azeredo R., Machado M., Kreuz E., Wuertz S., Oliva-Teles A., Enes P., Costas B. (2017). The European seabass (*Dicentrarchus labrax*) innate immunity and gut health are modulated by dietary plant-protein inclusion and prebiotic supplementation. Fish Shellfish Immunol., 60: 78–87.

Bell J.G., Waagbo R. (2008). Safe and nutritious aquaculture produce: benefits and risks of alternative sustainable aquafeeds. In: Aquaculture in the Ecosystem, Holmer M., Black K., Duarte C., Marba N., Karakassis I. (eds). Kluwer Academic Publishers Group, pp. 185–225.

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

Benítez-Dorta V., Caballero M., Izquierdo M., Manchado M., Infante C., Zamorano M., Montero D. (2013). Total substitution of fish oil by vegetable oils in Senegalese sole (*Solea senegalensis*) diets: effects on fish performance, biochemical composition, and expression of some glucocorticoid receptor related genes. Fish Physiol. Biochem., 39: 335–349.

Betiku O.C, Barrows F.T., Ross C., Sealey W.M. (2016). The effect of total replacement of fish oil with DHA-Gold® and plant oils on growth and fillet quality of rainbow trout (*Oncorhynchus mykiss*) fed a plant-based diet. Aquacult. Nutr., 22: 158–169.

Biga P.R., Meyer J. (2009). Growth hormone differentially regulates growth and growth-related gene expression in closely related fish species. Comp. Biochem. Physiol. Part A, 154: 465–473.

Bogevik A.S., Rathore R.M., Arjona Y., Atack T., Treasurer J., Rønnestad I., Kousoulaki K. (2014). Dietary plant oils delay early sexual maturation compared with marine fish oil in male European seabass (*Dicentrarchus labrax*) – Effects on testis histology and key reproductive hormones. Aquaculture, 431: 73–84.

Brum A., Pereira S.A., Owatari M.S., Chagas E.C., Chaves F.C.M., Mouriño J.L.P., Martins M.L. (2017). Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. Aquaculture, 468: 235–243.

Caballero-Solares A., Hall J.R., Xue X., Eslamloo K., Taylor R.G., Parrish C.C., Rise M.L. (2017). The dietary replacement of marine ingredients by terrestrial animal and plant alternatives modulates the antiviral immune response of Atlantic salmon (*Salmo salar*). Fish Shellfish Immunol., 64: 24–38.

Cabrita E., Martínez-Páramo S., Gavaia P.J., Riesco M.F., Valcarce D.G., Sarasquete C., Herráez M.P., Robles V. (2014). Factors enhancing fish sperm quality and emerging tools for sperm analysis. Aquaculture, 432: 389–401.

Carbone D., Faggio C. (2016). Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of *Sparus aurata* and *Dicentrarchus labrax*. Fish Shellfish Immunol., 54: 172–178.

Carmona-Osalde C., Rodríguez-Serna M., Hernández-Moreno H., Arredondo-Figueroa J.L. (2015). Total and partial substitution of dietary fish oil with palm oil to juvenile crayfish. *Procambarus llamasi*. J. Aquac. Res. Develop., 6: 367.

Chen N., Jin L., Zhou H., Qiu X. (2012). Effects of dietary arginine levels and carbohydrate-to-lipid ratios on mRNA expression of growth-related hormones in largemouth bass, *Micropterus salmoides*. Gen. Comp. Endocr., 179: 121–127.

Chen G., Liu Y., Jiang J., Jiang W., Kuang S., Tang L., Tang W., Zhang Y., Zhou X., Feng L. (2015). Effect of dietary arginine on the immune response and gene expression in head kidney and spleen following infection of Jian carp with *Aeromonas hydrophila*. Fish Shellfish Immunol., 44: 195–202.

Cowey C.B. (1986). The role of nutritional factors in the prevention of peroxidative damage to tissues. Fish Physiol. Biochem., 2: 171–178.

Culp B.R., Titus B.R., Lands W.E.M. (1979). Inhibition of prostaglandin biosynthesis by eicosapentaenoic acid. Prostagl. Med., 3: 269–278.

De Pablo M.A., De Cienfuegos G.Á. (2000). Modulatory effects of dietary lipids on immune system functions. Immunol. Cell Biol., 78: 31–39.

Duan C. (1998). Nutritional and developmental regulation of insulin-like growth factors in fish. J. Nutr., 128: 306–314.

Emre Y., Kurtoğlu A., Emre N., Güroy B., Güroy D. (2015). Effect of replacing dietary fish oil with soybean oil on growth performance, fatty acid composition and hematological parameters of juvenile meagre *Argyrosomus regius*. Aquac. Res., 47: 2256–2265.

Fabregat I., Fernando J., Mainez J., Sancho P. (2014). TGF-beta signaling in cancer treatment. Curr. Pharm. Des., 20: 2934–2947.

Fabregat I., Moreno-Càceres J., Sánchez A., Dooley S., Dewidar B., Giannelli G., ten Dijke P., the IT-LIVER Consortium. (2016). TGF-β signalling and liver disease. FEBS J., 283: 2219–2232.

Fernandes G., Chandrasekar B., Luan X., Troyer D.A. (1996). Modulation of antioxidant enzymes and programmed cell death by *n-3* fatty acids. Lipids, 31: 91–96.

Florini J.R., Ewton D.Z., Coolican S.A. (1996) Growth hormone and the insulin like growth factor system in myogenesis. Endocr. Rev., 17: 481–517.

Fonseca-Madrigal J., Karalazos V., Campbell P., Bell J.G., Tocher D.R. (2005) Influence of dietary palm oil on growth, tissue fatty acid compositions, and fatty acid metabolism in liver and intestine in rainbow trout (*Oncorhynchus mykiss*). Aquac. Nutr., 11: 241–250.

Fox B.K., Breves J.P., Davis L.K., Pierce A.L., Hirano T., Grau E.G. (2010). Tissue-specific regulation of the growth hormone/insulin-like growth factor axis during fasting and re-feeding: Importance of muscle expression of IGF-I and IGF-II mRNA in the tilapia. Gen. Comp. Endocr., 166: 573–580.

Funkenstein B., Olekh E., Jakowlew S.B. (2010). Identification of a novel transforming growth factor-β (TGF-β6) gene in fish: regulation in skeletal muscle by nutritional state. BMC Mol. Biol., 11: 37.

Futawaka K., Tagami T., Fukuda Y., Koyama R., Nushida A., Nezu S., Imamoto M., Kasahara M., Moriyama K. (2016). Growth hormone regulates the expression of UCP2 in myocytes. Growth Horm. IGF Res., 29: 57–62.

Gabillard J.C., Kamangar B.B., Nuria M. (2006). Coordinated regulation of the GH/IGF system genes during refeeding in rainbow trout (*Oncorhynchus mykiss*). J. Endocr., 191: 15–24.

Gatlin III D.M., Barrows F.T., Brown P. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res., 38: 551–579.

Geay F., Santigosa I., Culi E., Corporeau C., Boudry P., Dreano Y., Corcos L. (2010). Regulation of FADS2 expression and activity in European sea bass (*Dicentrarchus labrax*, L.) fed a vegetable diet. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 156: 237–243.

Geay F., Mellery J., Tinti E., Douxfils J., Larondelle Y., Mandiki S.N.M., Kestemont P. (2015). Effects of dietary linseed oil on innate immune system of Eurasian perch and disease resistance after exposure to *Aeromonas salmonicida achromogen*. Fish Shellfish Immunol., 47: 782–796.

González-Félix M.L., Maldonado-Othón C.A., Perez-Velazquez M. (2016). Effect of dietary lipid level and replacement of fish oil by soybean oil in compound feeds for the shortfin corvina (*Cynoscion parvipinnis*). Aquaculture, 454: 217–228.

Han T., Wang J., Hu S., Li X., Jiang Y., Wang C. (2015). Effects of different dietary lipid sources on growth performance and tissue fatty acid composition of juvenile swimming crab *Portunus trituberculatus*. Chin. J. Oceanol. Limnol., 33: 957–965.

Hoseinifar S.H., Esteban M.Á., Cuesta A., Sun Y.Z. (2015). Prebiotics and fish immune response: A review of current knowledge and future perspectives. Rev. Fish. Sci. Aquacult., 23: 315–328.

Hoseinifar S.H., Ringø E., Shenavar Masouleh A., Esteban M.Á. (2016). Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. Rev. Aquacult., 8: 89–102.

Hoseinifar S.H., Safari R., Dadar M. (2017) Dietary sodium propionate affects mucosal immune parameters, growth and appetite related genes expression: Insights from zebrafish model. Gen. Comp. Endocrinol., 243: 78–83.

Iwama G.K., Thomas P., Vijayan M.M., Forsyth R. (1998). Stress protein expression in fish. Fish Biol. Fish., 8: 35–56.

Jaya-Ram A., Kuah M.K., Lim P.S., Kolkovski S., Shu-Chien A.C. (2008). Influence of dietary HUFA levels on reproductive performance, tissue fatty acid profile and desaturase and elongase mRNAs expression in female zebrafish *Danio rerio*. Aquaculture, 277: 275–281.

Johansen K.A., Overturf K. (2005). Quantitative expression analysis of genes affecting muscle growth during development of rainbow trout (*Oncorhynchus mykiss*) Mar. Biotechnol., 7: 576–587.
Jordal A.O., Torstensen B.E., Tsoi S., Tocher D.R., Lall S.P., Douglas S.E. (2005). Dietary rapeseed oil affects the expression of genes involved in hepatic lipid metabolism in Atlantic salmon (*Salmo salar*). J. Nutr., 135: 2355–2361.
Kalmar B., Greensmith L. (2009). Induction of heat shock proteins for protection against oxidative stress. Adv. Drug Deliv. Rev., 61: 310–318.
Kim H.P., Wang X., Zhang J., Suh G.Y., Benjamin I.J., Ryter S.W., Choi A.M. (2005). Heat shock protein-70 mediates the cytoprotective effect of carbon monoxide: involvement of p38 beta MAPK and heat shock factor-1. J. Immunol., 175: 2622–2629.
Kocabas M., Kayim M., Can E., Ateş M., Kutluyer F., Aksu Ö. (2011). Spotting pattern features in the brown trout (*Salmo trutta macrostigma*, T., 1954) population, Sci. Res. Ess., 6: 5021–5024.
Lands W.E.M., LeTellier P.R., Rome L.H., Vanderhoek J.Y. (1973). Inhibition of prostaglandin synthesis. Adv. Biosci., 9: 15–27.
Leaver M.J., Villeneuve L.A.N., Obach A., Jensen L., Bron J.E., Tocher D.R., Taggart J.B. (2008). Functional genomics reveals increases in cholesterol biosynthetic genes and highly unsaturated fatty acid biosynthesis after dietary substitution of fish oil with vegetables oils in Atlantic salmon (*Salmo salar*). BMC Genomics, 9: 299.
Leroith D., Bondy C., Yakar S., Liu J.L., Butler A. (2001). The somatomedin hypothesis: 2001. Endocr. Rev., 22: 53–74.
Li P., Yin Y.L., Li D.F., Kim S.W., Wu G. (2007). Amino acids and immune function. Br. J. Nutr., 98: 237–252.
Martınez-Alvarez R.M., Morales A.E., Sanz A. (2005). Antioxidant defenses in fish: Biotic and abiotic factors. Rev. Fish Biol. Fish., 15: 75–88.
Martinez-Llorens S., Vidal A.T., Moñino A.V., Torres M.P., Cerdá M.J. (2007). Effects of dietary soybean oil concentration on growth, nutrient utilization and muscle fatty acid composition of gilthead sea bream (*Sparus aurata* L.). Aquac. Res., 38: 76–81.
Mente E., Pierce G.J., Antonopoulou E., Stead D., Martin S.A.M. (2017). Postprandial hepatic protein expression in trout *Oncorhynchus mykiss* a proteomics examination. Biochem. Biophys. Rep., 9: 79–85.
Moldal T., Løkka G., Wiik-Nielsen J., Austbø L., Torstensen B.E, Rosenlund G., Dale O.B., Kaldhusdal M., Koppang E.O. (2014). Substitution of dietary fish oil with plant oils is associated with shortened mid intestinal folds in Atlantic salmon (*Salmo salar*). BMC Vet. Res., 10: 60.
Montero D., Kalinowski T., Obach A., Robaina L., Tort L., Caballero M.J., Izquierdo M.S. (2003). Vegetable lipid source for gilthead seabream (*Sparus aurata*): effects on fish health. Aquaculture, 225: 353–370.
Moriyama S., Ayson F.G., Kawauchi H. (2000). Growth regulation by insulin-like growth factor-I in fish. Biosci. Biotechnol. Biochem., 64: 1553–1562.
Musaro A., McCullagh K.J., Naya F.J., Olson E.N., Rosenthal N. (1999). IGF-1 induces skeletal myocyte hypertrophy through calcineurin in association with GATA-2 and NF-ATcl. Nature., 400: 581–585.
Nasopoulou C., Zabetakis I. (2012). Benefits of fish oil replacement by plant originated oils in compounded fish feeds. A review. Food Sci. Technol., 47: 217–244.
Navarro-Guillen C., Engrola S., Castanheira F., Bandarra N., Hachero-Cruzado I., Tocher D.R. (2014). Effect of varying dietary levels of LC-PUFA and vegetable oil sources on performance and fatty acids of Senegalese sole post larvae: puzzling results suggest complete biosynthesis pathway from C18 PUFA to DHA. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 167: 51–58.
Nayak M., Saha A., Pradhan A., Samanta M., Giri S.S. (2017). Dietary fish oil replacement by linseed oil: Effect on growth, nutrient utilization, tissue fatty acid composition and desaturase gene expression in silver barb (*Puntius gonionotus*) fingerlings. Comp. Biochem. Physiol. Part B, 205: 1–12.
Panserat S., Kolditz C., Richard N., Plagnes-Juan E., Piumi F., Esquerré D., Mé-

dale F., Corrze G., Kaushik S. (2008). Hepatic gene expression profiles in rainbow trout (*Oncorhynchus mykiss*) fed fish meal or fish oil-free diets. Br. J. Nutr., 100: 953–967.

Panserat S., Hortopan G.A., Plagnes-Juan E., Kolditz C., Lansard M., Skiba--Cassy S., Esquerré D., Geurden I., Médale F., Kaushik S., Corraze G. (2009). Differential gene expression after total replacement of dietary fish meal and fish oil by plant products in rainbow trout (*Oncorhynchus mykiss*) liver. Aquaculture, 294: 123–131.

Pelham H.R.B. (1986). Speculations on the functions of the major heat shock and glucose-regulated proteins. Cell, 46: 959–961.

Peng M., Xu W., Mai K., Zhou H., Zhang Y., Liufu Z., Zhang K., Ai Q. (2014). Growth performance, lipid deposition and hepatic lipid metabolism related gene expression in juvenile turbot (*Scophthalmus maximus* L.) fed diets with various fish oil substitution levels by soybean oil. Aquaculture, 433: 442–449.

Pfaffl M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res., 29: 2003–2007.

Rajeshkumar S., Mini J., Munuswamy N. (2013). Effects of heavy metals on antioxidants and expression of HSP70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island, Chennai, India. Ecotox. Environ. Safe., 98: 8–18.

Ravagnan L., Gurbuxani S., Susin S.A., Maisse C., Daugas E., Zamzami N., Mak T., Jaattela M., Penninger J.M., Garrido C., Kroemer G. (2001). Heat-shock protein 70 antagonizes apoptosis-inducing factor. Nat. Cell. Biol., 3: 839–843.

Reinecke M., Björnsson B.T., Dickhoff W.W., McCormick S.D., Navarro I., Power D.M., Gutiérrez J. (2005). Growth hormone and insulin-like growth factors in fish: Where we are and where to go. Gen. Comp. Endocr., 142: 20–24.

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

Rolland M., Dalsgaard J., Holm J., Gómez-Requeni P., Skov P.V. (2015). Dietary methionine level affects growth performance and hepatic gene expression of GH–IGF system and protein turnover regulators in rainbow trout (*Oncorhynchus mykiss*) fed plant protein-based diets. Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol., 181: 33–41.

Rueda-Jasso R., Conceicao L.E.C., Dias J., De Coen W., Gomes E., Rees J.F., Soares F., Dinis M.T., Sorgeloos P. (2004). Effect of dietary non-protein energy levels on condition and oxidative status of Senegalese sole (*Solea senegalensis*) juveniles. Aquaculture, 231: 417–433.

Saddick S., Afifi M., Abu Zinada O.A. (2015). Effect of Zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zillii*. Saudi J. Biol. Sci., doi: 10.1016/j.sjbs.2015.10.021

Safari R., Hoseinifar S.H., Nejadmoghadam S., Jafar A. (2016). Transciptomic study of mucosal immune, antioxidant and growth related genes and non-specific immune response of common carp (*Cyprinus carpio*) fed dietary Ferula (*Ferula assafoetida*). Fish Shellfish Immunol., 55: 242–248.

Safari R., Hoseinifar S.H., Nejadmoghadam S., Khalili M. (2017). Non-specific immune parameters, immune, antioxidant and growth-related genes expression of common carp (*Cyprinus carpio* L.) fed sodium propionate. Aquac. Res., doi:10.1111/are.13272.

Sales J., Glencross B. (2011). A meta-analysis of the effects of dietary marine oil replacement with vegetable oils on growth, feed conversion and muscle fatty acid composition of fish species. Aquac. Nutr., 17: 271–287.

Sankar Giri S., Woo Jun J., Sukumaran V., Chang Park S. (2016). Dietary administration of banana (*Musa acuminata*) peelflour affects the growth, antioxidant status, cytokineresponses, and disease susceptibility of rohu, *Labeo rohita*. J. Immunol. Res., 2016: 1–11.

Santacruz H., Vriz S., Angelier N. (1997). Molecular characterization of a heat shock cognate cDNA of zeabrafish, hsc70, and developmental expression of the corresponding transcripts. Dev. Comp. Immunol., 21: 223–233.

Sargent J.R., Tacon A.G.J. (1999). Development of farmed fish: a nutritionally necessary alternative to meat. Proc. Nutr. Soc., 58: 377–383.

Teoh C.Y., Ng W.K. (2016). The implications of substituting dietary fish oil with vegetable oils on the growth performance, fillet fatty acid profile and modulation of the fatty acid elongase, desaturase and oxidation activities of red hybrid tilapia, *Oreochromis* sp. Aquaculture, 465: 311–322.

Teoh C.Y., Turchini G.M., Ng W.K. (2011). Erratum to "Genetically improved farmed Nile tilapia and red hybrid tilapia showed differences in fatty acid metabolism when fed diets with added fish oil or a vegetable oil blend. Aquaculture, 316: 144–154.

Terova G., Rimoldi S., Chini V., Gornati R., Bernardini G., Saroglia M. (2007). Cloning and expression analysis of insulin-like growth factor I and II in liver and muscle of sea bass (*Dicentrarchus labrax*, L.) during long-term fasting and refeeding. J. Fish Biol., 70(Supplement B): 219–233.

Tocher D.R. (2010). Fatty acid requirement in ontogeny of marine and freshwater fish. Aquac. Res., 41: 717–732.

Tocher D.R. (2015). Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture, 449: 94–107.

Tocher D.R., Agaba M., Hastings N., Bell J.G., Dick J.R., Teale A.J. (2001). Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio)* and tilapia (*Oreochromis niloticus*), Fish Physiol. Biochem., 24: 309–320.

Tocher D.R., Bell J.G., MacGlaughlin P., McGhee F., Dick J.R., (2001). Hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition of liver in salmonids: effects of dietary vegetable oil, Comp. Biochem. Physiol. B Biochem. Mol. Biol., 137: 257–270.

Tocher D.R., Zheng X., Schlechtriem C., Hastings N., Dick J.R., Teale A.J. (2006). Highly unsaturated fatty acid synthesis in marine fish: cloning, functional characterization, and nutritional regulation of fatty acyl delta 6 desaturase of Atlantic cod (*Gadus morhua* L.). Lipids, 41: 1003–1016.

Tovar-Ramírez D., Mazuraisa D., Gatesoupe J.F., Quazuguel P., Cahu C.L., Zambonino-Infantea J.L. (2010). Dietary probiotic live yeast modulates antioxidant enzyme activities and gene expression of sea bass (*Dicentrarchus labrax*) larvae. Aquaculture, 300: 142–147.

Trushenski J.T., Schwarz M., Lewis H., Laporte J., Delbos B., Takeuchi R., Sampaio L.A. (2011). Effect of replacing dietary fish oil with soybean oil on production performance and fillet lipid and fatty acid composition of juvenile cobia *Rachycentron canadum*. Aquac. Nutr., 17: 437–447.

Turchini G.M., Francis D.S. (2009). Fatty acid metabolism (desaturation, elongation and β-oxidation) in rainbow trout fed fish oil- or linseed oil-based diets. Br. J. Nutr., 102: 69–81.

Turchini G.M., Mentasti T., Frøyland L., Orban E., Caprino F., Moretti V.M., Valfré F. (2003). Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo salar*). Aquaculture, 225: 251–267.

Venkatraman J.T., Chandrasekar B., Kim J.D., Femandes G. (1994). Effects of *n-3* and *n-6* fatty acids on the activities and expression of hepatic antioxidant enzymes in autoimmune-prone NZB´NZW F_1 mice. Lipids, 29: 561–568.

Vong Q.P., Chan K.M., Cheng C.H. (2003). Quantification of common carp (*Cyprinus carpio*) IGF-I and IGF-II mRNA by real-time PCR: differential regulation of expression by GH. J. Endocrinol., 178: 513–521.

Voznesensky M., Lerz D.M., Spanings-Pierrot C., Towle D.H. (2004). Genomic approaches for detecting thermal stress in *Calanus finmarchicus* (Copepoda: Calanoida). J. Exp. Mar. Biol. Ecol., 311: 37–46.

Yarahmadi P., Miandare H.K., Farahmand H., Mirvaghefi A., Hoseinifar S.H. (2014). Dietary fermentable fiber upregulated immune related genes expression, increased innate immune response and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas hydrophila*. Fish Shellfish Immunol., 41: 326–331.

Wacyk J., Powell M., Rodnick K., Overturf K., Hill R.A., Hardy R. (2012). Dietary protein source significantly alters growth performance, plasma variables and hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) fed amino acid balanced diets. Aquaculture, 356–357: 223–234.

Wang W., Sun J., Liu C., Xue Z. (2016). Application of immunostimulants in aquaculture: current knowledge and future perspectives. Aquacult. Res., 48: 1–23.

Wang L., Xu Q., Wang C., Li J., Chen D., Zhao Z., Luo L., Du X. (2016). Effects of dietary α-ketoglutarate supplementation on the antioxidant defense system and HSP 70 and HSP 90 gene expression of hybrid sturgeon *Acipenser schrenckii*♀ × *A. baerii*♂ exposed to ammonia-N stress. Aquacult. Res., 2016: 1–12.

Webster C., Lim C., Lee S.C. (2007). Use of alternative protein sources in aquaculture diets. The Haworth Press, Inc. NY, USA, 626 pp.

Wood A.W., Duan C., Bern H.A. (2005). Insulin-like growth factor signaling in fish. Int. Rev. Cytol., 243: 215–285.

Whyte S.K. (2007). The innate immune response of finfish – a review of current knowledge. Fish Shellfish Immunol., 23: 1127–1151.

Zdunczyk Z., Pareek C.S. (2009). Application of nutrigenomics tools in animal feeding and nutritional research. J. Anim. Feed Sci., 18: 13–16.

Zheng X., Tortensen B.E., Tocher D.R., Dick J.R., Henderson R.J., Bell J.G. (2005). Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo salar*). Biochim. Biophys. Acta, 1734: 13–24.

Zheng X., Leaver M.J., Tocher D.R. (2009) Long-chain polyunsaturated fatty acid synthesis in fish: comparative analysis of Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua* L.) Delta6 fatty acyl desaturase gene promoters. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 154: 255–263.

Received: 10 II 2017
Accepted: 26 V 2017