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Growth performance, antioxidant and immune status of Siberian sturgeon (*Acipenser baeri*) fed diets containing green tea extract and oxidized fish oil

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Summary

The present study was conducted to determine the effects of dietary oxidized fish oil (OFO) and green tea extract (GTE) on growth performance, antioxidant status and immunological parameters in Siberian sturgeon (*Acipenser baeri*). Fish weighing about 108 g were randomly assigned to one of nine dietary treatments (control diet without OFO and GTE; diets containing only 25 or 50 mg kg⁻¹ of GTE; diets containing 50 mg kg⁻¹ of OFO combined with 0, 25 or 50 mg kg⁻¹ of GTE; diets containing 100 mg kg⁻¹ of OFO combined with 0, 25 or 50 mg kg⁻¹ of GTE) with three replicates of 15 fish stocked in fiberglass tanks using a completely randomized design. Growth performance indices, immunological parameters and antioxidant status of all treatment groups were determined using appropriate assays and compared. Results showed that GTE could enhance growth performance. Feeding GTE alone or combined with the OFO could decrease the hepatosomatic index (HSI) and viscerosomatic index (VSI). Serum lysozyme activity and antibody level were increased significantly by GTE alone or combined with OFO compared to the oxidized diets ($P<0.05$). Conversely, lipid peroxidation products and antioxidant enzyme activity were significantly lower in GTE treatment groups and those that received combination of GTE and OFO compared to the oxidized diets. Low-density lipoprotein (LDL) level in GTE groups was lower than other treatment groups ($P<0.05$) however, in OFO treatment groups the LDL level in fish serum was significantly increased in comparison with the other treatment groups ($P<0.05$). GTE and OFO alone or combined together significantly decreased the levels of triglyceride and cholesterol compared to the control group ($P<0.05$). In conclusion, immunological and biochemical parameters were improved by inclusion of GTE into Siberian sturgeon rancid diets which can protect fish from the oxidative damage challenged by dietary oil oxidation.

KEY WORDS: Siberian sturgeon, immunity, antioxidant activity, fish oil, green tea.

Introduction

Overexploitation of natural sturgeon stocks for eggs (caviar) along with habitat destruction has resulted in the decline of sturgeons in the Caspian Sea to a level where some are at risk of extinction in the near future (Bronzi et al. 2011). Consequently, the Iranian government along with the other neighboring countries have attempted to increase the stocks of sturgeons by artificial reproduction and release of produced fingerlings into the rivers flowing into the Caspian Sea (Bartley and Rana 1998). In addition, sturgeon fish farming has been encouraged, which might result in lower fishing pressure and restoring the wild stocks (Hasanalipour et al. 2013).

Siberian sturgeon (*Acipenser baerii*), a migratory fish tolerant of cool water, lives in freshwater water and eat different kinds of food as an omnivore species. These characteristics make this species a suitable fish candidate for aquaculture purposes (Hasanalipour et al. 2013). Like most fish species, sturgeon fish need different fatty acids, especially n-3 highly unsaturated fatty acids (n-3 HUFA) in their diet (Nieminen et al. 2014). However, these fatty acids are at high risk of oxidation especially during storage and feed processing. In fish, dietary oxidized lipid could affect growth, survival and health by lipid oxidation, damaging different biomolecules and inducing oxidative stress and finally disease occurrence (Huang and Huang 2004; Martins et al. 2007; Dong et al. 2011). In fish species, antioxidant system comprising both enzymatic and non-enzymatic antioxidants has defensive effects during the oxidative stresses caused by oxidized oil (Chen et al. 2013).

Green tea has long been known as a dietary source of antioxidant nutrients (Crespy and Williamson 2004; Cabrera et al. 2006). Green tea extract (GTE) is a main source of polyphenols, different carotenoids, vitamin C, minerals and some phytochemical compounds with antioxidant potential (Cabrera et al. 2006). Several studies also showed the anti-tumorigenic, anti-proliferative, anti-bacterial, anti-viral, and anti-parasitic properties of green tea in laboratory animals and human (Crespy & Williamson 2004; Cabrera et al. 2006). Although the application of green tea on growth, immunological and biochemical parameters and disease resistance in some fish species has been shown (Cho et al. 2007; Abdel-Tawwab et al. 2010; Thawonsuwan et al. 2010; Harikrishnan et al. 2011; Sheikhzadeh et al. 2011; Hwang et al. 2013; Nootash et al. 2013), little information is available on the protective role of green tea against different oxidative stresses in fish species (Hasanpour et al. 2017a,b; Sayed & Soliman 2018). Therefore, in this study the protective role of GTE against depletion of immunological and antioxidant defenses imposed by dietary oxidized fish oil (OFO) of Siberian sturgeon were evaluated.

Materials and methods

Lipid oxidation and experimental diets

Kilkafish oil with peroxidase value 1.02 ± 0.01 meq kg⁻¹ (Mean $\pm$ SE) was oxidized according to Dong et al. (2011) by bubbling with air while stirring and heating at 50°C. Peroxide value was monitored for 24 h, until the final peroxidase value 426.36 ± 8.2 meq kg⁻¹ (Mean $\pm$ SE) was reached. The basal diet was formulated to contain about 45.6% protein, 19.4% lipid, 11.3% ash and 9.1% moisture (Hasanpour et al. 2017a). Three levels of GTE in diet (Soha Jissa Company, Salmashahr, Iran) including 0 mg kg⁻¹, 25 mg kg⁻¹ (low green tea: LG) and 50 mg kg⁻¹ (high green tea: HG) combined with three levels of OFO including fresh fish oil, 50% OFO (low OFO: LO) and 100% OFO (high OFO: HO) were employed (Hasanpour et al. 2017a) in a completely randomized design of 3×3 with three replicates for each treatment groups. Diet formulation and chemical composition of each group are shown in Table 1. The experimental diets were prepared according to the previous study (Hasanpour et al. 2017a) and sealed in plastic bags before storing at -4°C.

Experimental conditions

Siberian sturgeon obtained from Shahid Beheshti sturgeon fish propagation and rearing complex (Rasht, Iran) were distributed into twenty seven 100 Lit circular fiberglass tanks. Fifteen fish with an average body weight of 108.13 ± 0.18 g were randomly assigned to each tank. Tanks were supplied with flow through well water and continuous aeration with water flow rate (1.7 l s⁻¹), temperature ($17.04 \pm 0.2^\circ\text{C}$), pH (8 ± 0.4), nitrite (0.03 ± 0.011 mg L⁻¹), and dissolved oxygen (6.9 ± 0.05 mg L⁻¹) maintained in normal range for Siberian sturgeon, under a 10 h light: 14 h dark photoperiod. During two-week acclimation period, all fish were fed with the same basal diet. After this period, for each of nine treatment diets, three tanks of fish were randomly assigned. Fish were fed with the described diets three times daily by hand at 8.00, 14.00 and 18.00 for the next 8 weeks.

Growth performance

At the beginning and the end of the experiment, the fish were not fed for 24 h before harvesting. Five fish from each tank were randomly selected and anesthetized with 2-phenoxy-ethanol (2%), and biomass was determined. Additionally, viscera and liver were also removed and weighed for estimating the hepatosomatic index (HSI) and viscerosomatic index (VSI). Feed conversion ratio (FCR), specific growth rate (SGR) and condition factor (CF) were also calculated as follows:

$$\text{FCR} = \text{dry feed intake (g)} / \text{weight gain (g)}$$

$$\text{SGR (\%)} = 100 \times [\ln (\text{final body weight}) - \ln (\text{initial body weight})] / \text{number of days in the feeding period}$$

$$\text{CF (\%)} = 100 \times \text{weight (g)} / \text{length (cm)}^3$$

HSI (%) = $100 \times [\text{liver weight (g)} / \text{wet weight (g)}]$

VSI (%) = $100 \times [\text{viscera weight (g)} / \text{wet weight (g)}]$

Fillet composition

At the end of the trial, three fish in each tank were randomly selected. After anesthetizing with 2-phenoxy-ethanol (2%), fish fillets were separately autoclaved at 105°C for 20 min, homogenized and oven-dried at 60°C. Crude protein, lipid, ash and energy were determined for fish samples as well as diets. Crude protein was measured via Kjeldahl method (using Kjeltex Auto Analyser, 2300 Tecator, Sweden). Crude fat was determined by extraction with oil ether using Soxhlet apparatus; ash by incineration at 600°C for 6 h; and energy through bomb calorimeter (Parr Instrument Co, Moline, IL, USA). All these parameters were determined based on the conventional methods (AOAC 1995).

Serum sample collection

At the end of the feeding trial, the other three fish not those used for fillet composition were selected from each tank and anaesthetized with 2-phenoxy-ethanol (2%). Blood sample was collected from the caudal vein of each fish with a non-heparinized syringe. Fish bloods were allowed to clot at room temperature for 1 h and then stored in a refrigerator overnight. The clot was then centrifuged at 1500 g for 5 min. and the serum was collected and stored in sterile Eppendorf tubes at -80°C until they analyzed.

Immunological parameters

Serum lysozyme activity was measured using a turbidimetric technique according to Tukmechi et al. (2011) with slight modification. Briefly, a standard suspension of *Micrococcus lysodeikticus* (75 µg mL⁻¹) was prepared with 0.1 M phosphate citrate buffer, pH 5.8. Fish serum (25 µL) was added to 75 µL of *Micrococcus lysodeikticus* suspension and the decrease in absorbance OD was measured after 4 and 9 min at 450 nm. A unit of lysozyme activity was defined as the amount of enzyme that caused a decrease in the absorbance at 450 nm of 0.001 min⁻¹ and was expressed as U mL⁻¹ serum.

Total antibody level in the serum was determined according to the method described by Siwicki et al. (1994). The difference in total protein content prior to and after precipitation of the antibody component with 12% polyethylene glycol (PEG; Sigma Aldrich) was determined using the Bradford method (Kruger 1996).

Alternative complement serum activity was determined using previously reported method (Andani et al. 2012). Rabbit red blood cells (RaRBC) were washed three times in ethylene glycol tetra acetic acid–magnesium–gelatin veronal buffer (0.01 M EGTA–Mg–GVB, pH 7) and resuspended in this buffer to a concentration of 2×10^8 cells mL⁻¹. A 100-μL suspension of RaRBC was added to 250 μL of serially diluted serum in buffer. Samples were incubated at 20°C for 90 min with regular shaking. Approximately 3.15 mL of 0.85% NaCl solution was added and the tubes were centrifuged at 1600 g for 10 min at 4°C. The extent of haemolysis was estimated by measuring the optical density of the supernatant at 414 nm using a spectrophotometer (Awarness, Palm, FA). ACH50 units were defined as the concentration of serum giving 50% haemolysis of RaRBC.

Biochemical parameters

Antioxidant activity

Total superoxide dismutase (SOD) activity was determined by measuring the inhibition of the oxygen-dependent oxidation of adrenalin (epinephrine) to adenochrome using xanthine oxidase plus xanthine (Panchenko et al. 1975). The reaction was followed at 480 nm and one unit of superoxide dismutase activity was described as the amount of the enzyme that decreased the production of adenochrome by 50%.

Glutathione peroxidase (GPX) was assayed by following the rate of NADPH oxidation at 340 nm with specific activity determined using the extinction coefficient of 6.22 mM⁻¹ cm⁻¹ (Bell et al. 1985). Catalase (CAT) activity was measured by reduction of hydrogen peroxide at 240 nm ($\epsilon = 40$ M⁻¹ cm⁻¹) using 50 mM H₂O₂ as substrate (Aebi 1974).

Lipid peroxidation

Malondialdehyde (MDA) level in fish serum was determined using the thiobarbituric acid (TBA) method (Buege and Aust 1978). After preparing 20% w/v serum in a cold 0.1 M phosphate buffer (pH 7.2), 200 μL serum was added to one mL trichloroacetic acid (TCA)-TBA solution (0.67% TBA in 2% TCA). Samples were shaken and incubated for 20 min in a boiling water bath. After cooling and centrifugation for 10 min at 1500 g, the absorbance of the supernatant was measured calorimetrically at 531 nm on a Unico UV-2100 PC spectrophotometer.

Serum organic compositions

Triglyceride and cholesterol levels of fish serums were estimated according to the enzymatic colorimetric method of Fossati and Prencipe (1982) and Allain et al. (1974), respectively. Serum high-density lipoprotein (HDL) level was estimated after precipitation of apolipoprotein B-containing proteins with magnesium phosphotungstate (Lopes-Virella et al. 1977). Low-density lipoprotein (LDL) level was calculated using the Friedewald formula (Friedewald et al. 1972).

Statistical analysis

Data were presented as mean $\pm$ standard error of mean (SEM). The Kolmogorov-Smirnov test was used to assess data normality and data transformation (e.g. square root) was performed where needed. One-way analysis of variance (ANOVA) was conducted to compare the means of treatment groups followed by Tukey HSD post-hoc test. All data analyses were performed using SPSS software version 24.0 (IBM Corp. NY, USA).

Results

Growth performance

Weight gain increased significantly by the dietary GTE (ANOVA; $F=4.07$; $df=18, 27$; $P=0.035$). The administration of GTE decreased the FCR level compared to other treatment groups ((ANOVA; $F=97.35$; $df=18, 27$; $P<0.001$), however, in fish fed with OFO diet, FCR was significantly higher than GTE-fed treatment groups. Adding GTE to rancid diet especially in LGLO and HGLO groups was also enhanced FCR compared to the OFO fed group (ANOVA; $F=29.95$; $df=18, 27$; $P<0.001$). Meanwhile there were no significant differences in SGR and CF between all treatment groups (CF: ANOVA; $F=0.509$; $df=18, 27$; $P=0.609$; SGR: ANOVA; $F=2.10$; $df=18, 27$; $P<0.001$). GTE significantly decreased the HSI and VSI levels in the examined fish (HSI: ANOVA; $F=964.23$; $df=18, 27$; $P=0.609$; VSI: ANOVA; $F=1036.35$; $df=18, 27$; $P<0.001$), whereas, OFE increased these values (HSI: ANOVA; $F=1322.85$; $df=18, 27$; $P<0.001$; VSI: ANOVA; $F=1544.08$; $df=18, 27$; $P<0.001$). Supplementation of the rancid diet with GTE alleviated the effects of oxidation by decreasing the HSI and VSI levels. Additionally, no mortality occurred in different groups during the feeding trial (Table 2).

Fillet composition

Moisture in groups fed with different levels of GTE was significantly higher than the other groups (ANOVA; $F=5.92$; $df=18, 27$; $P=0.011$). Meanwhile, lipid content in fish that received high dose of OFO showed higher level of lipid than the other groups (ANOVA; $F=5.71$; $df=18, 27$; $P=0.012$). Feeding fish with the high dose of GTE significantly increased the protein content in comparison with the HO and LGHO treatment groups (ANOVA; $F=3.73$; $df=18, 27$; $P=0.044$). No significant changes were noted in ash content between all examined groups (Table 3).

Immunological parameters

Serum lysozyme activity was significantly increased by the inclusion of GTE in fish diet (ANOVA; $F=343.79$; $df=18, 27$; $P<0.001$). In fish fed OFO diets, significant decrease in lysozyme activity was observed (ANOVA; $F=850.08$; $df=18, 27$; $P<0.001$). When GTE was added into the rancid diets, lysozyme activity was increased in comparison with OFO (low and high doses) groups (ANOVA; $F=11.85$; $df=18, 27$; $P<0.001$). Total antibody level in fish fed GTE was higher than those received OFO (ANOVA; $F=49.63$; $df=18, 27$; $P<0.001$). Meanwhile, the addition of GTE to oxidized diet was able to significantly increase the total antibody level compared to the oxidized diet (ANOVA; $F=4.53$; $df=18, 27$; $P=0.010$). The complement activity in groups that received OFO especially in HO group was lower than green tea-fed groups (ANOVA; $F=14.50$; $df=18, 27$; $P<0.001$) while the administration of GTE alone or combined with the OFO did not change the complement activity (ANOVA; $F=2.38$; $df=18, 27$; $P=0.121$) (Table 4).

Biochemical parameters

Antioxidant activity and Lipid peroxidation

In fish fed GTE, CAT activity was significantly lower than the other treatment groups (ANOVA; $F=524.94$; $df=18, 27$; $P<0.001$). The administration of OFO increased CAT activity (ANOVA; $F=126.50$; $df=18, 27$; $P<0.001$), however, in fish that received a combination of GTE and OFO, CAT activity was lower than fish fed with OFO (ANOVA; $F=31.79$; $df=18, 27$; $P<0.001$). Fish fed with GTE (High and low doses) showed a significant decrease in their SOD activity (ANOVA; $F=56.28$; $df=18, 27$; $P<0.001$). In contrast, in fish received OFO the activity of SOD was higher than those received GTE (ANOVA; $F=23.43$; $df=18, 27$; $P<0.001$). However, no changes were observed in SOD activity by adding GTE in rancid diet. GPX activity in fish that received GTE was significantly lower than fish received other treatment diets (ANOVA; $F=122.60$; $df=18, 27$; $P<0.001$), however higher GPX activity was noted in fish received OFO than those received GTE alone or in combination with OFO (ANOVA; $F=219.80$; $df=18, 27$; $P<0.001$). In fish fed both GTE and OFO, GPX activity was

still lower than the OFO fed groups (ANOVA; $F=13.60$; $df=18, 27$; $P<0.001$). MDA activity followed a similar pattern to CAT and GPX enzyme activities in the examined fish (Table 5).

Serum organic compositions

In fish received GTE, LDL level was significantly lower than all the other treatment groups including the control group (ANOVA; $F=61.45$; $df=18, 27$; $P<0.001$), while the use of OFO increased the LDL level compared to all other treatment groups (ANOVA; $F=136.45$; $df=18, 27$; $P<0.001$). HDL level in fish received high dose of GTE in their diet was higher than other treatment groups (ANOVA; $F=14.77$; $df=18, 27$; $P<0.001$), whereas HDL level in fish received OFO was significantly decreased in comparison with the fish received GTE (ANOVA; $F=7.80$; $df=18, 27$; $P=0.004$). In all treatment groups, especially those that received OFO alone or combined with GTE, cholesterol levels were lower than the control group (ANOVA; $F=414.07$; $df=18, 27$; $P<0.001$). Similar trend was also observed for the triglyceride level. In all treatment groups especially, those that received OFO in their diet and combination of GTE and OFO, triglyceride level was significantly lower than the control group (ANOVA; $F=166.54$; $df=18, 27$; $P<0.001$) (Table 6).

Discussion

The results showed that Siberian sturgeon fed diets with GTE displayed improved growth which was in parallel with previous studies in Nile tilapia, *Oreochromis niloticus*, and rainbow trout (Abdel-Tawwab et al. 2010; Thawonsuwan et al. 2010). Conversely, administration of green tea did not enhance the growth performance in yellowtail (*Seriola quinqueradiata*) and olive flounder, *Paralichthys olivaceus* (Ishihara et al. 2002; Cho et al. 2007). In the present study, higher FCR level was shown in group received OFO in comparison with the other groups which was in accordance with previous studies (Tocher et al. 2003; Huang and Huang 2004; Gao et al. 2012). It seems that decreased digestibility of some macronutrients in response to the dietary oil oxidation could impair the growth, feed utilization and survival in many fish species (Chen et al. 2013). The conflicting results obtained regarding the effects of green tea and rancid diet may originate from differences in digestion and absorption mechanism of different fish species, dose or sources of green tea or oxidized oil in the diet, and also duration of the experiment (Eder and Stangle 2000; Mourente et al. 2002; Tocher et al. 2003; Fontagne et al. 2006; Chen et al. 2011).

Supplementation of diet with GTE alone and combined with OFO reduced HSI and VSI in fish. Similarly, HSI was lower in gilthead sea bream (*Sparus aurata*) administrated with vitamin E (Mourente et al. 2002). Peng et al. (2009) also reported that addition of vitamin E to black sea bream (*Acanthopagrus schlegelii*) diet decreased the HSI level compared to rancid diet. In the present study,

fish fed OFO, especially in high dose, had high fillet fat content, and high HSI and VSI values. Green tea can enhance fat metabolism and prevent the cholesterol accumulation in fish body (Chen et al. 2011). This mechanism can result in lower HSI and VSI indices and fillet fat content in groups that received GTE. Meanwhile, fillet protein content in fish fed high dose of GTE was higher than fish received high dose of OFO. The changes in protein contents in fish fillet following the use of GTE and OFO could be associated with the changes in protein synthesis, rate of muscle deposition as well as differences in growth rate (Abdel-Tawwab et al. 2010; Gao et al. 2012).

In this study, higher lysozyme activity was shown in Siberian sturgeon administrated with GTE. The positive effects of green tea on immune system and disease resistance were shown (Abdel-Tawwab et al. 2010; Harikrishnan et al. 2011; Sheikhzadeh et al. 2011; Thawonsuwan et al. 2010). Meanwhile, peroxidized lipid in the absence of a good antioxidant could impair the fish immune system (Obach and Laurencin 1992; Obach et al. 1993; Martins et al. 2007). In this study, serum lysozyme activity decreased significantly in OFO fed fish groups compared to all other groups. It was previously shown that some oxidation bio-products, especially those with low molecular weight, have reaction with the thiol groups of some enzymes and inhibit them (Obach et al. 1993). Therefore, it is not surprising to note that inhibition of lysozyme activity in fish received OFO could be attributed to these oxidation products.

In the present study, complement activity decreased in sturgeon fed OFO. Macrophage is the site of producing complement proteins; therefore, impaired macrophage function caused by oxidation of the membrane phospholipid can change the synthesis of these proteins (Obach et al. 1993). The beneficial effects of GTE on immunological parameters was observed in fish received both GTE and OFO. In fact, membrane-related signaling and gene expression are important in maintaining the normal function of immune system and their ability to defend against different foreign antigens (Meydani et al. 1995). Therefore, augmenting the antioxidant system by some substances like green tea could positively affect the immune response.

In the current study, OFO caused significant increase in serum LDL of Siberian sturgeon. In contrast, GTE decreased the LDL and cholesterol levels. In accordance with this study, Liu and Pan (2004) showed that green tea supplement in gray mullet (*Mugil cephalus*) diet decreased the oxidation rate of LDL. Catechins in green tea have a hypocholesterolemic effect and prevent LDL oxidation to regenerate alpha-tocopherol in LDL particles (Zhu et al. 1999). In the present study, higher HDL level in GTE fed groups in comparison with the OFO treatment groups was noted, showing that green tea polyphenols exert an anti-atherosclerotic effect (Crespy and Williamson 2004). Meanwhile, fish fed OFO without GTE had lower triglyceride and cholesterol levels than the other groups. Triglyceride lowering effects of oxidized oil have been consistently observed in numerous experiments which was reviewed by Ringseis and Eder (2011). Several studies also reported the reduced cholesterol levels in

the plasma and liver following the use of oxidized oils (Ringseis and Eder 2011). In fact, transcription factors including sterol regulatory-element binding proteins are inhibited by oxidized fats which result in preventing the cholesterol homeostasis and fatty acid synthesis (Ringseis and Eder 2011). Our data also showed that supplementation of fish diet with GTE alone or combined with the oxidized oil, decreased serum triglyceride and cholesterol in Siberian sturgeon. Green tea intake decreases the absorption of triglycerides and cholesterol, which are in accordance with the increase in the excretion of fat (Crespy and Williamson 2004). Therefore, GTE combined with the OFO can decrease serum triglyceride and cholesterol levels synergistically but with different mechanisms.

In this study, serum CAT and GPX activities were markedly elevated by OFO which shows the enhanced antioxidant activity. Previously, Mourente et al. (2002) observed that liver antioxidant enzyme activity increased in gilthead sea after receiving different levels of OFO. SOD and CAT activities were also increased by administrating the dietary oxidized oil in sea bream (Tocher et al. 2003). Fontagne et al. (2006) also reported the increased activity of antioxidant enzymes GPX and SOD in Siberian sturgeon larvae received OFO. Parallel with enhanced antioxidant enzyme activity in OFO-fed groups, elevated MDA level were also noted. MDA is a biomarker which shows the lipid peroxidation levels. Conversely, in sturgeons fed with GTE supplemented diet alone or combined with OFO, the level of MDA was lower than fish fed with OFO. Similarly, lower lipid peroxidation product was also noted in rainbow trout that received the green tea (Nootash et al. 2013). GTE, as a source of polyphenols, could neutralize and scavenges reactive oxygen species by reducing the damage to cell content imposed by different oxidants (Sayed & Soliman 2018).

To conclude, our findings showed that oral administration of GTE resulted in (a) improving somatic parameters, (b) augmenting the immune parameters including the lysozyme activity and total antibody level, (c) enhancing the antioxidant system, (d) ameliorating the biochemical parameters in Siberian sturgeon challenged by dietary oil oxidation.

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Ingredients (g kg ⁻¹)	Control	LG	HG	LO	HO	LGLO	LGHO	HGLO	HGHO
Fish meal ^a	600.0	600.0	600.0	600.0	600.0	600.0	600.0	600.0	600.0
Meat meal ^b	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Soybean meal ^c	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0
Wheat flour ^d	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Fresh kila fish oil ^e	100.0	100.0	100.0	50.0	-	50.0	-	50.0	-
Oxidized kila fish oil	-	-	-	50.0	100.0	50.0	100.0	50.0	100.0
Green tea extract ^f	-	0.025	0.050	-	-	0.025	0.025	0.050	0.050
Molasses	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Clay	15.00	14.95	14.90	15.00	15.00	14.95	14.95	14.90	14.90
Vitamin premix ^g	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Mineral premix ^h	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0

Table 1. Formulation and chemical composition of the experimental diets

^{a,b,c,d,e} Behparvar Co, Karaj, Iran.

^f soha jissa Co, Salmashahr, Mazandaran, Iran.

^g Vitamin premix (Science Laboratories Company, Qazvin, Iran, composition per 1000 g): 160000 IU vitamin A, 400000 IU Vitamin D₃, 40 mg vitamin E, 2 g vitamin K₃, 6 g vitamin B₁, 8 g vitamin B₂, 12 g Pantothenate calcium, 40 g vitamin B₃, 4 g vitamin B₆, 2 g vitamin B₉, 8 g vitamin B₁₂, 0.24 g H₂, 60 g vitamin C, 20 mg Inositol, 20 mg BHT

^h Mineral premix (Science Laboratories Company, Qazvin, Iran, composition per 1000 g): 26mg Fe, 12.5g zinc, 2 mg Se, 480 mg Co, 2.4 g Cu, 8.15g Mn, 1 g I, 12g Chloride choline.

LG: low green tea; HG: high green tea; LO: low oxidized fish oil; HO: high oxidized fish oil; LGLO: low green tea combined with low oxidized fish oil; LGHO: low green tea combined with high oxidized fish oil; HGLO: high green tea combined with low oxidized fish oil; HGHO: high green tea combined with high oxidized fish oil.

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Table 2

Growth parameters and survival rate in juvenile Siberian sturgeon after feeding oxidized fish oil and green tea for eight weeks. All data are expressed as mean $\pm$ SE. Different letters in the same column represent significant differences ($p < 0.05$).

Treatments	Initial weight (g)	Weight gain (g)	Feed conversion ratio	Specific growth rate (%)	Condition factor (%)	Hepatosomatic index (%)	Viscerosomatic index (%)	Survival rate (%)
Control	108.33 $\pm$ 0.60	284.46 $\pm$ 1.00 ^a	1.225 $\pm$ 0.003 ^{cd}	2.153 $\pm$ 0.001 ^{ab}	0.524 $\pm$ 0.002	2.17 $\pm$ 0.01 ^b	6.06 $\pm$ 0.00 ^b	100
LG	108.30 $\pm$ 0.35	287.37 $\pm$ 1.00 ^b	1.092 $\pm$ 0.001 ^a	2.157 $\pm$ 0.000 ^b	0.536 $\pm$ 0.006	2.05 $\pm$ 0.01 ^a	5.40 $\pm$ 0.00 ^a	100
HG	107.96 $\pm$ 0.43	286.24 $\pm$ 0.7 ^b	1.090 $\pm$ 0.000 ^a	2.157 $\pm$ 0.000 ^b	0.525 $\pm$ 0.005	2.03 $\pm$ 0.00 ^a	5.36 $\pm$ 0.01 ^a	100
LO	107.06 $\pm$ 0.83	282.66 $\pm$ 1.96 ^{ab}	1.237 $\pm$ 0.007 ^d	2.155 $\pm$ 0.000 ^{ab}	0.498 $\pm$ 0.019	2.64 $\pm$ 0.00 ^e	6.40 $\pm$ 0.00 ^c	100
HO	107.63 $\pm$ 0.43	283.34 $\pm$ 1.00 ^{ab}	1.234 $\pm$ 0.003 ^d	2.155 $\pm$ 0.000 ^{ab}	0.507 $\pm$ 0.003	2.70 $\pm$ 0.00 ^f	6.69 $\pm$ 0.04 ^d	100
LGLO	108.33 $\pm$ 0.63	285.4 $\pm$ 1.47 ^{ab}	1.195 $\pm$ 0.007 ^{bc}	2.154 $\pm$ 0.001 ^{ab}	0.515 $\pm$ 0.000	2.42 $\pm$ 0.00 ^c	6.08 $\pm$ 0.00 ^b	100
LGHO	107.90 $\pm$ 1.00	284.13 $\pm$ 1.57 ^{ab}	1.210 $\pm$ 0.006 ^{bcd}	2.155 $\pm$ 0.002 ^{ab}	0.500 $\pm$ 0.003	2.48 $\pm$ 0.00 ^d	6.12 $\pm$ 0.00 ^b	100
HGLO	109.10 $\pm$ 0.25	286.23 $\pm$ 0.57 ^a	1.190 $\pm$ 0.005 ^b	2.152 $\pm$ 0.000 ^{ab}	0.516 $\pm$ 0.010	2.20 $\pm$ 0.00 ^b	6.06 $\pm$ 0.00 ^b	100
HGHO	107.26 $\pm$ 1.21	283.27 $\pm$ 2.02 ^a	1.210 $\pm$ 0.012 ^{bcd}	2.152 $\pm$ 0.000 ^a	0.510 $\pm$ 0.016	2.22 $\pm$ 0.00 ^b	6.11 $\pm$ 0.00 ^b	100

Factorial

analysis								
Green tea	N.S.	*	*	N.S.	N.S.	*	*	N.S.
Oxidized oil	N.S.	N.S.	*	N.S.	N.S.	*	*	N.S.
Interaction	N.S.	N.S.	*	N.S.	N.S.	*	*	N.S.

N.S.: not significant, *: significant

Table 3 Fillet composition (%) in juvenile Siberian sturgeon after feeding oxidized fish oil and green tea for eight weeks. All data are expressed as mean $\pm$ SE. Different letters in the same column represent significant differences ($p < 0.05$).

Treatments	Moisture	Lipid	Protein	Ash
Control	74.93 $\pm$ 0.15 ^a	6.53 $\pm$ 0.33 ^a	14.17 $\pm$ 0.17 ^{ab}	3.12 $\pm$ 0.15
LG	75.33 $\pm$ 0.16 ^b	5.93 $\pm$ 0.10 ^a	14.57 $\pm$ 0.07 ^{ab}	3.06 $\pm$ 0.05
HG	75.35 $\pm$ 0.20 ^b	5.78 $\pm$ 0.12 ^a	14.73 $\pm$ 0.07 ^b	3.03 $\pm$ 0.04
LO	74.85 $\pm$ 0.10 ^a	6.74 $\pm$ 0.01 ^{ab}	14.09 $\pm$ 0.08 ^{ab}	3.06 $\pm$ 0.07
HO	74.79 $\pm$ 0.02 ^a	6.97 $\pm$ 0.39 ^b	13.97 $\pm$ 0.23 ^a	3.02 $\pm$ 0.10
LGLO	75.16 $\pm$ 0.17 ^a	6.54 $\pm$ 0.19 ^a	14.10 $\pm$ 0.16 ^{ab}	3.04 $\pm$ 0.12

LGHO	75.13 ± 0.15 ^a	6.71 ± 0.29 ^a	14.00 ± 0.01 ^a	3.02 ± 0.02
HGLO	75.14 ± 0.11 ^a	6.45 ± 0.20 ^a	14.22 ± 0.15 ^{ab}	3.02 ± 0.03
HGHO	75.09 ± 0.05 ^a	6.52 ± 0.28 ^a	14.25 ± 0.18 ^{ab}	3.03 ± 0.19
Factorial analysis				
Green tea	*	N.S.	*	N.S.
Oxidized oil	N.S.	*	*	N.S.
Interaction	N.S.	N.S.	N.S.	N.S.

N.S.: not significant, *: significant

Table 4

Immune parameters in juvenile Siberian sturgeon after feeding oxidized fish oil and green tea for eight weeks. All data are expressed as mean ± SE. Different letters in the same column represent significant differences (p<0.05).

Treatments	Lysozyme activity (U mL⁻¹)	Antibody level (g dL⁻¹)	Alternative complement activity (mg dL⁻¹)
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Control	37.49±0.46 ^c	11.45±0.20 ^c	0.42±.07 ^{abc}
LG	41.25±0.25 ^e	12.16±0.17 ^c	0.47±.06 ^c
HG	39.72±0.38 ^d	12.09±0.15 ^c	0.45±.04 ^{bc}
LO	31.57±0.31 ^a	8.64±0.41 ^a	0.32±.04 ^{ab}
HO	30.72±0.25 ^a	9.16±0.31 ^a	0.31±.05 ^a
LGLO	35.12±0.25 ^b	10.36±0.36 ^b	0.38±.04 ^{abc}
LGHO	35.47±0.39 ^b	10.49±0.37 ^b	0.36±.03 ^{abc}
HGLO	34.62±0.37 ^b	10.03±0.22 ^b	0.34±.04 ^{abc}
HGHO	35.20±0.31 ^b	10.15±0.15 ^b	0.35±.04 ^{abc}
Factorial analysis			
Green tea	*	*	N.S.
Oxidized oil	*	*	*
Interaction	*	*	N.S.

N.S.: not significant, *: significant

Table 5 Antioxidant activity and malondialdehyde (MDA) level in juvenile Siberian sturgeon serum after feeding oxidized fish oil and green tea for eight weeks. All data are expressed as mean $\pm$ SE. Different letters in the same column represent significant differences ($p < 0.05$).

Treatments	CAT (U mL ⁻¹)	SOD (U mL ⁻¹)	GPX (μ mol mL ⁻¹)	MDA (mmol mL ⁻¹)
Control	9.82 $\pm$ 0.03 ^b	5.20 $\pm$ 0.05 ^{defc}	0.15 $\pm$ 0.00 ^a	4.49 $\pm$ 0.01 ^a
LG	9.37 $\pm$ 0.01 ^a	4.33 $\pm$ 0.05 ^{ab}	0.14 $\pm$ 0.00 ^a	4.45 $\pm$ 0.02 ^a
HG	9.29 $\pm$ 0.05 ^a	4.14 $\pm$ 0.03 ^a	0.13 $\pm$ 0.00 ^a	4.16 $\pm$ 0.00 ^a
LO	10.42 $\pm$ 0.03 ^c	5.34 $\pm$ 0.08 ^{ef}	0.24 $\pm$ 0.00 ^c	7.86 $\pm$ 0.12 ^{cd}
HO	10.74 $\pm$ 0.04 ^d	5.60 $\pm$ 0.11 ^f	0.26 $\pm$ 0.00 ^c	8.95 $\pm$ 0.07 ^c
LGLO	9.70 $\pm$ 0.03 ^b	4.90 $\pm$ 0.10 ^{cde}	0.19 $\pm$ 0.00 ^b	7.46 $\pm$ 0.02 ^c
LGHO	9.81 $\pm$ 0.02 ^b	5.17 $\pm$ 0.09 ^{def}	0.19 $\pm$ 0.00 ^b	8.03 $\pm$ 0.09 ^d
HGLO	9.43 $\pm$ 0.04 ^a	4.36 $\pm$ 0.12 ^{abc}	0.18 $\pm$ 0.00 ^b	6.52 $\pm$ 0.01 ^b
HGHO	9.35 $\pm$ 0.02 ^a	4.55 $\pm$ 0.05 ^{bcd}	0.18 $\pm$ 0.00 ^b	6.92 $\pm$ 0.25 ^b
Factorial analysis				
Green tea	*	*	*	*
Oxidized oil	*	*	*	*
Interaction	*	N.S.	*	*

N.S.: not significant, *: significant

Table 6

Serum organic compositions in juvenile Siberian sturgeon after feeding oxidized fish oil and green tea for eight weeks. All data are expressed as mean $\pm$ SE. Different letters in the same column represent significant differences ($p < 0.05$).

Treatments	LDL (mg dL⁻¹)	HDL (mg dL⁻¹)	Cholestreol (mg dL⁻¹)	Triglycerides (mg dL⁻¹)
Control	28.33 $\pm$ 0.33 ^b	13.00 $\pm$ 0.57 ^{ab}	131.66 $\pm$ 0.33 ^e	871.00 $\pm$ 4.93 ^g
LG	26.33 $\pm$ 0.33 ^a	14.66 $\pm$ 0.33 ^{bc}	128.33 $\pm$ 0.33 ^d	775.33 $\pm$ 2.02 ^f
HG	25.66 $\pm$ 0.33 ^a	15.33 $\pm$ 0.33 ^c	127.66 $\pm$ 0.33 ^d	742.33 $\pm$ 2.60 ^e
LO	32.33 $\pm$ 0.33 ^{de}	12.00 $\pm$ 0.57 ^a	122.33 $\pm$ 0.33 ^{bc}	644.33 $\pm$ 4.48 ^c
HO	33.33 $\pm$ 0.33 ^e	11.66 $\pm$ 0.33 ^a	120.66 $\pm$ 0.33 ^{ab}	623.33 $\pm$ 3.38 ^b
LGLO	30.66 $\pm$ 0.33 ^{cd}	13.33 $\pm$ 0.33 ^{ab}	123.00 $\pm$ 0.57 ^c	597.33 $\pm$ 5.36 ^a
LGHO	31.66 $\pm$ 0.33 ^{de}	13.00 $\pm$ 0.57 ^{ab}	121.00 $\pm$ 0.57 ^{ab}	623.01 $\pm$ 2.64 ^b
HGLO	29.33 $\pm$ 0.33 ^{bc}	14.66 $\pm$ 0.33 ^{bc}	120.33 $\pm$ 0.33 ^a	673.00 $\pm$ 4.35 ^d
HGHO	29.00 $\pm$ 0.57 ^{bc}	14.33 $\pm$ 0.33 ^{bc}	119.66 $\pm$ 0.33 ^a	692.01 $\pm$ 5.50 ^d
Factorial analysis				

Green tea	*	*	*	*
Oxidized oil	*	*	*	*
Interaction	N.S.	N.S.	*	*

N.S.: not significant, *: significant

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