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PROOF RESEARCH PAPER

Effect of Green Tea (Camellia sinensis L.) on Growth, Blood and Immune Parameters in Sturgeon Hybrid (Huso huso ♂ × Acipenser ruthenus ♀) Fed Oxidized Fish Oil

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Abstract

Effects of green tea in fish based oxidized oil was evaluated in this study. Lipid of diet was replaced by oxidized fish oil (OFO) at 0, 50 and 100% levels. Green tea extract (GTE) was added to diet in 3 levels, 0, 5 and 100 mg/kg giving a total of 9 experimental diets. Two hundred and seventy sturgeon hybrid (*Huso huso* ♀ × *Acipenser ruthenus* ♂), mean weight of 211.71 ± 0.23 g randomly divided in 27 fiberglass tanks with 700 l volume after 2 weeks adaptation. After 6 weeks, biometry was done to evaluate growth performance and blood samples were taken. The result showed that feeding with OFO had no effects on condition factor and specific growth rate. However, in fish fed GTE feed efficiency ratio and protein efficiency ratio improved slightly. Red blood cells were not affected by OFO and GTE while in fish received GTE and both GTE and OFO white blood cells increased significantly ($P<0.05$). Immunological parameters, (IgM, ACH50 and Lysozyme) showed significant increase in compare to control ($P<0.05$). Our findings showed that feeding of sturgeon hybrid with OFO had no effects on growth but GTE improved feeding performance. Also OFO had no effects on immune function.

Keywords: sturgeon hybrid, hematology, green tea, lysozyme, oxidized fish oil.

Introduction

Fish oil is widely used in fish diet because of its high content of polyunsaturated fatty acids (PUFA) (Chen, Zhu, Han, Yang, Lei, & Xie, 2010) as amends of fish deficiency in desaturation and elongation pathways crucial for biosynthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Sargent, Tocher, & Bell, 2002). Lipid oxidation occurrences in fish diets is common, especially when food is stored under aerobic conditions. Oxidation of lipids result to production of hydroperoxides as primary products of oxidation and secondary oxidation products including aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids and epoxy compounds (Shahidi & Zhong 2005).Ingestion of diets including oxidized lipid could be detrimental through modification of fatty acid profiles and vitamin E depletion (Baker & Davies, 1997). The adverse effects of dietary oxidized fish oil (OFO) including the depression of appetite and decrease of growth (Fontagné, Bazin, Brèque, Vachot, Bernarde, Rouault, & Bergot, 2006; Peng, Chen, Qin, Hou, Yu, Long, LI, & YE, 2009) anemia and depletion of vitamins E and C (Sargent *et al*., 2002), increase in intensity of stress responses (Alves Martins *et al*., 2007) Vit. E had been used in different studies to counteract with adverse effects of OFO on fish immune system (Zhong, Lall, & Shahidi, 2008; Wang, Lee, Rha, Yoon, Park, Han, & Kim, 2015).

Green tea as food additive in fish diet had been reported to increase disease resistance, improved survival rate, growth rate, stress responses and antioxidant system (Cho, Lee, Park, Ji, Lee, Bae, & Oh, 2006; Abdel-Tawwab, Ahmad, Seden & Sakr, 2010; Sheikhzadeh, Nofouzi, Delazar, & Oushani, 2011; Ebrahimi, Salati, Azarm, & Hasanpour, 2015). Main compositions of green tea, a product that made up from leaf and buds of the plant *Camellia sinensis* are tea polyphenols, vitamins, nitrogenous compounds, caffeine, inorganic elements, lipids and carbohydrates (Ebrahimi *et al*., 2015). It had been showed that green tea could improve immune responses in *Oncorhynchus mykiss* (Nootash *et al*., 2013), and, its efficiency in improving immune system was similar to Vit. E (Thawonsuwan, Kiron, Satoh, Panigrahi, & Verlhac, 2009).

Sturgeon aquaculture is growing fast because of their valuable meat and caviar, while little information is available regarding to nutritional requirements and feeding. Despite deleterious effects of OFO found in some aquatic animals (Fontagné *et*

al., 2006; Peng *et al*., 2009; Sargent *et al*. 2002) less is known about its action in sturgeon. For evaluating protective effects of green tea against OFO, this study was done to evaluate the effects of green tea extract in promotion of growth and immune status of sturgeon hybrid (*Huso huso* ♂ × *Acipenser ruthenus* ♀) fed dietary OFO.

Materials and Methods

Diet Preparation

Three levels of green tea extract (GTE) (0, 50 and 100 mg/kg diet) in combination with three degrees of oxidized fish oil (OFO) replacement (0, 50, 100%), respectively (Table 1). Ingredients and nutrient contents of the experimental diets are presented in Table 1. Alcoholic extract of GT prepared by Solvent Extraction was purchased from Sohajissa Co (Salmanshahr, Mazandaran, Iran) and was added to diet by replacing the Clay, as filler. Kilka fish meal (Ghaem Sahel Co, Rasht, Iran) and Kilka fish oil (Ghaem Sahel Co, Rasht, Iran) were used as the primary protein and lipid source, respectively. For preparing oxidized oil, fresh fish oil without any antioxidant was heated at 50 °C for 24 h as described by Dong *et al*. (2011) until its peroxide value reached to 410 meq kg^{-1} versus 2.70±0.05 meq kg^{-1} preheating. During this process stirring was also done. All ingredients were thoroughly mixed with distilled water (300 ml kg^{-1} dry ingredients mixture), and 4-5 mm pellets were prepared. The pellets were dried at room temperature for 24 h and broken into desirable particle sizes. All diets were stored at -20 °C until feeding.

Experimental Design

Two hundred and seventy sturgeon hybrids (*Huso huso* ♂ × *Acipenser ruthenus* ♀) were used in this study. Fish acclimated to laboratory condition for 2 weeks before starting the experiment, then divided into 700 L circular fiberglass tanks (10 fish in each tank) randomly. Each treatment was done in triplicate for 6 weeks. Fish in experimental groups (initial mean weight 211.71 ± 0.23 g) were hand-fed to apparent satiation three times a day (9:00, 14:00 and 20:00). Uneaten food was collected and dried to determine feed intake (Gao *et al*., 2012). During the period of study, the mean water temperature was 18 ± 1°C, dissolved oxygen was 9.7 ± 0.3 mg L^{-1} and the pH was about 7.9 ± 0.2. The photoperiod was 10L: 14D.

Estimation of Growth Criteria

Fish were deprived from food one day before sampling procedure. Fifteen fish from each treatment (5 fish from each replicate) were randomly captured and were anesthetized with 2-phenoxy-ethanol (2%). The blood samples were taken by 5mL non-heparinized syringe through the severing the caudal peduncle and was divided into two parts. One part was discharged into heparinized microtubes and used fresh to determine cellular components of blood. Another part centrifuged (5 min at 5000 g, Hettich, Germany) at room temperature, then serum separated, and stored at -80°C until analysis. After blood sampling, in order to calculate the growth indices of fish for comparing the effects of experimental diets, all fish in each experimental group were weighed at the end of study. Growth performance and feed utilization were calculated by using the below

Table 1. Composition of the experimental diets

	Experimental diets								
Ingredients diets(g/1000g)	Control	OFO_1	OFO_2	GT_1	GT_2	GT_1OFO_1	GT_1OFO_2	GT_2OFO_1	GT_2OFO_2
Fish meal[a]	600	600	600	600	600	600	600	600	600
Meat meal	50	50	50	50	50	50	50	50	50
Soybean meal	80	80	80	80	80	80	80	80	80
Wheat flour	100	100	100	100	100	100	100	100	100
Green tea extract[c]	0	0	0	0.05	0.1	0.05	0.05	0.1	0.1
Molasses	15	15	15	15	15	15	15	15	15
Clay	15	15	15	14.95	14.90	14.95	14.95	14.90	14.90
Vitamin premix [d]	25	25	25	25	25	25	25	25	25
Mineral premix [e]	15	15	15	15	15	15	15	15	15
Fresh fish oil[b] (% of total diet oil)	100	0	0	100	100	50	0	50	0
Oxidized fish oil (% of total diet oil)	0	50	100	0	0	50	100	50	100

[a] Ghaem Sahel Co, Rasht, Iran

[b] Kilka oil, Ghaem Sahel Co, Rasht, Iran

Soha Jissa Co, Mazandaran, Iran [c]

[d] Vitamin premix (Science Laboratories Company, Qazvin, Iran, composition per 1000 g): 160000 IU vitamin A, 400000 IU Vitamin D_3, 40 mg vitamin E, 2 g vitamin K_3, 6 g vitamin B_1, 8 g vitamin B_2, 12 g Pantothenate calcium, 40 g vitamin B_3, 4 g vitamin B_6, 2 g vitamin B_9, 8 g vitamin B_{12}, 0.24 g H_2, 60 g vitamin C, 20 mg Inositol, 20 mg BHT

[e] 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.

formulas:

CF = fish weight (g) · 100/body length3 (cm).

Specific growth ratio = 100 · ln (final weight (g)/initial weight (g))/days of feeding.

Feed efficiency ratio (FER) = wet WG (g)/ dry feed consumed (g)

Protein efficiency ratio (PER) = fish wet weight gain (g)/protein intake (g)

Hematological Analysis

Red blood cells and white blood cells were counted after dilution with Natt & Herrick's diluting solution using neubauer slide. Hemoglobin and hematocrit were estimated by photometric assay of cyanomethemoglobin and microhematocrit method, respectively. Differential WBCs counts (neutrophils and monocytes) were conducted on Giemsa stained blood smears prepared from fresh blood (Salati, Baghbanzadeh, Soltani, Peyghan & Riazi, 2010).

ACH50 was determined using the method of Sunyer and Tort (1995) with little modifications described by Yeh, Chang, Chang, Liu, & Cheng (2008). The volume of supernatant complement producing 50% hemolysis (ACH50) was determined, and the number of ACH50 units/ml was calculated for the sample.

Serum lysozyme activity was assayed according to the method of Demers & Bayne (1997), based on the lysis of the lysozyme sensitive Gram positive bacterium, *Micrococcus lysodeikticus* (Sigma). The dilutions of hen egg white lysozyme (Sigma) ranging from 0 to 20 mL ml^{-1} (in 0.1 M phosphate citrate buffer, pH 5.8) were taken as the standard. This along with the undiluted serum sample (25 mL) was placed into wells of a 96-well plate in triplicate. One hundred and seventy five microliter of *M. lysodeikticus* suspension (75 mg ml^{-1}) prepared in the same buffer was then added to each well. After rapid mixing, the change in turbidity was measured every 30 s for 5 min at 450 nm at approximately 20 ºC using a microplate reader.

Immunoglobulin M (IgM) concentration in supernatants was determined by an ELISA assay and using mouse anti-rainbow trout serum (Aquatic Diagnostics Ltd.) as described by Reyes-Becerril *et al*. (2008).

Total protein and albumin were assayed by biuret and Bromocresol green binding method by commercial kits (Pars Azmoon, Karaj, Iran) and globulin were calculated by distracting albumin from total protein. Alanine amino transferase (ALT) and Aspartate amino transferase (AST) were determined with commercial kits (Pars Azmoon, Karaj, Iran). ALT plasma enzyme activity was calculated depending on the concentration of pyruvate hydrazone in 2, 4-dinitrophenyl-hydrazone formula. AST plasma enzyme activity was determined by using AST kit depending on the concentration of oxaloacetate hydrazone in 2, 4-dinitrophenyl-hydrazine. Absorbance of ALT and AST were measured by spectrophotometer at 340 nm.

Statistical Analyses

All data have been expressed as mean ± standard error. All statistical procedures were done using SPSS software (SPSS 18.0, Chicago, IL). Normality of data and homogeneity of variances was checked by the Shapiro–Wilk and Levene's tests, respectively. After that, Two-Way analysis of variance (ANOVA) was followed by Duncan post-hoc was used to determine significant differences between experimental groups. The value $P<0.05$ was used as the criterion for statistical significance.

Results

Growth indices in hybrid sturgeon fed by dietary OFO and GTE are described in table 2. CF was no affected by OFO ($P>0.05$) and only increased in fish received 100 mg GTE in compare to control ($P<0.05$). No interaction was recorded for GTE and OFO on CF. SGR was not changed significantly in OFO groups ($P>0.05$), while GTE increased this index ($P<0.05$). Also an increase in SGR in groups received both OFO and GTE was recorded ($P<0.05$). Statistical analysis showed that PER influenced by OFO and GTE ($P<0.01$). PER decreased in groups fed by OFO while an increase in GTE groups was recorded

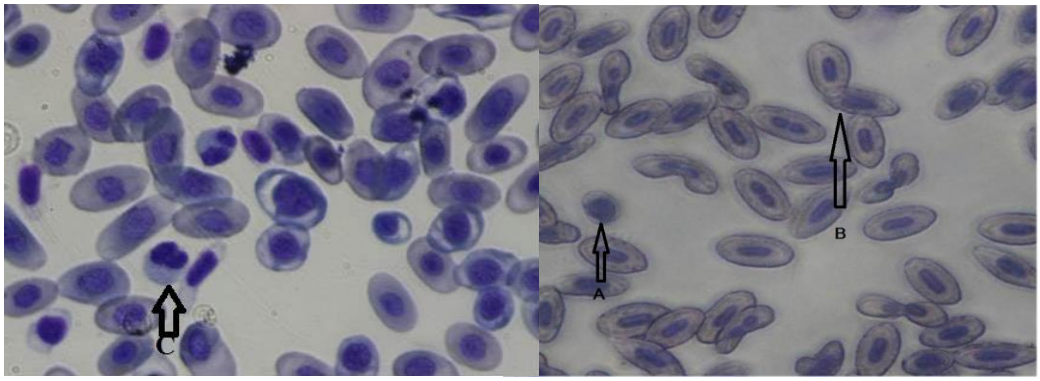

Figure1. Blood smear stained with giemsa. A: lymphocytes, B: red blood cells, C: neutrophil.

(P<0.05). An incrementary pattern was seen in groups received both OFO and GTE (P<0.05). FER decreased in the groups fed diets containing OFO, but increased in GTE groups (P<0.05). GTE counteracted with OFO effects and improved FER (P<0.05).

Effects of OFO and GTE on hematological parameters in sturgeon hybrid is shown in table 3. Dietary OFO and GTE increased RBCs significantly in compare to control (P<0.05). No interaction was recorded between OFO and GTE on RBCs (P>0.05). Hematocrit (Hct) in the groups fed with OFO, showed no significant changes compared to the control group (P>0.05), while in the groups fed by GTE a significant increase was observed (P<0.05), as the highest value of Hct was recorded in fish received 50 mg GTE. Hct was not affected by inclusion of both OFO and GTE in diet. Hemoglobin (Hb) did not show significant difference in experimental groups. Blood indices including MCV, MCH and MCHC did not change significantly in all experimental groups (P>0.05). WBCs in the fish fed only OFO and GTE showed a significant increase in compare to control (P<0.05). Positive interaction of GTE and OFO on WBCs count was recorded in groups received both of them (P<0.05). Differential count of WBC was affected by GTE and OFO content of diet (P<0.05). Dietary GTE caused an increase in neutrophil percentage but OFO resulted in decrease in proportion of neutrophils (P<0.05). A significant increase in lymphocyte percent was shown in OFO and GTE groups (P<0.05), but in groups received both OFO and GTE a significant difference was not recorded (P>0.05).

Changes in immune parameters in fish fed by experimental diets are presented in Table 4. GTE and OFO both increased serum IgM content (P>0.05). This increase was dose dependent in fish received OFO as in OFO100 group, IgM was lower in compare to OFO50 (34 ± 1.52 in compare to 43 ± 0.57 mg/dl). In GTE group an opposite relation was seen as IgM was higher in GT50 in compare to GT100 (45.66 ± 1.45 in compare to 42 ± 1.15 mg/dl). Significant interaction of GTE and OFO on serum IgM was recorded (P<0.05). OFO and GTE both increased ACH50 significantly in compare to control (P<0.05), also incrementory pattern was recorded in groups received both GTE and OFO (P<0.05). Similar pattern was recorded for lysozyme activity, as increase in lysozyme activity was recorded in fish fed by OFO and GTE in compare to control (P>0.05). Interaction between OFO and GTE on serum lysozyme activity was recorded (P<0.05).

The results of biochemical parameters illustrated in table 5. Serum total protein was not affected by OFO or GT, as no significant change was recorded in experimental groups in compare to control (P>0.05). Like total protein, blood albumin and globulin values were not affected by dietary OFO and GTE (P>0.05). Experimental diets had no effect on serum ALT and AST activity (P>0.05). Statistical analysis did show interaction between OFO and GTE only for AST (P<0.05).

Discussion

In this study, was observed that the dietary OFO had no effect on CF, SGR, FER and PER of hybrid sturgeon (P>0.05) but groups fed by diet containing GTE, showed significant increase and fed by diet containing GTE and OFO improvement growth

Table 2. Growth performance and feed utilization in sturgeon hybrid (*Huso huso* ♂ × *Acipenser ruthenus* ♀) fed oxidized fish oil (OFO) and green tea (GT) for 6 weeks.

	W_0 (g)	W_1 (g)	CF	SGR	FER	PER
Control	211.7 ± 0.52	366.15 ± 1.15^{ab}	0.44 ± 0.01^{b}	1.30 ± 0.06^{b}	0.88 ± 0.03^{cd}	1.99 ± 0.05^{cd}
OFO_1	213.4 ± 0.43	369 ± 0.86^{ab}	0.45 ± 0.02^{b}	1.30 ± 0.07^{b}	0.88 ± 0.04^{cd}	1.98 ± 0.03^{cd}
OFO_2	210.3 ± 0.20	364 ± 0.75^{b}	0.46 ± 0.0^{b}	1.30 ± 0.08^{b}	0.87 ± 0.05^{d}	1.97 ± 0.02^{d}
GT_1	211.26 ± 1.1	367.7 ± 2.86^{ab}	0.53 ± 0.02^{a}	1.31 ± 0.06^{a}	0.97 ± 0.03^{a}	2.10 ± 0.02^{b}
GT_2	212.3 ± 0.69	369.9 ± 1.73^{a}	0.45 ± 0.01^{b}	1.31 ± 0.07^{ab}	0.90 ± 0.002^{b}	2 ± 0.01^{cd}
GT_1OFO_2	211.16 ± 0.5	366.13 ± 1.46^{ab}	0.44 ± 0.01^{b}	1.30 ± 0.08^{ab}	0.89 ± 0.01^{c}	2.02 ± 0.06^{c}
GT_1OFO_2	211.9 ± 0.61	367.30 ± 1.46^{ab}	0.45 ± 0.01^{b}	1.32 ± 0.07^{a}	0.98 ± 0.04^{a}	2.16 ± 0.02^{a}
GT_2OFO_1	211.43 ± 0.6	367.33 ± 1.53^{ab}	0.44 ± 0.01^{b}	1.31 ± 0.05^{ab}	0.97 ± 0.04^{a}	2.15 ± 0.01^{ab}
GT_2OFO_2	211.93 ±0.58	367.75 ± 1.45^{ab}	0.43 ± 0.01^{b}	1.31 ± 0.06^{ab}	0.97 ± 0.03^{a}	2.13 ± 0.02^{ab}
Two way ANOVA						
OFO		n. s.	*	*	**	**
GTE		n. s.	n. s.	n. s.	**	**
GT×OFO		n. s.	n. s.	n. s.	**	n. s.

Values are means ± S.E. (n = 3). Asterisks indicate significant differences as * P<0.05 and ** P<0.01. n. s. indicates nonsignificant differences.

Table 3. Serum blood chemical parameters in sturgeon hybrid (*Huso huso* ♂ × *Acipenser ruthenus* ♀) fed oxidized fish oil (OFO) and green tea (GTE) for 6 weeks

	RBC(*1000 in mm^3)	Ht(%)	Hb(g/dl)	WBC(mm^3)	MCV(fi)	MCH(pg)	MCHC(mg/dl)	Monocyte(%)	lymphocyte(%)	Neutrophil (%)
Control	980 ± 5.37^{e}	31.16 ± 0.47^{bcd}	6.9 ± 0.16	6233.3 ± 49.44^{e}	270.67 ± 4.66	65.83 ± 0.79	24.16 ± 0.30	3.66 ± 0.21	69 ± 0.36	26.16 ± 0.30
OFO_1	1196.25 ± 19.83^{bc}	31 ± 0.40^{cd}	7.9 ± 0.42	6325 ± 85.39^{e}	269.5 ± 1.04	66 ± 0.40	25 ± 0.40	3.5 ± 0.22	69.16 ± 0.9	25.83 ± 0.60
OFO_2	1153.83 ± 10.15^{cd}	28.33 ± 0.84^{e}	7.73 ± 0.33	6500 ± 203.3^{e}	273.5 ± 1.89	65.83 ± 0.47	25.33 ± 0.42	2.82 ± 0.16	69.33 ± 0.33	27.33 ± 0.42
GT_1	1141.67 ± 35.36^{cd}	32.16 ± 1.72^{abc}	7.03 ± 0.41	7416.7 ± 98.03^{b}	267.5 ± 4.47	65.5 ± 0.34	24.5 ± 0.34	3 ± 0.40	69.5 ± 0.28	27.25 ± 1.1
GT_2	1280.5 ± 21.61^{a}	33.5 ± 1.22^{a}	7.51 ± 0.34	8050 ± 56.27^{a}	274.5 ± 5.85	66 ± 0.93	24 ± 0.44	3.28 ± 0.28	69.71 ± 0.28	26 ± 0.61
GT_1OFO_1	1253.33 ± 12.71^{ab}	32.83 ± 0.47^{ab}	7.93 ± 0.36	6850 ± 145.48^{d}	267.83 ± 4.36	65 ± 0.81	24.16 ± 0.30	3.2 ± 0.2	70.8 ± 0.2	25.6 ± 0.24
GT_1OFO_2	1109.33 ± 33.13^{d}	30 ± 0.68^{de}	7.33 ± 0.36	7000 ± 57.73^{c}	276.67 ± 2.56	66.66 ± 0.49	24 ± 0.25	3.83 ± 0.40	69.66 ± 0.42	26 ± 0.68
GT_2OFO_1	1138.67 ± 5.11^{cd}	29.83 ± 0.60^{de}	7.08 ± 0.16	7033.3 ± 61.46^{c}	271.17 ± 5.22	65.5 ± 0.84	24 ± 0.36	3 ± 0.36	70 ± 0.25	26 ± 0.57
GT_2OFO_2	1088.33 ± 18.19^{d}	29.66 ± 0.21^{de}	7.03 ± 0.28	8000 ± 51.63^{a}	274 ± 5.18	66.5 ± 0.61	24.16 ± 0.40	3.33 ± 0.33	68.33 ± 0.42	27.33 ± 0.49
Two way ANOVA										
OFO	n. s.	n. s.	n. s.	n. s.	**	n. s.	n. s.	n. s.	*	n. s.
GTE	n. s.	**	n. s.	*	n. s.	n. s.	*	n. s.	n. s.	n. s.
GT×OFO	n. s.	n. s.	n. s.	*	**	n. s.	**	n. s.	n. s.	n. s.

Values are means ± S.E. (n = 3). Asterisks indicate significant differences as * P<0.05 and ** P<0.01. n. s. indicates nonsignificant differences.

Table 4. Hematological indices in sturgeon hybrid (*Huso huso* ♂ × *Acipenser ruthenus* ♀) fed oxidized fish oil (OFO) and green tea (GTE) for 6 weeks

	IgM (mg/dl)	ACH50 (U%)	Lysozyme Activity (u/ml/min)
Control	27 ± 2^{d}	120 ± 1.15^{c}	27.33 ± 2.18^{c}
OFO_1	43 ± 0.57^{ab}	140.67 ± 2.96^{ab}	42.67 ± 1.45^{a}
OFO_2	34 ± 1.52^{c}	134 ± 2.08^{ab}	34.33 ± 2.02^{b}
GT_1	45.66 ± 1.45^{a}	137.33 ± 1.76^{ab}	38 ± 2.08^{ab}
GT_2	42 ± 1.15^{ab}	136 ± 2.64^{b}	43.66 ± 2.33^{a}
GT_1OFO_2	44.66 ± 0.88^{a}	141.67 ± 0.88^{a}	42.66 ± 1.85^{a}
GT_1OFO_2	33 ± 1.15^{bc}	134.67 ± 1.45^{b}	42.33 ± 0.88^{a}
GT_2OFO_1	41.33 ± 2.02^{abc}	135 ± 2.88^{ab}	39 ± 1.73^{ab}
GT_2OFO_2	37 ± 4.72^{bc}	136 ± 1.52^{ab}	39.33 ± 4.25^{ab}
Two way ANOVA			
OFO	**	**	n. s.
GTE	*	n. s.	n. s.
GT×OFO	**	n. s.	*

Values are means ± S.E. (n = 3). Asterisks indicate significant differences as * P<0.05 and ** P<0.01. n. s. indicates nonsignificant differences.

Table 5. Biochemical parameters in sturgeon hybrid (Huso huso ♂ × Acipenser ruthenus ♀) fed oxidized fish oil (OFO) and green tea (GTE) for 6 week

	Total Protein(g/dl)	Albumin(g/dl)	ALT(u/l)	AST(u/l)
Control	2.73 ± 0.35	1.46 ± 0.23^{ab}	3.66 ± 0.33	252.33 ± 18.19^{bc}
OFO_1	2.9 ± 0.35	1.43 ± 0.08^{ab}	4 ± 0.57	270 ± 9.01^{bc}
OFO_2	3.06 ± 0.18	1.26 ± 0.08^{b}	4.33 ± 0.33	340.33 ± 43.7^{a}
GT_1	2.73 ± 0.35	1.53 ± 0.12^{ab}	3.66 ± 0.88	247.33 ± 14.19^{bc}
GT_2	2.33 ± 0.23	1.8 ± 0.15^{a}	3 ± 0.57	224 ± 12.74^{c}
GT_1OFO_2	2.86 ± 0.17	1.53 ± 0.08^{ab}	3.33 ± 0.33	259.67 ± 10.26^{bc}
GT_1OFO_2	2.53 ± 0.29	1.4 ± 0.20^{ab}	4 ± 0.01	265.33 ± 7.51^{bc}
GT_2OFO_1	2.73 ± 0.14	1.46 ± 0.03^{ab}	3.66 ± 0.33	292.33 ± 20.88^{ab}
GT_2OFO_2	2.4 ± 0.15	1.26 ± 0.08^{b}	3.66 ± 0.66	232.67 ± 10.47^{bc}
Two way ANOVA				
OFO	n. s.	*	n. s.	n. s.
GTE	n. s.	n. s.	n. s.	n. s.
GT×OFO	n. s.	n. s.	n. s.	*

Values are means ± S.E. (n = 3). Asterisks indicate significant differences as * P<0.05 and ** P<0.01. n. s. indicates nonsignificant difference

performance in compare to groups which fed received only OFO. This effect of OFO on growth performance is disagreement with the findings in sea bream, *Sparus aurata* (Tocher *et al*. 2003), Atlantic cod, (Zhong *et al*., 2008) and black sea bream, *Spondyliosoma cantharus* (Peng *et al*., 2009) fed different levels of oxidized oils for 8 weeks or more. Tocher *et al*. (2003) reported that OFO slightly stimulated growth of sea bream for 8 weeks. However, in other species including Atlantic salmon (*Salmo salar*) (Koshio, Ackman, & Lall, 1994), African catfish, *Clarias gariepinus* (Baker & Davies, 1997), turbot and halibut (Tocher *et al*. 2003) reduced growth performance caused by dietary OFO after 8 weeks had been reported. Difference in effects of OFO on growth could be related to the amount of oxidized oils in the diet, the level of lipid oxidation, compounds used in the diet as antioxidant (Fontagné *et al*., 2006), fish species, fish size (Tocher *et al*., 2003), duration and tolerance to oxidized oils in the diet (Chen *et al*., 2011) and fat oxidation temperature (Eder & Stangle., 2000). For example, when the POV of oxidized oils in the diet of red sea bream (*Pagrus major*), was higher than 83.8 meq/kg growth was decreased (Gao *et al*., 2012). In other study when that three species of sea fish fed with the same food diets with levels of oxidized oil, sea bream was most resistant to the diets in compare to turbot (*Scophthalmus maximus*), and halibut (*Hippoglossus hippoglossus*), which express the different sensitivities in different species to oxidized oil content of diet (Tocher *et al*., 2003).

In this study GTE in experimental diets, improved growth performance. This phenomena had been showed in some previous studies. Increase in protein retention and improvement of absorption and digestion are possible mechanisms of effects of GTE on growth parameters (Abdel-Tawwab *et al*., 2010; Hwang *et al*., 2012).

Body composition of hybrid sturgeon was not affected by dietary OFO and GTE. Similar results has been reported previously in other species. Hung, Cho, & Slinger (1981) reported that dietary OFO did not affect carcass composition of Rainbow trout,

Oncorhynchus mykiss. Decrease in whole-body crude protein content in black sea bream fed by OFO had been reported, while Vit. E abrogated this phenomena (Peng *et al*., 2009). Dietary GTE could lower lipid content of liver in flounder, *Paralichthys olivaceus* although the body lipid of the fish, without the livers, did not show any change in compare to control (Cho *et al*., 2006). Clinical signs of fatty liver was not seen in experimental groups while after dissection increase in liver weight was recorded in our study.

There is a growing interest in the study of hematological parameters of fish, as an important tool in assessing the physiological conditions for aquaculture purposes (Karimi, Kochinian & Salati, 2013). RBCs and HCT values increased in experimental groups by OFO. There had been a controversy in effects of OFO on RBCs in various studies. Zhong *et al*. (2008) in Atlantic cod, *Gadus morhua*, Lewis-McCrea & Lall (2007) in Atlantic halibut, *Hippoglossus hippoglossus* and Hung *et al*. (1981) in rainbow trout, found that OFO had no effect on these parameters after a long period of feeding. Decreased HCT in fish undergoing oxidative stress had been reported in sea bass (*Dicentrarchus labrax*) and rainbow trout fingerlings (Messager, Stéphan, Quentel, & Baudin Laurencin, 1992). They concluded that feeding with OFO may be resulted to impairment of prooxidant-antioxidant balance in RBCs and therefore decreases the ability of blood oxygen transportation capacity. Increase in number of RBCs in this study could be a compensatory response to impairment of oxygen delivery capacity of RBCs. Also, the controversy in reports could be related to different sensitivities in different species to oxidized oil content of diet, so oxidative stress and therefore changes in RBC related parameters appear in higher value of OFO in some species in compare to other species. GTE in diets increased the HCT, RBC and Hb that was dose-dependent. Similar results were obtained by Abdel-Tawwab *et al*. (2010) in Nile tilapia *Oreochromis niloticus*, which found that RBCs, HCT, and Hb increased significantly with increasing dietary green tea levels compared to control fish. Increase in lymphocytes was seen in fish received OFO and GTE. This could be related to immunostimulatory effects of these compounds which result to increase in number of WBCs.

Humoral immunity such as lysozyme activity functions as a primary defense factor in the preference to cellular defense mechanism when attacked by an invader (Ren *et al*., 2007). In this study, serum lysozyme activity, IgM and ACH50 and serum biochemical parameters including total protein, albumin and ALT and AST showed a significant increase in fish fed with OFO and GTE, respectively. Our finding is in contrast with previous findings. Decrease in serum lysozyme activity in sea bass plasma was recorded when fish fed by OFO and α-tochopherol (Obach, Quentel, & Baudin Laurencin, 1993). Similar results had been recorded in Japanese sea bass, *Lateolabrax japonicas* fed by OFO and palm oil (Han *et al*., 2012). In vertebrates lipid peroxides may not only influence cell membrane function but also lead to the inhibition of certain enzymes that involved in process like immunity (Kaneda & Miyazawa, 1987). Kanazawa, Danno, & Natake (1975) showed that linoleic acid oxidation products may inhibit lysozyme, *in vitro*, through selective destruction of certain amino-acids, including methionine.

There are limited reports that diet containing GTE resulted to enhancement of immune function in rainbow trout (Sheikhzadeh *et al*., 2011) and tilapia (Abdel-Tawwab *et al*., 2010). Thawonsuwan *et al*. (2009) found that Epigallocatechin-3-gallate derivated from green tea could enhance immune function in rainbow trout. They reported that EGCG could possibly be more effective in enhancing serum lysozyme activity and the alternative complement activity.

In Fish fed OFO decrease in ACH50 was recorded. Macrophages are the site of production of some of the complement proteins suggests that an alteration of macrophage function caused by peroxidation of the membrane phospholipid fatty acids may alter the synthesis of some of these proteins (Obach *et al*., 1993). IgM level is determined for evaluation of the humoral immune responses, low levels of IgM related to immunodeficiency (Buckley, 1986), while higher IgM values is associated with inflammatory and pathological conditions (Redman, 1979). There is no information about effects of OFO on fish IgM, but Liu *et al*. (2014) found synthesis of antibodies in pigs fed OFO helped to improve defense system for eliminating the endotoxin. Increase in IgM was in parallel with changes in lymphocyte percentage, as the primary source of IgM secretion in our study.

The mechanism of immunostimulation of GTE is still not clear but it may related to one or more of components, especially catechins, flavonols, flavanones, phenolic acids, glycosides, and the aglycones of plant pigments (Farhoosh *et al*. 2007). These components have powerful natural antioxidants (Farhoosh *et al*. 2007). The usefulness of antioxidants in protecting cellular components against oxidative stress is well established (Mohan *et al*. 2007).

Our findings showed that dietary OFO caused no effects on growth performance, but dietary GTE improved growth performance. OFO and GTE increased WBCs significantly and also the same pattern was recorded for IgM and ACH50. These findings showed that GTE could be used as immunostimulants in sturgeon hybrid and also protected sturgeon hybrid from deleterious effects of OFO.

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References

Abdel-Tawwab, M., Ahmad, M.H., Seden, M.E.A., & Sakr, S.F.M. (2010). Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, (*Oreochromis niloticus*), against *Aeromonas hydrophila* infection. *Journal World Aquaculture Society*, 41, 203-213. http://dx.doi.org/10.1111/j.1749-7345.2010.00360.x

Alves Martins, D., Afonso, L.O.B., Hosoya, S., Lewis-McCrea, L.M., Valente, L.M.P., & Lall, S.P. (2007). Effects of moderately oxidized dietary lipid and the role of vitamin E on the stress response in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture*, 272, 573-580. http://dx.doi.org/10.1016/j.aquaculture.2007.08.044

Baker, R.T.M., & Davis, S.J. (1997). Muscle and hepatic fatty acid profiles and alpha-tocopherol status in African cat fish (*Clarias gariepinus*) given diets varying in oxidative state and vitamin E inclusion level. *Animal Science*, 64, 187-195. http://dx.doi.org/10.1017/S1357729800015708

Buckley, R.H. (1986). Humoral immunodeficiency. *Clinical Immunology and Immunopathology*, 40, 13–24. http://dx.doi.org/10.1016/0090-1229(86)90065-6

Chen, J., Zhu, X., Han, D., Yang, Y., Lei, W., & Xie, S. (2011). Effect of dietary n-3 HUFA on growth performance and tissue fatty acid composition of gibel carp *Carassius auratus gibelio*. *Aquaculture Nutrition*, *17*(2), e476-e485. http://dx.doi.org/10.1111/j.1365-2095.2010.00784.x

Cho, S., Lee, S., Park, B., Ji, S., Lee, J., Bae, J., & Oh, S. (2006). Effect of dietary inclusion of various sources of green tea on growth, body composition and blood chemistry of the juvenile olive flounder, *Paralichthys olivaceus*. *Fish Physiology and Biochemistry*, *33*(1), 49-57. http://dx.doi.org/10.1007/s10695-006-9116-3

Dong, X.L.; Lei, W.; Zhu, X.M.; Han. D.; Yang, Y.X. & Xie, S.Q. (2011). Effects of dietary oxidized fish oil on growth performance and skin color of Chinese longsnout catfish (*Leiocassis longirostris*). *Aquaculture Nutrition* 17, 861-868. http://dx.doi.org/10.1111/j.1365-2095.2011.00854.x

Ebrahimi, V., Salati, A., Azarm, H., & Hasanpour, S. (2015). Effects of dietary green tea (*Camellia sinensis* L) on acute stress responses in sturgeon hybrid (Huso huso ♂ ×Acipenser ruthenus ♀). *Aquaculture Research*, n/a-n/a. http://dx.doi.org/10.1111/are.12908

Eder, K., & Stangle, G.I. (2000). Plasma thyroxine and cholesterol concentrations of miniature pigs are influenced by thermally oxidized dietary lipids. *Journal of Nutrition*, 130, 116-121.

Farhoosh, R., Golmovahhed G.A., & Khodaparast, M.H.H. (2007). Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). *Food Chemistry*, 100, 231-236. http://dx.doi.org/10.1016/j.foodchem.2005.09.046

Fontagné, S., Bazin, D., Brèque, J., Vachot, C., Bernarde, C., Rouault, T., & Bergot, P. (2006). Effects of dietary oxidized lipid and vitamin A on the early development and antioxidant status of Siberian sturgeon (*Acipenser baeri*) larvae. *Aquaculture*, *257*(1-4), 400-411. http://dx.doi.org/10.1016/j.aquaculture.2006.01.025

Gao, J.; Koshio, S.; Ishikawa, M.; Yokoyama S.; Mamauag, R.E.P.; Han, Y. (2012). Effects of dietary oxidized fish oil with vitamin E supplementation on growth performance and reduction of lipid peroxidation in tissues and blood of red sea bream (*Pagrus major*). *Aquaculture* 356, 73-79. http://dx.doi.org/10.1016/j.aquaculture.2012.05.034

Han, Y.Z., Ren, T.J., Jiang, Z.Q., Jiang, B.Q., Gao. J., Koshio. S., & Komilus, C.F. (2012). Effects of palm oil blended with oxidized fish oil on growth performances, hematology, and several immune parameters in juvenile Japanese sea bass, (*Lateolabrax japonicas*). *Fish Physiology and Biochemistry*, 38, 1785-1794. http://dx.doi.org/10.1007/s10695-012-9675-4

Hung, S.S.O., Cho, C.Y. & Slinger, S.J. (1981). Effect of oxidized fish oil, DL-a-tocopheryl acetate and ethoxyquin supplementation on the vitamin E nutrition of rainbow trout (*Salmo gairdneri*) fed practical diets. *Journal of Nutrition*, 111, 648-654.

Hwang, J., Lee, S., Rha, S., Yoon, H., Park, E., Han, K., & Kim, S. (2012). Dietary green tea extract improves growth performance, body composition, and stress recovery in the juvenile black rockfish, *Sebastes schlegeli*. *Aquaculture International*, *21*(3), 525-538. http://dx.doi.org/10.1007/s10499-012-9586-5

Kanazawa, K., Danno, G.I., & Natake, M. (1975). Lysozyme damage caused by secondary degradation products during the autoxidation process of linoleic acid. Journal of Nutritional Science and Vitaminology, 21, 373-382.

Kaneda, T. & Miyazawa, T. (1987). Lipid peroxides and nutrition. *World Review of Nutrition and Dietetics*, 50, 186-214.

Karimi, S., Kochinian, P. & Salati, A.P. (2013). The effect of sexuality on some haematological parameters of the yellowfin seabream, *Acanthopagrus latus* in Persian Gulf. *Iranian Journal of Veterinary Research*, 14(1), 65-68.

Koshio, S., Ackman, R., & Lall, S. (1994). Effects of Oxidized Herring and Canola Oils in Diets on Growth, Survival, and Flavor of Atlantic Salmon, *Salmo salar*. *Journal of Agricultural Food Chemistry*, *42*(5), 1164-1169. http://dx.doi.org/10.1021/jf00041a022

Lewis-McCrea, L.M. & Lall, S.P. (2007) Effects of moderately oxidized dietary lipid and the role of vitamin E on the development of skeletal abnormalities in juvenile Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture*, 262, 142–155. http://dx.doi.org/10.1016/j.aquaculture.2006.09.024

Mohan, K.V., Gunasekaran, P., Varalakshmi, E., Hara, Y., & Nagini, S. (2007). In vitro evaluation of the anticancer effect of lactoferrin and tea polyphenol combination on oral carcinoma cells. *Cell Biology International*, 31, 599–608. http://dx.doi.org/10.1016/j.cellbi.2006.11.034

Messager, J., Stéphan, G., Quentel, C., & Baudin Laurencin, F. (1992). Effects of dietary oxidized fish oil and antioxidant deficiency on histopathology,

haematology, tissue and plasma biochemistry of sea bass *Dicentrarchus labrax*. *Aquatic Living Resources*, *5*(3), 205-214. http://dx.doi.org/10.1051/alr:1992020

Nootash, S.; Sheikhzadeh, N.; Baradaran, B.; Oushani, A.K.; Maleki Moghadam, M.R.; Nofouzi, K.; Monfaredan, A.; Aghebati, L.; Zare, F. & Shabanzadeh, S. (2013). Green tea (*Camellia sinensis*) administration induces expression of immune relevant genes and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology* 35, 1916-1923. http://dx.doi.org/10.1016/j.fsi.2013.09.030

Obach, A., Quentel, C., & Baudin Laurencin, F. (1993). Effects of alpha-tocopherol and dietary oxidized fish oil on the immune response of sea bass *Dicentrarchus labrax*. *Diseases of Aquatic Organisms*, *15*, 175-185. http://dx.doi.org/10.3354/dao015175

Peng, S., Chen, L., Qin, J., Hou, J., Yu, N., Long, Z., LI, E., & YE, J. (2009). Effects of dietary vitamin E supplementation on growth performance, lipid peroxidation and tissue fatty acid composition of black sea bream (*Acanthopagrus schlegeli*) fed oxidized fish oil. *Aquaculture Nutrition*, *15*(3), 329-337. http://dx.doi.org/10.1111/j.1365-2095.2009.00657.x

Redman, D.R. (1979). Parenatal influence on immunocompetence of the neonate. *Journal of Animal Science*, 49, 258-267. http://dx.doi.org/10.2527/jas1979.491258x

Ren, T., Koshio, S., Ishikawa, M., Yokoyama, S., Micheal, F., Uyan, O., & Tung, H. (2007). Influence of dietary vitamin C and bovine lactoferrin on blood chemistry and non-specific immune responses of Japanese eel, Anguilla japonica. *Aquaculture*, *267*(1-4), 31-37. http://dx.doi.org/10.1016/j.aquaculture.2007.03.033

Reyes-Becerril, M., Tovar-Ramírez, D., Ascencio-Valle, F., Civera-Cerecedo, R., Gracia-López, V., & Barbosa-Solomieu, V. (2008). Effects of dietary live yeast *Debaryomyces hansenii* on the immune and antioxidant system in juvenile leopard grouper Mycteroperca rosacea exposed to stress. *Aquaculture*, *280*, 39-44. http://dx.doi.org/10.1016/j.aquaculture.2008.03.056

Salati, A.P., Baghbanzadeh, A., Soltani, M., Peyghan, R. & Riazi, G. (2010). The response of plasma glucose, lactate, protein and hematologic parameters to osmotic challenge in common carp (*Cyprinus carpio*). *International Journal of Veterinary Medicin*e, 4 (1), 49-52

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002). The lipids. In: J.E. Halver & R.W. Hardy (Eds.), *Fish Nutrition* (pp. 181–257), 3rd edition. San Diego, USA, Academic Press.

Shahidi F. & Zhong Y. (2005). Lipid oxidation: measurement methods. In: F. Shahidi (ed.), *Bailey's Industrial Oil and Fat Products*, (pp. 357-386) Vol.1., Hobiken, USA, JohnWiley & Sons.

Sheikhzadeh, N., Nofouzi, K., Delazar, A., & Oushani, A. (2011). Immunomodulatory effects of decaffeinated green tea (Camellia sinensis) on the immune system of rainbow trout (Oncorhynchus mykiss). *Fish & Shellfish Immunology*, *31*(6), 1268-1269. http://dx.doi.org/10.1016/j.fsi.2011.09.010

Thawonsuwan, J., Kiron, V., Satoh, S., Panigrahi, A., & Verlhac, V. (2009). Epigallocatechin-3-gallate (EGCG) affects the antioxidant and immune defense of the rainbow trout, Oncorhynchus mykiss. *Fish Physiology and Biochemistry*, *36*(3), 687-697. http://dx.doi.org/10.1007/s10695-009-9344-4

Tocher, D.R., Mourente, G., Van der Eecken, A., Evjemo, J.O., Diaz, E., Wille, M., Bell, J.G. & Olsen, Y. (2003). Comparative study of antioxidant defence mechanisms in marine fish fed variable levels of oxidized oil and vitamin E. *Aquaculture International*, 11,195-216. http://dx.doi.org/10.1023/a:1024127003997

Yeh, S.P., Chang, C.A., Chang, C.Y., Liu, C.H. & Cheng, W. (2008). Dietary sodium alginate administration affects fingerling growth and resistance to Streptococcus sp. and iridovirus, and juvenile non-specific immune responses of the orange-spotted grouper, *Epinephelus coioides*. *Fish and Shellfish Immunology*, 25, 19-27. http://dx.doi.org/10.1016/j.fsi.2007.11.011

Zhong, Y., Lall, S., & Shahidi, F. (2008). Effects of dietary oxidized oil and vitamin E on the growth, blood parameters and body composition of juvenile Atlantic cod *Gadus morhua* (Linnaeus 1758). *Aquaculture Research*, 1647-1657. http://dx.doi.org/10.1111/j.1365-2109.2008.02038.x