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Research Article

Effects of dietary olive oil and butylated hydroxytoluene (BHT) on growth, blood, and immunity indices in juvenile Persian sturgeon (*Acipenser persicus*)

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Abstract

The present study aimed to investigate the physiological effects of a natural antioxidant, olive oil, and a synthetic antioxidant, butylated hydroxytoluene (BHT), on growth indices, blood and biochemical parameters, and immunity of farmed Persian sturgeons (*Acipenser persicus*). Three hundred and fifteen juveniles Persian sturgeon, with a mean weight of 108±0.02 g, were randomly assigned to seven treatments each with three replicates (15 fish in each replicate) following the adaptation to brackish water of the Caspian Sea. The fish in three replicates were fed with diets containing 1%, 3%, and 5% olive oil (only as an antioxidant) and other treatments were fed with diets containing 100, 150, and 200 (mg per kg diet) of BHT. A group of fish was fed with the basal diet (containing no olive oil or BHT) as the control. The fish were fed 2-3 times a day for two months. At the end of the trial, blood sampling and biometry were done to determine the growth indices and blood indices. The results showed that the lowest FCR and the highest SGR, BWI, and GR were found in fish fed with 3% olive oil. The highest hemoglobin (Hb) concentration and the lowest hematocrit (Hct) level were observed in olive oil 1%, whereas the highest Hct was observed in 200 mg/kg BHT. The results indicated that there was no significant difference between experimental treatments and the control group in terms of MCV, MCH, and MCHC. The highest percent of neutrophil and monocyte and the lowest percent of lymphocyte were observed in olive oil 5%. Eosinophils were observed in groups of 3% and 5% olive oil, and 200 mg/kg BHT in diet, and its highest level was related to 3% olive oil. The highest cholesterol and triglyceride levels were observed in 200 and 150 mg/kg BHT in diet, respectively. The results of the study demonstrated that albumin levels significantly increased in 5% and 100 mg/kg BHT in diet compared to the others and the control group (p<0.05). The lowest glucose level was found in olive oil 1% and 3% and the highest protein level was observed in 5% olive oil and 100 mg/kg BHT in diet. The results showed that the mean blood plasma IgM of fish in diet containing 5% olive oil was significantly higher than the other treatments and control. In addition, CH50 level in 5% olive oil was significantly higher than the other treatments and control group (p<0.05). The study findings generally suggested that the addition of olive oil in the diet of juvenile Persian sturgeon can positively improve their growth indices, blood and biochemical parameters, and immunity.

Keywords: Olive oil, Butylated hydroxytoluene (BHT), Diet, Growth, Biochemical and blood indices, Persian sturgeon

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Introduction

Sturgeons are among the most valuable fish which are found in the world, especially in the Caspian Sea. Sturgeons have been physiologically adapted to grow in the brackish water of the Caspian Sea. Because of unique ecological and biological features, Persian sturgeon (*Acipenser persicus*) is abundantly found and caught in the southern shores of the Caspian Sea (Keyvan, 2003).

Improvement of the quality of diets to fit the nutritional requirements of farmed fish plays a major role in growth performance, prevention of pathogens, and reduction of farming costs (Gabor *et al*., 2012). Antioxidants play a major role in protecting organisms against oxidative stresses. Synthetic chemicals such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used to control of food products spoilage (Jeon, 2002). However, there are great concerns about the use of synthetic antioxidants as food additives (Payghambari *et al*., 2009) because of their adverse effects such as genetic mutations, toxicity, gastrointestinal problems, and carcinogenicity (He and Ackman 2000; Cadun, 2008), as well as their accumulation in the muscles of aquatic organisms (Harikrishnan *et al*., 2010). That is why there is an increasing tendency to use natural antioxidants (Burt, 2004). Nowadays, synthetic antioxidants such as BHT are used as additives in aquaculture to prevent the formation of peroxide radicals of the lipids used as the main source of energy in the feeding of aquatic organisms. BHT is a phenolic and hydrophobic antioxidant that is widely used in the food industry (Sudagar and Zakariaei, 2015). Hydrophilic phenols are the main antioxidant components in olive oil. Olive oil also contains tocopherol and carotenes. The hydrophilic phenols found in olive oil are alcohols and phenolic acids, flavonoids, lignans, and steroids. Olive oil contains strong antioxidants, such as polyphenols and flavonoids, which reduce lipid peroxidation and then oxidative stresses (Servili *et al.*, 2014).

Considering the economic importance of Persian sturgeon and its caviar in aquaculture, the present study aims to compare the effects of different levels of olive oil, as a natural antioxidant, and BHT, as a synthetic antioxidant, on growth, blood and immunity indices in farmed Persian sturgeon.

Materials and methods

Sampling

This study was carried out in Guilan Sturgeon Research Center, located in Chaboksar, using brackish water of the Caspian Sea. To do this, 315 juveniles of Persian sturgeon, with an initial mean weight of 108±0.02 g and an initial length of 32.9±0.01 cm, were selected and distributed in twenty-one 500-liter fiberglass tanks. Fish were fed with a diet containing 11% fat, 18% carbohydrates, and 42% protein during 60 days. The brackish water of the Caspian Sea was transferred to a reservoir pond using an electric pump and then transferred to tanks through a water pipeline. After adaptation of fish and measuring the weight and length,

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the fish were randomly distributed to six experimental treatments and a control group with three replicates (15 fish in each replicate) under the same experimental conditions. Totally, 7 treatments were designed. The tanks were also equipped with a canopy and an aeration system. Olive oil was added to food ingredients, mixed and pletted. The fish in treatments 1, 2, and 3 were fed with the basal diet containing 1%, 3%, and 5% olive oil (Sicuro *et al*., 2009) and those in treatments 4, 5, and 6 were fed with the basal diet containing 100, 150, and 200 mg of BHT per kg of diet (Sudagar *et al.*, 2015). BHT dissolved in canola oil and then added to food ingredients. The fish in the control group were fed with the basal diet (without olive oil and, BHT). All diets were prepared in Guilan Sturgeon Research Center, located in Chaboksar. Considering the environmental conditions, water temperature, and biomass at different times (usually after each biometry), the fish were manually fed 2-3 times per day by 1-3% of their biomass (Faridpak, 2008). In order to maintain the proper water physiochemical conditions, some water parameters such as temperature, dissolved oxygen, salinity, and pH were measured every day. In addition, biometric parameters were measured once a month to determine the feed conversion ratio and growth of fish. For this, a number of fish from each tank were selected for biometry. Feeding was discontinued for 12 hours before and after the biometry to reduce the stress. Moreover, the volume of water in the tanks was reduced by half and all fish were carefully removed out of the tank under hygienic considerations without causing any damage to them.

Growth measurements

After anesthetizing the fish with clove powder (300 mg/liter) (Mohseni *et al*., 2002), they were weighted by a digital scale with an accuracy of 0.01 g (Mehrabi, 2000) and their length was measured on a biometry board. The data were inserted into Excel to form a data bank. At the end of the trial, these data were used to calculate growth parameters and FCR based on the following formula:

1- Feed conversion ratio (FCR) (Ronyai *et al*., 1990): $FCR=F/ (W_t – W_o)$
F= the amount of food consumed by fish
W_o= the mean initial biomass (g)
W_t= the mean final biomass

2- Specific growth rate (SGR) (Ronyai *et al*., 1990): $SGR= (L_{nwt} – L_{nwo}) / t \times 100$
W_o= the mean initial biomass (g)
W_t= the mean final biomass
T = duration of the trial (days)

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3- Body weight increase (BWI%) (Hung *et al*., 1989): $\%BWI = (Bwf - Bwi) / Bwi \times 100$
B_{wi} = the mean initial weight per tank
B_{wf} = the mean final weight per tank

4- Growth rate (GR) (g/day) (Hung *et al*., 1989): $GR = (B_{wf} - B_{wi}) / n$
B_{wi} = the mean initial weight per tank
B_{wf} = the mean final weight per tank
N = duration of the trial (days)

5- Condition factor (CF or K) (Hung and Lutes, 1987): $CF = (B_w / TL^3) \times 100$
B_w = the mean final weight (g)
TL = the mean final total length (cm)

Blood indices measurement

For blood sampling at the end of the trial, two fish were randomly selected from each replicate, and then 2.5 ml of blood was taken from caudal vein by using a 5 ml syringe (Raida *et al*., 2003) and poured into the heparinized Eppendorf tubes. Blood samples were carefully transferred to a laboratory in an ice container. To prepare blood plasma, 1 ml of the blood sample from each replicate was poured into a vial and placed at room temperature for 2 hours to coagulate. Then, it was centrifuged at 3,000 rpm for 10 minutes (Multi-Speed, ALC PK-131) (Panigrahi *et al*., 2005). The plasma was poured into a tube by a micropipette (Transferpett100-1000 µL). The plasma was stored in a freezer at -20°C until further tests (Nafisi Bahabadi *et al*., 2014). Blood biochemical indices were measured by the kits purchased from Pars Azmun and a spectrophotometer (DR600, HACH, made in the US). Red blood cells or erythrocytes were counted using a red melange pipette, Lewis solution, and a neobaur chamber (Simmons, 1997). In addition, white blood cells or leukocytes were counted by using a white melange pipette, Lewis solution, and a neobaur chamber (Simmons, 1997). The hemoglobin concentration was measured by the cyanmethemoglobin method (a spectrophotometric method) at a wavelength of 540 nm (Kazemi *et al*., 2010). The hemoglobin measurement kit of Zistshimi Co. was used for this purpose. The hematocrit level was measured by the microhematocrit method, which is obtained by determining the volume of red blood cells in a certain volume of blood in percentage (Kazemi *et al*., 2010). The mean corpuscular volume (MCV) which refers to the mean volume of red blood cells in femtoliter (fl) calculated by the following equation: MCV=HCT/RBC×100. The mean corpuscular hemoglobin (MCH) denotes the average mass of hemoglobin (Hb) per red blood cell (RBC) in a sample of blood in pictogram per cell (pg/cell) and calculated by the following equation:

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MCH=Hb/RBC×100. The mean corpuscular hemoglobin concentration (MCHC) is a measure of the average concentration of hemoglobin inside a single red blood cell (grams per deciliter). MCHC is calculated by the following equation: MCHC=Hb/hct×100. For differential white blood cell count, appropriate blood smears were prepared and stained by Giemsa staining method. Then the samples were observed under a microscope with a magnification of 100X to count and examine the morphology of white blood cells (Kazemi *et al*., 2010).

Plasma total protein was measured based on the Biuret method at a wavelength of 540 nm (Kazemi *et al*., 2010). The peroxidase-glucose oxidase reaction method (Trinder, 1969) was employed to measure the glucose level (mg/dl) at a wavelength of 500 nm and 37 °C. In addition, cholesterol, triglycerides, and albumin were measured by the cholesterol oxidase method (Burtis and Ashwood, 1994) at a wavelength of 510 nm (Kazemi *et al*., 2010), lipase, glycerol kinase, and peroxidase enzymes (Burtis and Ashwood, 1994) at a wavelength of 510 nm, and bromocresol green (BCG) (Wootton, 1962) at a wavelength of 630 nm (as a fraction of total protein and globulin albumin) (Kumar *et al*., 2005), respectively. The IgM level was measured by the immunoturbidimetry method in a spectrophotometer at a wavelength of 340 nm with distilled water as a blank. Nephelometry was employed to measure CH50. In this technique, parallel monochromatic light is exposed to a solution to disperse antibodies and antigens. The rate of dispersion is directly associated with CH50 (Bahmani and Yousefi Jourdehi, 2017).

Statistical analysis

The normal distribution of data in different treatments and replicates was assessed by the Shapiro-Wilk test. The One-Way analysis of variance (ANOVA) was employed to statistically compare the experimental treatments. After examining the homogeneity of the data in experimental groups, Duncan's Multiple Range Test was used to compare the groups. All statistical analyses were performed in SPSS-20 and all graphs were plotted in Excel-2007.

Results

The results showed that FCR, SGR, BWI and GR were significantly changed after the addition of olive oil to the diet (p<0.05). Accordingly, the lowest FCR and the highest final weight, SGR, BWIand GR were observed in 3% olive oil and exhibited a significant difference with other treatments and the control group. However, there was no significant difference between the experimental treatments and the control in CF (p>0.05) (Table 1).

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Table 1: Mean values (±SD) of growth indices of juveniles Persian sturgeon in different experimental treatments at the end of the trial.

Growth indices	Groups						
		Olive oil (%)			BHT (mg/kg diet)		
	Control	1	2	3	4	5	6
Final weight (g)	168.62±0.76^{g}	212.48±1.42^{b}	238.99±0.68^{a}	207.77±1.27^{c}	178.56±0.77^{f}	193.66±1.86^{cd}	187.43±0.14^{e}
FCR	2.59±0.03^{a}	1.49±0.02^{d}	1.25±0.007^{e}	1.55±0.011^{d}	2.33±0.002^{b}	2.04±0.041^{c}	2.06±0.001^{c}
SGR (%/day)	0.74±0.006^{g}	1.13±0.01^{b}	1.32±0.005^{a}	1.09±0.01^{c}	0.83±0.007^{f}	0.97±0.02^{d}	0.92±0.001^{e}
BWI(%)	56.16±0.56^{g}	96.72±1.30^{b}	120.82±0.68^{a}	93.18±0.72^{c}	65.53±0.66^{f}	78.86±1.58^{d}	73.57±0.02^{e}
GR (g/day)	0.93±0.01^{g}	1.61±0.02^{b}	2.02±0.011^{a}	1.54±0.02^{c}	1.09±0.011^{f}	1.32±0.03^{d}	1.22±0.002^{e}
CF	0.28±0.03	0.30±0.02	0.29±0.005	0.30±0.001	0.32±0.03	0.32±0.01	0.28±0.006

Mismatched letters in each row indicate a significant difference at the 0.05 level of significance. Data are presented as mean ± standard deviation

The results related to blood indices indicated that WBC and RBC were significantly higher in 5% and 1% of olive oil, respectively, than the other treatments (p<0.05). The hemoglobin and hematocrit levels were significantly higher in 1% olive oil than the other treatments. In addition, the lowest hematocrit level was observed in 200 mg/kg BHT in diet and the control group. The highest neutrophils and the lowest lymphocytes percentage were related to 5% olive oil, which exhibited a significant difference with other treatments and the control group. The highest and lowest monocytes percentage were observed in 5% olive oil and 200 mg/kg BHT in diet, respectively. Eosinophils were observed in 3% and 5% olive oil, and 200 mg/kg BHT in diet and the highest level was related to 3% olive oil, which was significantly different from the other treatments and the control (Table 2).

Table 2: Mean values (± SD) of blood parameters of juveniles Persian sturgeon in different experimental treatments at the end of the trial.

Blood parameters	Groups						
		Olive oil (%)			BHT (mg/kg diet)		
	Control	1	2	3	4	5	6
WBC (n/mm^3)	3083.33± 44.09^{d}	3386.67± 46.67^{Cd}	3713.33± 59.26^{c}	3713.33± 59.26^{C}	4210± 58.59^{b}	4210± 58.59^{b}	3420± 41.63^{cd}
RBC (n/mm^3)	510000± 5773.50^{ab}	631666.7± 6009.25^{ab}	496333.3± 4666.67^{b}	609000± 4932.88^{a}	587666.7± 64 89.31^{ab}	534000± 23895.61^{ab}	481333.3± 6333.33^{ab}
Hb (g/dl)	4.23±0.37^{c}	6.3±0.34^{a}	5.33±0.46^{abc}	6.03±0.34^{ab}	5.53±0.52^{abc}	5.46±0.63^{abc}	4.5±0.67^{bc}
Hct (%)	23.33±0.88^{e}	32.33±1.20^{a}	24.33±0.88^{de}	30.33±0.88^{ab}	27.675±0.88^{bc}	26.67±0.88^{cd}	22.33±0.88^{e}
MCV (fl)	480.67±0.88	485.33±0.88	472.33±1.20	486±0.58	479.67±0.88	489.67±0.88	452.67±0.88
MCH (Pg)	94.67±1.20	93.67±0.88	94.67±0.88	94.67±1.20	93.67±0.88	93.33±0.33	90.67±1.20
MCHC (%)	18.37±0.63	20.57±0.89	21.57±0.19	20.67±0.75	19.17±0.77	18.40±0.88	21.23±0.69
Neutrophil (%)	12.67±0.88^{bc}	13.67±0.88^{bc}	14.67±0.88^{ab}	16±0.58^{a}	13±0.58^{bc}	11.67±0.33^{c}	14±0.58^{abc}
Lymphocyte (%)	84.67±0.88^{a}	86.67±0.88^{a}	84.33±1.20^{a}	79±0.58^{b}	82.33±0.88^{a}	83.33±0.88^{a}	82.33±0.88^{a}
Monocyte (%)	3.33±0.88^{c}	4.67±0.88^{ab}	5.33±088^{b}	7±0.58^{a}	5±0.58^{ab}	5.67±0.66^{b}	3±0.58^{c}
Eosinophil (%)	3083.33±44.09^{d}	3386.67±46.67^{Cd}	3713.33±59.26^{C}	3713.33±59.26^{C}	4210±58.59^{b}	-	1.33±0.33^{b}

Mismatched letters in each row indicate a significant difference at the 0.05 level of significance. Data are presented as mean ± standard deviation

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The results indicated that the plasma total protein was significantly higher in 5% olive oil and 100 mg/kg BHT in diet than the other treatments and the control group. In addition, plasma total protein was significantly lower in 200 mg/kg BHT in diet than the other treatments and the control group. The results also demonstrated that the plasma albumin level was significantly higher in 5% olive oil and 100 mg/kg BHT in diet than the other treatments and the control. The lowest plasma albumin level was observed in 200 mg/kg BHT in diet.

The plasma glucose level was significantly lower in 1% and 3% olive oil than the other treatments and the control. The lowest plasma glucose level was found 100 mg/kg BHT in diet and the control group. The plasma cholesterol level was significantly lower in 3% olive oil than all the other groups except the control group and the highest plasma cholesterol level was observed in 200 mg/kg BHT in diet. The study results showed that the plasma triglycerides level was significantly higher in 150 mg/kg BHT in diet than the other treatments and the control (p<0.05) and the lowest plasma triglycerides level was related to treatments 2% and 200 mg/kg BHT in diet. Table 3 presents changes in plasma total protein, cholesterol, triglycerides, glucose, and albumin in different treatments.

Table 3: Mean values (±SD) of blood biochemical parameters of juveniles Persian sturgeon in different experimental treatments at the end of the trial.

Biochemical indices	Groups						
	Olive oil (%)				BHT (mg/kg diet)		
	Control	1	2	3	4	5	6
Total protein (mg/dl)	1.53±0.03 ^{d}	1.79±0.02 ^{c}	1.57±0.03 ^{d}	2.23±0.03 ^{c}	2.21±0.02 ^{b}	1.83±0.03 ^{b}	1.19±0.03 ^{a}
Cholesterol (mg/dl)	75.67±2.33 ^{d}	85.67±2.33 ^{c}	73±1.73 ^{d}	84.67±2.33 ^{c}	95±1.73 ^{b}	97.33±1.76 ^{b}	105.33±1.20 ^{a}
Triglycerides (mg/dl)	124.67±2.91 ^{f}	284±2.64 ^{b}	136.67±2.03 ^{e}	265.67±2.40 ^{c}	246.33±2.33 ^{d}	295.67±2.60 ^{a}	110.33±2.40 ^{g}
Glucose (mg/dl)	55.67±1.45 ^{a}	33±1.73 ^{c}	34.67±2.03 ^{c}	42.33±1.45 ^{b}	55.33±1.76 ^{a}	46.67±2.03 ^{b}	44.33±2.03 ^{b}
Albumin (mg/dl)	0.56±0.02 ^{c}	0.64±0.02 ^{b}	0.56±0.01 ^{c}	0.85±0.02 ^{a}	0.84±0.01 ^{a}	0.68±0.02 ^{b}	0.43±0.02 ^{d}

Mismatched letters in each row indicate a significant difference at the 0.05 level of significance. Data are presented as mean ± standard deviation

The results of immunological indices showed that the plasma IgM level of fish was significantly higher in 5% olive oil than the other treatments and the control. In addition, the plasma CH50 level was significantly higher in 5% than the other treatments and the control (Table 4).

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Table 4: Mean values (±SD) of immunological parameters of juveniles Persian sturgeon in different experimental treatments at the end of the trial.

Biochemical indices	Groups						
	Olive oil (%)				BHT (mg/kg diet)		
	Control	1	2	3	4	5	6
IgM (mg/dl)	45.33±0.88 ^{cd}	43.67±1.20 ^{d}	48.67±0.88 ^{bc}	66.67±0.88 ^{a}	52±2.51 ^{b}	^{b} 51.33±0.88	44±0.58 ^{d}
CH50 (mg/dl)	119.67±1.50 ^{c}	124.67±0.60 ^{bc}	127±0.50 ^{ab}	131.33±3.50 ^{a}	124.33±1 ^{bc}	126±1 ^{ab}	121.67±1 ^{bc}

Mismatched letters in each row indicate a significant difference at the 0.05 level of significance. Data are presented as mean ± standard deviation

Discussion

Feed efficiency and growth are two major economic factors affecting the commercial production of fish species. For the economic justification of feeding, it is necessary to determine the feeding rate and FCR. Water temperature, environmental conditions, and fish selection are among the most important factors affecting fish nutrition and growth and should be taken into the account in determining the feeding rate (Brett, 1979; Bertt and Groves, 1979). Fish farming conditions such as density, temperature, water quality and nutrition can dramatically affect fish growth. Therefore, the use of a proper diet plays an important role in increasing production. Nowadays, dietary supplements are commonly being used in the aquaculture industry to improve health, growth, immunity, and resistance of aquatic organisms (Gatlin and Li, 2004).

Growth parameters

The results of this study demonstrated that the addition of olive oil to the diet of Persian sturgeon improved growth performance and FCR, as it significantly increased the growth indices of fish compared to the control group and BHT treatments. The highest values of growth indices were found in treatment 2 (3% olive oil). This can be attributed to the existence of natural compounds in olive oil that are known as stimulators of digestion, appetite, and immunity. Gisbert *et al*. (2017) reported that the addition of olive oil to the diet of bream increased the growth rate of this species. Sanchez-Muros *et al*. (2003) and Sadek *et al*. (2004) reported the antioxidant effects of olive oil on growth indices of sea bream (*Sparus aurata*) and found that the addition of olive oil increased their growth. Increased growth and disease resistance are among the important factors in aquaculture (Li *et al*., 2005).

Blood indices

Since blood parameters are considered an indicator of health in different species of aquatic organisms, measurement of blood parameters plays a major role in aquaculture (Soliman and Badeaa, 2002). Improvement of physiological parameters in aquatic organisms caused by the enhancement of diet formulation can be traced by the measurement of blood parameters (Nayak *et al*., 2007). Although the study findings showed that the addition of olive oil to the diet was effective in changing blood parameters. It is not possible to accurately and completely decide on the health of individuals of

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the same species based on the available results (Kazemi *et al*., 2012). This is because physiological characteristics of blood and blood plasma, sample size, skill and experience of the sampler (Bani and Haghi, 2011), and sampling stress (being the first or last subject to sample in each tank) can easily affect the values of blood parameters. In this study, the highest mean WBC was observed in fish fed with diets containing olive oil, especially in treatment 3 (5% olive oil). The increased number of WBC actually indicates a better status of the general immune system. It seems that diets containing olive oil positively affect the composition of fatty acids, the production site of WBC, growth performance, and immune system of Persian sturgeon and increased the number of WBC. By contrast, Liberia *et al*. (2020) reported no significant difference between the diets containing 2%, 4%, 6%, and 8% olive pomace in terms of WBC and RBC and stated that the number of blood cells reduced compared to the control group. Harmantepe *et al*. (2015) also observed no significant difference between the fish fed with diets containing olive pomace in WBC and RBC of rainbow trout and juveniles of hybrid tilapia, respectively. This can be attributed to the fact that the active ingredients of olive pomace are eliminated or reduced during the olive oil production process. RBCs, which are responsible for carrying oxygen to respiratory organs of fish, account for the largest group of blood cells in the blood plasma. In this study, the mean number of RBCs was significantly higher in treatments containing 1% and 5% olive oil compared to the other treatments and the control group. This was also true for the mean percentage of hematocrit and hemoglobin level because these two indices are dependent on the number of RBCs (Kazemi *et al*., 2010). Increased number of RBCs, hematocrit, and hemoglobin levels are considered a response to increased metabolic demand in the body (Kazemi *et al*., 2012). The increased number of RBCs also indicates a greater need for oxygen for metabolism (Satheeshkumar *et al*., 2011). As a result, a proper and nutritionally valuable diet can increase RBCs, hemoglobin, and hematocrit (Kori Siakpere *et al*., 2005). Many factors such as age, gender, size, environment, and physiological conditions affect hematological responses in fish (Sowunmi, 2003). Some studies have shown that the hematocrit level in fish increases under the influence of physical stresses (Wendelaar Bonga, 1997), probably due to water absorption in RBCs (Milligan and Wood, 1982). The findings of Malakpour Kolbadinezhad *et al*. (2012) showed that the hematocrit level increased on the first day after stress in the Caspian roach (*Rutilus caspicus*). Yildiz and Uzbilek (2001) also reported that increased water salinity led to an increase in the hematocrit level in grass carp. In this study, the hematocrit level was significantly higher in treatment 1 than the other treatments and the control group. The increased hematocrit level can be attributed to the increased demand for oxygen supply to major

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organs in response to increased metabolism during the stresses (Ruane *et al*., 2001). It seems that the addition of appropriate concentrations of olive oil to the diet provided suitable conditions for metabolism by stimulating the production of RBCs, which was corroborated by the results of growth indices. Based on previous studies, there is a direct relationship between the number of RBCs, hemoglobin level, and hematocrit percentage (Garcia *et al*., 2007; Kazemi *et al*., 2012). This means that the hemoglobin level and hematocrit percentage increase with the increase of RBCs. All of these changes will increase the capacity of carrying dissolved oxygen in the blood to regulate fish oxygen demand (Kazemi *et al*., 2012). The results of this study also confirmed the findings of other researchers. It is noteworthy that all RBC-dependent blood parameters, including MCV, MCH, and MCHC, also change with any change in RBCs, hematocrit percentage, and hemoglobin level based on specific mathematical relations. The reduced number of RBCs increases MCV because MCV is the result of the growth and development of red blood cells and reflects their size and normal or abnormal status of their division. Moreover, the reduced hematocrit percentage reduces MCV and increases of MCHC (Kazemi *et al*., 2012). Lebria *et al*. (2019) studied the effects of different levels of olive pomace (2, 4, 6 and 8%) on growth indices and blood parameters of the goldfish (*Carassius auratus*) and reported that there was a significant difference between the fish fed with the diet containing 2% olive pomace with the other groups in terms of protein and fat efficiency. However, there was no significant difference between treatments in blood parameters such as RBC, WBC, differential percentage of WBC, hemoglobin, hematocrit, MCV, MCH, and MCHC. Considering that the increased MCV, MCH, and MCHC is regarded as a sign of disruption and dysfunction of hematopoietic organs, such as spleen and liver, and the occurrence of poisoning (Munker *et al*., 2007), but no change in the above-mentioned parameters suggests proper conditions of vital organs of the fish fed with diets containing olive oil. Bransden *et al*. (2001) reported that there was no significant difference between the fish fed with plant-based protein sources and the control group in the number of neutrophils in the Atlantic salmon (*Salmo salar*). Jalili *et al*. (2013) also observed no significant difference between individuals of rainbow trout fed with different levels of plant-based proteins in the number of lymphocytes. Total protein is a parameter for assessing the physiological, nutritional, and health status of fish species (Svetina *et al*., 2002). The present results showed that total protein was significantly higher in fish fed with different levels of olive oil and BHT, except for treatment 6, than the control group. The important point here is that the reduced total protein can occur following a decrease in albumin (Tietz, 1986). Based on the study findings, changes in serum total protein were consistent with changes in

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albumin. As the most abundant plasma protein that is made in the liver, albumin is the source of the body's amino acids and contributes to the maintenance of osmotic pressure. Additionally, albumin carries various substances such as bilirubin, calcium, and long-chain fatty acids. Albumin can absorb toxic heavy metals and drugs and thus counteract their toxic effects (Doumas, 1971). Total protein, globulin, and albumin are three major indices used to control and monitor the course of diseases, immune system disorders, and hepatic and renal dysfunctions (Nafisi Bahabadi *et al*., 2014). The results showed that the addition of different levels of olive oil and BHT to the diet of Persian sturgeon significantly increased total protein and albumin compared to the control group. The highest plasma total protein was observed in 5% olive oil and 100 mg/kg BHT in diet, which was significantly different from the other treatments and the control group. The highest albumin level was related to the treatment containing 5% olive oil, which was significantly different from the other treatments (except for the treatment containing 100 g BHT per kg of diet) and the control group. It can be concluded that the increasing plasma albumin level improves the distribution of active ingredients of this antioxidant in fish blood. It has been shown that the addition of the extracts of purple coneflower (*Echinacea purpurea*) (Oskoii *et al*., 2012) to the diet of rainbow trout resulted in a significant increase in the serum level of globulin, albumin, and total protein, which is consistent with the findings of the present study. The glucose level is another blood serum biochemical parameter that can be used as one of the important indicators in determining the physiological status of fish species. The liver acts as an important buffering system for blood glucose. When blood glucose rises to a high concentration after feeding, the insulin secretion also increases and the glucose absorbed from the intestine is immediately converted to glycogen and stored in the liver. During the following few hours when the blood glucose concentration as well as the insulin secretion reduce, the liver breaks down glycogen into the glucose (Kazemi *et al*., 2010). The glucose level is the most variable blood parameter that is greatly affected by stress, manipulation, nutritional status, and sexual maturity (Khanna and Singh, 1971). The glucose levels in this study were more appropriate in treatments containing olive oil compared to BHT treatments and the control group, because the serum glucose level increases in fish suffering from environmental or nutritional stress (Kazemi *et al*., 2010). This indicates the high ability of Persian sturgeon to maintain blood glucose during feeding from a diet containing olive oil. In this study, there was no significant difference between 100 mg/kg BHT in diet and the control group in terms of the blood glucose level. Generally, the blood glucose level significantly reduced in the fish fed with diets containing olive oil compared to those fed with diets containing BHT. Abdelwahab and El-Bahr and (2012)

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studied the effects of black cumin and turmeric, as two plant supplements, on the Asian Sea bass and reported that there was no significant difference between treatments in terms of cholesterol level. Plasma cholesterol is carried by lipoproteins, which are a set of lipids and apolipoproteins. In this study, the highest levels of cholesterol and total protein were observed in treatments with the lowest values of growth indices. These findings showed that proper nutritional factors can reduce stressful conditions and lead to optimal growth. Overally, the cholesterol level significantly reduced in olive oil treatments than BHT treatments. The highest plasma triglyceride level was observed in treatment 5 (150 mg of BHT per kg of diet). Alikhan and Zeb (2019) reported that the serum biochemical parameters and blood parameters improved in the poisoned mice fed with diets containing oxidized olive oil, as they observed a significant decrease in the serum levels of cholesterol and triglyceride. Xiaotao *et al*. (2006) compared the triglyceride levels in Chinese sturgeon (*Acipenser sinensis*) and Amur sturgeon (*Acipenser schrenckii*) and stated that there was a significant difference between these two species. They attributed this difference to the evolution of these two species in different environments; the Chinese sturgeon is an anadromous species, whereas Amur sturgeon is a catadromous fish. As a result, differences in the serum levels of blood parameters can be attributed to the type of fish response to environmental changes. The basal values of each biochemical parameter depend on the individual's adaptation to the environment during the evolution.

Immunological indices

In this study, the IgM level was significantly higher in the fish fed with diets containing 5% olive oil compared to the other treatments and the control group. It seems that certain levels of olive oil and BHT caused to increase the plasma immunoglobulin levels and improve the immune system and growth performance of fish by stimulating the production of WBCs (Gannam and Schrock., 1999). In fact, the serum immunoglobulins are the main component of the humoral immune system and IgM is the major immunoglobulin in fish species (Wilson *et al*., 1997). The complement system (CH50) is one of the major humoral components of non-specific immune system that plays an important role in alerting the immune system and clearing the body of invasive pathogens (Sun *et al*., 2010). The bactericidal activity of the complement system is well known as a key bacterial clearance mechanism in fish and other animals (Ellis, 2001). The activity of the complement alternative pathway is considered a powerful non-specific defense mechanism to protect fish species against a wide range of pathogenic organisms such as bacteria, fungi, viruses, and parasites (Chiu *et al*., 2010). In this study, the CH50 level was significantly higher in 5% olive oil treatment than the other treatments. Although this parameter in the treatment with the optimal growth

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indices was lower than that the 5% olive oil treatment, and there was no significant difference between these two treatments in this regard. In fact, the value of this parameter confirmed the proper performance of diets containing olive oil. This study findings indicated that olive oil has positive effects on the growth and nutritional performance of Persian sturgeon. Since the increasing growth, nutritional efficiency, and immunity are the major goals of aquaculture, it seems that the addition of olive oil to the diet of Persian sturgeon can help to approach these aims.

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