# Partial replacement of wheat flour and corn meal with olive pomace in diet of rainbow trout (*Oncorhynchus mykiss*): effects on growth performance, body composition, hematological parameters and sensory evaluation

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## Abstract

This study was aimed to assess the possibility of partial replacement of wheat flour and corn meal with Olive pomace (OP) in the rainbow trout diet through evaluating the growth, immunological, and hematological indices as well as the flesh quality. To this end, a total of 3600 rainbow trout (weighting 184±0.7 g) were fed with differecnt levels of OP (2, 4, 6, 8 and 10 wt %) for 63 days, besides a control group without OP treatment. The findings exhibited no significant change in the growth indices of the experimental fish groups when compared to the control group. Among the exprimetnal groups, in general, the fish received 10% OP demonstrated the highest alterations. whereas the activity of superoxide dismutase, lysozyme, monocyte and neutrophil considerably increased when compare to the control treatment. Generally, OP inclusion decreased and increased, respectively, the saturated and unsaturated fatty acid contents of both liver and carcass tissues, especially at the higher levels. Taken together, OP could improve both health state and nutritional values of fish and the findings suggested the feasibility of partial OP replacement in the diet of rainbow trout.

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**Keywords:** Olive pomace, dietary replacement, fatty acid, Rainbow trout

## Introduction

Todays, fish and shellfish cultivation is an ever-developing industry with considerable proportion in annual human food production, globally over one hundred million tons per year (Pahlow *et al.,* 2015). In the near future, the development of aquaculture will be depend on the indentification and utilization of easy available and sustainable food resources for aquatics (Kamalam *et al.,* 2017).





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Wheat comprises about 17-20% of the annual worldwide cereal production and is highly included in farmed animals diets (http://www.wheatinitiative.org). It has been estimated that wheat production with the current rate will fall seriously below the human and animal demands by 2050. Therefore, to reduce the dependency of livestock producing sectors on cereal grains such as wheat and corn, agricultural by-products are strongly suggested to be used as substitutional feed component in animal diets (Zangeneh and Torki, 2011).

Olive pomace (OP) is the major by-product of the olive oil extraction industry. This by-product possesses several valuable compounds, including polysaccharides, fatty acids, proteins, and polyphenols (Karantonis *et al*., 2007). Its phenolic compounds have exhibited antimicrobial, antioxidant, and anti-inflammatory features and ,thus, attracted the attention of nutritionists and microbiologists (Pahlow *et al*., 2015). For example, OP was included in the diet of rainbow trout (*Oncorhynchus mykiss*) and its immunological and biological effects were assessed (Khoshkholgh et al., 2013; Sicuro *et al*., 2010). Further studies demonstrated that replacement of OP with wheat flour in the diet of yearling Siberian sturgeon (*Acipenser baerii*) increased the polyunsaturated fatty acid contents (especially C22:6n-3) in the the muscle of the fish recieved 75 g/kg and 100 g/kg OP (Banavreh *et al.,* 2019a) and improved the immunological status of the fish (Banavreh *et al.,* 2019c). Furthermore, OP was utilized as a substitute ingredient for wheat bran in the diet of tilapia (*Oreochromis niloticus*) and findings suggested the feasibility of up to 25% substitution without compromising the growth performance and feed utilization efficiency (Al-asgah *et al.,* 2011).

Given that OP possesses low protein contents (5-10%), like wheat flour and corn meal, it could be used in animal diets to reduce the dependency on wheat flour and corn meal as well as to make cost-effective meat production (Sansoucy, 1985). Hence, the current study was aimed to assess the feasibility OP replacement at different levels with wheat flour and corn meal in a formulated diet for rainbow trout, and the purpose was pursued through analyzing the growth indices, chemical body composition, fatty acid profile, and hematological indices.

## Materials and methods

### Experimental diets

Commercial olive pomace (OP-2000) was purchased from the Hekmatdan Zeyton Morsalin and the utilized fish meal and fish oil were prepared from the Gil Powder Co. (Guilan, Iran). An isonitrogenous (400.94 g/kg) and isolipidic (170.59 g/kg crude lipid) diet was formulated with different partial substitution of OP (0, 20, 40, 60, 80, and 100 g/kg) with wheat flour and corn meal as follows (Table 1). The diets were prepared in the Gilan Vahdat Co. (Rasht, Iran). The feed ingredients were ground and mixed with fish oil before water being added to produce stiff dough (Gatlin *et al.,* 2007). The wet mixture was passed through a grinder and sieved (4 mm in diameter) and dried with an oven at 40°C for 48 hr. The dried pellets were kept in sealed bags at −10°C until




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utilization. Table 2 and 3 illustrate the chemical composition of the experimental OP and fatty acid profile of the trial diets, respectively.

**Table 1.** Formulation and proximate composition of the experimental diets

| Components (%) | Diets | | | | | |
|---|---|---|---|---|---|---|
| | 0%OP | 2%OP | 4%OP | 6%OP | 8%OP | 10%OP |
| Kilka meal | 37 | 37 | 37 | 37 | 37 | 37 |
| Soybean meal | 12 | 12 | 12 | 12 | 12 | 12 |
| Wheat flour | 10 | 9 | 8 | 7 | 6 | 5 |
| Olive pomace | 0 | 2 | 4 | 6 | 8 | 10 |
| Corn meal | 10 | 9 | 8 | 7 | 6 | 5 |
| Meat bone meal | 15 | 15 | 15 | 15 | 15 | 15 |
| Kilka oil | 6.75 | 6.55 | 6.35 | 6.15 | 5.95 | 5.75 |
| Molasses | 2 | 2 | 2 | 2 | 2 | 2 |
| Vitamin premix | 2 | 2 | 2 | 2 | 2 | 2 |
| Mineral mix | 1 | 1 | 1 | 1 | 1 | 1 |
| Vitamin C (coated) | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| L-methionine | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| lysine | 1 | 1 | 1 | 1 | 1 | 1 |
| Sand (filler) | 2.15 | 2.35 | 2.55 | 2.75 | 2.95 | 3.15 |
| **Proximate composition (% as fed)** | | | | | | |
| Moisture | 9.66 | 10.58 | 10.91 | 10.42 | 10.60 | 10.52 |
| Crude protein | 40.94 | 42.24 | 41.81 | 41.75 | 41.78 | 41.75 |
| Crude lipid | 17.59 | 17.27 | 18.00 | 17.96 | 18.03 | 17.79 |
| Ash | 9.44 | 9.03 | 8.69 | 8.72 | 9.50 | 9.014 |

**Table 2.** Chemical composition of the commercial Olive Pomace (OP-2000) included in the experimental diets

| Chemical composition (%) | |
|---|---|
| Dry Matter | 93.57 |
| Crude protein | 11.7 |
| Crude Fat | 11.50 |
| Crude Fiber | 35.00 |
| Crude Ash | 6.20 |

**Table 3.** Fatty acid composition of the experimental diets containing different percentage of OP

| Fatty acids (%) | Diets | | | | | |
|---|---|---|---|---|---|---|
| | 0% OP | 2% OP | 4% OP | 6% OP | 8% OP | 10% OP |
| C14:0 | 3.33 | 2.06 | 2.24 | 2.42 | 2.28 | 2.28 |
| C14:1 | 0.348 | 0.217 | 0.200 | 0.234 | 0.175 | 0.221 |
| C15:0 | 0.901 | 0.478 | 0.503 | 0.551 | 0.500 | 0.551 |
| C16:0 | 23.750 | 22.150 | 23.108 | 22.695 | 23.089 | 22.977 |
| C16:1 | 6.311 | 4.108 | 4.438 | 4.624 | 4.438 | 4.572 |
| C17:0 | 0.825 | 0.717 | 0.644 | 0.657 | 0.639 | 0.755 |
| C17:1 | 0.372 | 0.248 | 0.303 | 0.296 | 0.269 | 0.509 |
| C18:0 | 5.628 | 5.640 | 5.725 | 5.532 | 5.738 | 5.812 |
| C18:1(n-9)Cis | 36.751 | 40.283 | 37.955 | 37.843 | 37.539 | 38.624 |
| C18:1(n-9)Trans | 0.052 | 0.000 | 0.000 | 0.000 | 0.020 | 0.000 |
| C18:2(n-6)Cis | 13.424 | 10.305 | 9.509 | 7.942 | 8.342 | 7.488 |
| C18:2(n-6)Trans | 0.144 | 0.057 | 0.055 | 0.094 | 0.000 | 0.092 |
| C18:3(n-3) ALA | 1.926 | 1.159 | 1.134 | 1.204 | 1.082 | 1.054 |
| C20:0 | 0.590 | 0.334 | 0.382 | 0.335 | 0.310 | 0.344 |
| C20:1 | 1.198 | 0.657 | 0.742 | 0.895 | 0.691 | 0.766 |
| C20:2 | 0.468 | 0.288 | 0.257 | 0.282 | 0.250 | 0.286 |





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| | | | | | | |
|---|---|---|---|---|---|---|
| C20:3 n-9 | 0.258 | 0.159 | 0.137 | 0.182 | 0.109 | 0.143 |
| C20:3 n-3 | 0.309 | 0.188 | 0.129 | 0.225 | 0.185 | 0.238 |
| C21:0 | 0.098 | 0.047 | 0.000 | 0.075 | 0.000 | 0.077 |
| C20:4(n-6) ARA | 0.642 | 0.393 | 0.434 | 0.454 | 0.437 | 0.453 |
| C22:0 | 0.419 | 0.219 | 0.217 | 0.251 | 0.195 | 0.252 |
| C22:1 | 0.138 | 0.115 | 0.065 | 0.127 | 0.107 | 0.134 |
| C20:5(n-3) EPA | 4.595 | 2.512 | 2.808 | 3.194 | 2.830 | 2.989 |
| C22:4 (n-6) DTA | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| C24:0 | 0.496 | 0.222 | 0.281 | 0.339 | 0.247 | 0.312 |
| C22:4 (n-6) | 0.846 | 0.450 | 0.498 | 0.518 | 0.445 | 0.568 |
| C22:5 (n-6) DPA | 0.416 | 0.181 | 0.224 | 0.262 | 0.149 | 0.246 |
| C22:6(n-3) DHA | 12.763 | 8.812 | 8.002 | 8.767 | 7.486 | 8.254 |
| Cholesterol (mg/100 g lipid) | 0.67 | 1.02 | 1.03 | 1.01 | 1.0 | 0.92 |

C18:1n-9, Oleic acid; ALA, alpha-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. (n = 3).

**Experimental fish and feeding management**

The study was conducted at a local fish farm (Dornab Fish Farm, Rudkhan Castle, Iran). A total of 3600 rainbow trout (184±0.7 g) were assigned into six treatments with three replicates, according to the prepared experimental diets. The ponds were supplied with a flow-through natural freshwater with a temperature and a flow rate of 10 ± 3°C and 10 L/s, respectively. Befor starting the main experiment, the fish were fed with the control diet (free of OP) for 14 days and then fasted for two days. For the main expriment, the fish were individually anesthetized with clove powder (300 mg/L; Barij Essence, Iran), weighed, and assigned into eighteen rectangle concrete ponds (n=200 per each one and with a stocking density of 1.1 kg/$m^3$). Three ponds were used as replicates for each treatment. The experimental groups were fed three times a day to an *ad libitum* for 63 days. The trial was carried out according to a guideline accepted by the institutional review board of Guilan University on the care and use of fish in research and testing.

**Evaluation of growth performance and somatic indices**

At the termination of the feeding trial, twenty fish were collected randomly from each replicate, and the following growth indices were determined:

Weight gain (WG, %): 100 × (final weight (g)-initial weight (g))/initial weight (g)

Food conversion ratio (FCR): (dry feed intake (g))/(wet weight gain (g))

Specific growth rate (SGR %/day): [Ln (final body weight, g)-[Ln (initial body weight, g)]/63 × 100

Survival rate (SR, %)= 100 × (final fish number)/(initial fish number)

Condition factor (CF)= final weight (g)/final body length (cm)

Body weight index (BWI, %)= 100 × (final body weight (g)-initial body weight (g))/initial body weight (g)

Hepatosomatic index (HSI, %)= 100 × [liver weight (g)]/[body weight (g)]

Viscera somatic index (VSI, %)= 100 × [viscera weight (g]/[body weight (g)]





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**Hematological assays**

To assess hematological indices, the feeding was stopped for 24 h and then the blood was colleted from nine fish per treatment (three fish per replicate). Briefly, the fish were anesthetized with clove powder and the blood was collected from the caudal vein with heperinized and unheparinized syrings and centrifuged (3000*g*, 5 min) at room temperature. The separated serum was kept at -20 °C until the following analyses. The number of white blood cells (WBCs) and red blood cells (RBCs) were determined according to a previous described method (Blaxhall and Daisley, 1973).

Differential leukocyte counts such as lymphocyte, monocyte, and neutrophil were counted using the Giemsa staining method and blood smears with a light microscope. Hematocrit (Hct) value was calculated using the standard microhematocrit method and described in percentage. Hemoglobin (Hb) was determined using a spectrophotometer (Unico 2100-UV, USA) at 540 nm on (Stoskoph 1993). Moreover, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) were determined using the following equations:

MCHC (g/L)= Hb (g/L)/Hct (%)

MCV (fl)= Hct (%)×10/RBC ($10^{12}$/L)

MCH (pg)= Hb (g/L)/RBC ($10^{12}$/L)

**Blood biochemistry analysis**

The blood biochemical indices including glucose, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, total protein (TP), aspartate transaminase (AST), superoxide dismutase (SOD), and lysozyme were measured using diagnostic commercial kits (Pars Azmoon, Iran) following the manufacturer's protocols.

**Proximate and sensory analyses**

After blood sampling, nine fish per treatment (three fish per replicate) were randomly captured and anesthetized with clove powder (1000 mg/L). To proximate analysis, the fish were deheaded and eviscerated, and the crude protein, crude lipid, moisture, and ash were analyzed according to the standard procedures of AOAC (1990). Brifely, the carcass was minced and oven dried at 105 °C until to reach a constant weight, and the moisture was measured. Ash content was calculated by combusting dry samples in a muffle furnace at 550 °C for 6 hr. Crude protein and lipid were determined using Kjeldahl (Bakhshi, Iran) and Soxhlet (Bakhshi, Iran), respectively. Crude fibre was measured by Fiber Analyzer (Gerhardt, Germany). In addition, the eviscerated and deheaded fish was cooked in an oven for 15 min at 200 °C and immediately presented to a ten panelists. Sensory evaluation was conducted in the same controlled conditions of temperature, light, and humidity. The panelists were asked to score odor, taste, and texture of the fish using a 4-pint hedonic score (Score 4= like extremely, 3= like, 2= dislike, 1= dislike extremely) (Martinsdóttir, 2010; O'Mahony, 1986).

**Fatty acid composition**

The fatty acid profile of the experimental diets, homogenized carcass, and liver tissue were determined according to the Folch method





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(Folch *et al.,* 1957). Briefly, chloroform: methanol (2:1, v/v) as well as butylated hydroxyl (0.01%) were added to the samples to extract the lipids. The fatty acids were transmethylated using methanolic boron trifluoride (Merck, Germany). The FA methyl esters (FAME) were analyzed with a Phillips GC-PU4400 (Cambridge, UK) which was equipped with a capillary column (0.25-µm film thickness, BPX70, 60 m × 0.32 mm ID, SGM, Australia), and a flame ionization detector was adjusted to identify fatty acids. Different FAs was recognized and quantified through comparing the retention time and absorbance area of each FA in FAME standards. Data Apex software was employed to analyze the peak position.

### Statistical analysis

The results were analyzed using the SPSS software version no 16.0 (SPSS, Chicago IL, USA), and the differences were analyzed significant at $p < 0.05$. Variance normality and heterogeneity were tested, and then one-way analysis of variance (ANOVA) was applied. Significant differences between the experimental groups were determined by Tukey, and also Kruskal-Wallis test was used to compare the sensory data. Data are presented as mean ± standard deviation (SD).

## Results

### Growth performance

The growth performance and feed utilization of rainbow trout fed with diets containing various levels of OP are presented in Table 4. No significant difference was observed between the fish fed with the treated diets when compared to the control group. No mortality was recorded during the feeding trial.

**Table 4.** Mean individual somatic and growth indices of the fish fed the diets with different substitutional levels of OP

| Somatic and growth indices | Dietary treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | 2%OP | 4%OP | 6%OP | 8%OP | 10%OP |
| Wi (g) | 181.20±3.18 | 186.20±3.39 | 187.00±3.64 | 182.40±3.63 | 185.00±4.67 | 186.40±5.91 |
| Wf (g) | 397.95±8.30 | 395.05±9.42 | 403.62±9.73 | 397.62±8.83 | 393.87±7.58 | 403.00±8.98 |
| BG (g) | 216.75±5.74 | 208.85±6.40 | 216.62±6.68 | 215.22±6.23 | 208.87±6.12 | 216.60±7.44 |
| SR (%) | 100 | 100 | 100 | 100 | 100 | 100 |
| CF | 1.23±0.28 | 1.30±0.22 | 1.28±0.18 | 1.26±0.25 | 1.31±0.37 | 1.27±0.23 |
| SGR (%) | 1.269±0.11 | 1.213±0.15 | 1.241±0.13 | 1.257±0.19 | 1.219±0.17 | 1.243±0.16 |
| BWI | 119.62±7.20 | 112.16±6.40 | 115.84±5.73 | 117.99±9.42 | 112.90±8.37 | 116.20±6.57 |
| FCR | 1.476±0.05 | 1.532±0.09 | 1.477±0.07 | 1.487±0.07 | 1.532±0.07 | 1.492±0.07 |
| HSI (%) | 1.26±0.03 | 1.49±0.04 | 1.48±0.08 | 1.31±0.04 | 1.51±0.28 | 1.37±0.16 |
| VSI (%) | 11.44±0.20 | 10.47±0.16 | 11.08±0.72 | 11.06±0.31 | 11.93±0.23 | 12.18±0.99 |

Data are means of triplicate determinations. Values are expressed as means ± S.D. Values in the same row with same superscript letters are not significantly different ($p > 0.05$).

### Hematological and biochemical indices

TP, MCV, MCH, and MCHC were uninfluenced by dietary OP ($p > 0.05$; Table 5). However, the levels of Hct, Hb, RBC, LDL, and lymphocyte significantly decreased in fish groups received higer levels of OP ($p < 0.05$), whereas lysozyme, SOD, WBC, monocyte and neutrophil significantly increased ($p < 0.05$). The number of thrombocytes and activity of AST showed fluctuations in response to different levels of OP (i.e., decreased and then increased). However, the amount of triglyceride




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significantly decreased in all of the fish groups received OP. The other parameters showed no change, except for HDL which increased in the group received 8% OP.

**Table 5.** Hematological characteristics and levels of some blood constituents for rainbow trout fed the diets containing different OP levels

| | Dietary treatment | | | | | |
|---|---|---|---|---|---|---|
| | **0%OP** | **2%OP** | **4%OP** | **6%OP** | **8%OP** | **10%OP** |
| Hematocrit (%) | 55.33±1.53$^{a}$ | 53.00±2.00$^{ab}$ | 53.67±2.52$^{ab}$ | 52.67±2.52$^{ab}$ | 51.33±2.08$^{ab}$ | 47.33±2.08$^{b}$ |
| Hemoglobin (g/dL) | 9.01±0.41$^{a}$ | 8.50±0.50$^{ab}$ | 8.47±0.31$^{ab}$ | 8.67±0.25$^{ab}$ | 8.67±0.32$^{ab}$ | 8.01±0.25$^{b}$ |
| Red blood cell ($\times10^{4}$mm$^{3}$) | 159.33±3.5$^{a}$ | 152.17±3.55$^{ab}$ | 153.67±8.50$^{ab}$ | 150.77±6.49$^{b}$ | 150.83±7.29$^{ab}$ | 141.20±3.0$^{c}$ |
| MCV (Fl) | 347.00±6.24 | 344.33±5.69 | 349.67±3.21 | 345.00±4.58 | 344.67±3.06 | 347.00±7.81 |
| MCH (pg) | 56.67±0.58 | 57.00±0.00 | 57.00±1.73 | 57.33±1.15 | 57.33±0.58 | 57.67±0.58 |
| MCHC (g/dL) | 16.00±0.00 | 16.67±0.58 | 16.33±0.58 | 16.00±0.00 | 16.67±0.58 | 16.33±0.58 |
| Cholesterol (mg/dL) | 412.33±12.86 | 379.00±14.42 | 353.67±26.08 | 356.00±36.72 | 349.00±9.00 | 332.67±10.79 |
| Triglyceride (mg/dL) | 304.33±27$^{a}$ | 262.33±10.50$^{b}$ | 263.00±25.24$^{b}$ | 272.00±18.5$^{b}$ | 264.67±8.74$^{b}$ | 265.00±3.0$^{b}$ |
| LDL (mg/dL) | 69.33±3.21$^{a}$ | 62.00±2.00$^{b}$ | 59.00±2.00$^{ab}$ | 57.67±3.79$^{ab}$ | 58.33±1.53$^{ab}$ | 54.67±0.58$^{c}$ |
| HDL (mg/dL) | 73.67±1.53$^{a}$ | 79.00±6.00$^{ab}$ | 75.67±2.08$^{ab}$ | 77.67±2.52$^{ab}$ | 82.33±1.53$^{b}$ | 79.33±1.53$^{a}$ |
| TP (g/dL) | 3.6±0.10 | 3.93±0.15 | 3.80±0.10 | 4.00±0.10 | 3.97±0.15 | 4.03±0.15 |
| Glucose (mg/dL) | 77.67±2.08 | 74.00±3.61 | 73.33±3.79 | 70.33±4.04 | 69.67±1.53 | 71.33±3.21 |
| SOD (u/mL) | 37.83±0.76$^{a}$ | 41.43±2.00$^{ab}$ | 44.90±1.49$^{ab}$ | 49.03±3.91$^{b}$ | 46.80±2.81$^{ab}$ | 49.10±2.00$^{b}$ |
| AST (u/L) | 375.33±35$^{a}$ | 327.33±8.74$^{ab}$ | 289.33±6.03$^{ab}$ | 309.00±11.5$^{ab}$ | 327.33±41.3$^{ab}$ | 232.33±10$^{b}$ |
| Lysozyme (u/ml/min) | 40.00±7.00$^{a}$ | 48.67±10.34$^{a}$ | 53.00±9.17$^{ab}$ | 59.00±8.54$^{ab}$ | 56.00±9.17$^{ab}$ | 72.67±4.04$^{b}$ |
| White blood cell ($\times10^{2}$mm$^{3}$) | 60.67±6.11$^{a}$ | 62.67±6.51$^{ab}$ | 69.00±4.36$^{ab}$ | 70.33±1.53$^{ab}$ | 70.67±1.08$^{ab}$ | 78.67±4.51$^{b}$ |
| Lymphocytes (%) | 75.67±1.53$^{a}$ | 71.33±1.15$^{b}$ | 69.67±0.58$^{b}$ | 71.67±1.53$^{b}$ | 69.00±1.73$^{b}$ | 67.67±1.53$^{b}$ |
| Monocytes (%) | 2.33±0.58$^{a}$ | 3.33±0.58$^{ab}$ | 4.00±1.00$^{ab}$ | 3.67±0.58$^{ab}$ | 4.67±0.58$^{b}$ | 5.00±1.00$^{b}$ |
| Neutrophil | 21.67±1.53$^{a}$ | 24.00±1.00$^{ab}$ | 25.67±0.58$^{ab}$ | 23.67±0.58$^{ab}$ | 25.67±0.58$^{ab}$ | 26.67±0.58$^{b}$ |
| Thrombocytes ($\times10^{3}$mm$^{3}$) | 49.67±8.50$^{a}$ | 37.00±4.36$^{ab}$ | 34.67±5.51$^{ab}$ | 29.33±3.79$^{a}$ | 31.33±4.16$^{a}$ | 34.00±5.57$^{b}$ |

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TP, total protein; SOD, superoxide dismutase; AST, aspartate aminotransferase. Data are means of triplicate determinations. Values are expressed as means ± S.D. Values in the same row with different superscript letters are significantly different ($p < 0.05$). Absence of letters indicates no significant difference between treatments ($p > 0.05$).

### Proximate composition and sensory evaluation

The proximate composition of the fish carcass is presented in Table 6. No difference was observed between the fish fed the diets containing OP and the control group. Table 7 shows the analyzed scores obtained for the color, odor, and taste of the cooked meat. As the substitutional level of OP increase in the formulated fish food, so does the overall acceptability of taste and odor by panelists, with the highest scores for the meat of the experimental group received the highest level of OP (10%; $p < 0.05$). However, the apparent color of the cooked meat of all OP-received groups exhibited no conciderable change when compared to the control group.




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**Table 6.** Proximate composition of eviscerated, and deheaded (% of wet weight) of rainbow trout fed the diets containing different levels of OP

| Proximate composition | Dietary treatment | | | | | |
|---|---|---|---|---|---|---|
| | 0%OP | 2%OP | 4%OP | 6%OP | 8%OP | 10%OP |
| Moisture | 72.44±3.2 | 72.38±4.1 | 72.40±2.9 | 73.49±4.1 | 72.08±3.2 | 72.48±3.7 |
| Crude protein | 16.24±2.2 | 17.23±2.2 | 17.27±3.1 | 16.98±3.5 | 17.20±1.9 | 16.47±3.3 |
| Crude lipid | 9.44±1.5 | 8.65±2.2 | 9.26±2.0 | 8.99±2.9 | 9.64±2.5 | 9.36±2.4 |
| Ash | 2.69±2.7 | 3.07±1.2 | 3.22±1.6 | 3.08±2.1 | 3.62±2.1 | 3.48±1.9 |

Data are means of triplicate determinations. Values are expressed as means ± S.D. Absence of letters indicates no significant difference between treatments ($p > 0.05$).

**Table 7.** Sensory analysis scores for taste, odor, and color of the muscle cooked rainbow trout fed the diets containing different levels of OP (%)

| Diets | taste | odor | color |
|---|---|---|---|
| 0% OP | 22.0 | 23.00 | 29.50 |
| 2% OP | 25.00 | 26.00 | 29.50 |
| 4% OP | 28.00 | 29.00 | 29.50 |
| 6% OP | 34.00 | 32.00 | 29.50 |
| 8% OP | 37.00 | 35.00 | 29.50 |
| 10% OP | 37.00 | 38.00 | 29.50 |

**Fatty acid composition**

Fatty acid (FA) composition of the carcasse and liver samples are presented in Table 8 and 9, respectively. Among the measured saturated fatty acids (SFA), C18:0 showed a demonstrated an increasing trend in the liver tissue but did no regular change in the carcass. As to monounsaturated fatty acids (MUFA), the cis-form of oleic acid (18:1n-9) markedly increased in the liver of the fish treated with OP. Alpha-linoleic acid (C18:3n-3) increased in both carcass and liver of almost all experimental groups received OP compared to control, whereas arachidonic acid (C20:4n-6) significantly decreased ($p < 0.05$) in the liver but remained unchanged in the muscle ($p > 0.05$). The amount of eicosapentaenoic acid (C20:5n-3) tended to decrease in the carcasses of the fish received more than 4% OP, yet with no difference between the treatments. Likewise, no difference was observed in the liver ($p > 0.05$). The percentage of EPA+DHA in liver of the fish received higher OP significantly increased as compared to the control treatment.

**Table 8.** Carcasses (eviscerated and deheaded) fatty acid composition of the fishes fed on the experimental diets containing different levels of OP (%)

| Fatty acids (%) | Diets | | | | | |
|---|---|---|---|---|---|---|
| | 0% OP | 2% OP | 4% OP | 6% OP | 8% OP | 10% OP |
| C14:0 | 1.756±0.04 | 1.515±0.09 | 1.565±0.04 | 1.475±0.04 | 1.474±0.12 | 1.571±0.09 |
| C14:1 | 0.065±0.05 | 0.103±0.04 | 0.123±0.03 | 0.108±0.08 | 0.102±0.04 | 0.113±0.05 |
| C15:0 | 0.380±0.09 | 0.337±0.05 | 0.342±0.06 | 0.326±0.04 | 0.313±0.06 | 0.329±0.07 |
| C16:0 | 18.578±0.09 | 17.767±0.05 | 17.690±0.04 | 17.631±0.07 | 18.020±0.06 | 17.381±0.05 |
| C16:1 | 3.838±0.07 | 3.689±0.03 | 3.958±0.11 | 3.574±0.06 | 3.963±0.89 | 3.873±0.10 |
| C17:0 | 0.566±0.10 | 0.402±0.06 | 0.444±0.08 | 0.443±0.06 | 0.428±0.10 | 0.331±0.09 |
| C17:1 | 0.256±0.05 | 0.235±0.09 | 0.325±0.11 | 0.372±0.04 | 0.270±0.13 | 0.235±0.11 |
| C18:0 | 4.929±0.08 | 4.249±0.06 | 4.871±0.07 | 5.041±0.08 | 3.395±0.07 | 4.851±0.10 |
| C18:1(n-9)Cis | 34.222±0.05$^{b}$ | 34.926±0.07$^{a}$ | 35.982±0.06$^{a}$ | 35.656±0.09$^{a}$ | 36.303±0.05$^{a}$ | 36.381±0.06$^{a}$ |
| C18:1(n-9)Trans | 0.755±0.04$^{b}$ | 0.052±0.03$^{a}$ | 0.046±0.04$^{a}$ | 0.036±0.04$^{a}$ | 0.044±0.05$^{a}$ | 0.039±0.03$^{a}$ |
| C18:2(n-6)Cis | 15.643±0.33 | 17.152±0.08 | 16.869±0.08 | 17.138±0.06 | 15.817±0.11 | 16.970±0.06 |
| C18:2(n-6)Trans | 0.453±0.02 | 0.467±0.03 | 0.398±0.09 | 0.521±0.08 | 0.399±0.11 | 0.484±0.09 |
| C18:3 n-3 | 1.614±0.21 | 1.811±0.21 | 1.686±0.13 | 1.808±0.20 | 1.763±0.19 | 1.775±0.22 |




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| | | | | | | |
|---|---|---|---|---|---|---|
| C20:0 | 0.181±0.09 | 0.168±0.16 | 0.189±0.07 | 0.175±0.08 | 0.188±0.09 | 0.170±0.07 |
| C20:1 | 0.403±0.12 | 0.551±0.09 | 0.451±0.09 | 0.561±0.10 | 0.469±0.09 | 0.462±0.07 |
| C20:2 | 0.840±0.09 | 0.932±0.07 | 0.983±0.07 | 0.986±0.06 | 1.069±0.05 | 1.085±0.16 |
| C20:3 n-9 | 0.094±0.08 | 0.117±0.05 | 0.158±0.07 | 0.109±0.07 | 0.162±0.06 | 0.238±0.07 |
| C20:3 n-3 | 0.998±0.09 | 1.101±0.08 | 1.031±0.07 | 1.195±0.08 | 1.101±0.07 | 1.100±0.06 |
| C21:0 | 0.512±0.08 | 0.722±0.07 | 0.748±0.06 | 0.739±0.06 | 0.674±0.09 | 0.627±0.08 |
| C20:4 n-6 | 0.523±0.12 | 0.656±0.11 | 0.709±0.09 | 0.691±0.06 | 0.661±0.08 | 0.598±0.11 |
| C22:0 | 0.064±0.04$^{a}$ | 0.127±0.08$^{b}$ | 0.146±0.05$^{b}$ | 0.138±0.09$^{b}$ | 0.155±0.04$^{b}$ | 0.134±0.05$^{b}$ |
| C22:1 | 0.066±0.04$^{a}$ | 0.146±0.05$^{ab}$ | 0.211±0.04 $^{b}$ | 0.139±0.03$^{ab}$ | 0.149±0.05 $^{ab}$ | 0.163±0.02$^{b}$ |
| C20:5(n-3) EPA | 1.517±0.19 | 1.403±0.08 | 1.404±0.06 | 1.389±0.13 | 1.327±0.13 | 1.361±0.19 |
| C24:0 | 0.238±0.03$^{a}$ | 0.364±0.04$^{b}$ | 0.357±0.06$^{b}$ | 0.347±0.05$^{b}$ | 0.342±0.05$^{b}$ | 0.362±0.05$^{b}$ |
| C22:4 (n-6) | 0.196±0.09 | 0.266±0.08 | 0.287±0.08 | 0.228±0.07 | 0.269±0.08 | 0.306±0.07 |
| C22:5 (n-6) | 0.393±0.07 | 0.443±0.05 | 0.480±0.04 | 0.422±0.04 | 0.445±0.04 | 0.484±0.06 |
| C22:6(n-3) DHA | 8.156±0.08 | 8.208±0.07 | 8.560±0.09 | 7.787±0.09 | 7.870±0.09 | 8.581±0.09 |
| SFA | 27.204±0.25$^{b}$ | 25.651±0.19$^{a}$ | 26.352±0.24$^{a}$ | 26.315±0.17$^{a}$ | 24.989±0.20$^{a}$ | 25.756±0.21$^{a}$ |
| MUFA | 39.605±0.12 | 39.702±0.16 | 40.885±0.17 | 40.446±0.15 | 41.300±0.21 | 41.266±0.18 |
| PUFA | 30.427±0.12$^{b}$ | 32.556±0.21$^{a}$ | 32.565±0.16$^{a}$ | 32.174±0.13$^{a}$ | 30.883±0.14$^{a}$ | 32.982±0.14$^{a}$ |
| EPA+DHA | 9.673±0.08 | 9.611±0.05 | 9.964±0.07 | 9.176±0.11 | 9.197±0.11 | 9.942±0.14 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Data are means of triplicate determinations. Values are expressed as means ± S.D. Values in the same row with different superscript letters are significantly different ($p < 0.05$). Absence of letters indicates no significant difference between treatments ($p > 0.05$).

**Table 9.** Liver fatty acid composition of the fishes fed on the experimental diets containing different levels of OP (%)

| **Fatty acids** | **Diets** | | | | | |
|---|---|---|---|---|---|---|
| | **0% OP** | **2% OP** | **4% OP** | **6% OP** | **8% OP** | **10% OP** |
| C14:0 | 1.124±0.08 | 1.042±0.08 | 1.041±0.07 | 0.979±0.09 | 0.999±0.05 | 1.03±0.04 |
| C14:1 | ND | ND | ND | ND | ND | ND |
| C15:0 | 0.254±0.08 | 0.239±0.07 | 0.219±0.05 | 0.255±0.06 | 0.225±0.06 | 0.179±0.06 |
| C16:0 | 18.716±0.19$^{a}$ | 18.333±0.19$^{a}$ | 18.664±0.14$^{a}$ | 16.376±0.15$^{c}$ | 16.678±0.12$^{c}$ | 16.093±0.13$^{c}$ |
| C16:1 | 2.599±0.10$^{a}$ | 2.439±0.11$^{a}$ | 2.674±0.08$^{a}$ | 2.650±0.11$^{a}$ | 1.984±0.12$^{b}$ | 2.080±0.12$^{b}$ |
| C17:0 | 0.370±0.08$^{a}$ | 0.372±0.07$^{a}$ | 0.372±0.05$^{a}$ | 0.470±0.06$^{b}$ | 0.374±0.06$^{a}$ | 0.560±0.05$^{c}$ |
| C17:1 | 0.220±0.05 | 0.250±0.05 | 0.179±0.19 | 0.202±0.09 | 0.212±0.06 | 0.213±0.05 |
| C18:0 | 6.875±0.10$^{a}$ | 7.208±0.08$^{a}$ | 7.236±0.09$^{b}$ | 8.890±0.06$^{b}$ | 7.961±0.07$^{b}$ | 8.216±0.07$^{b}$ |
| C18:1(n-9)Cis | 17.832±0.06$^{a}$ | 18.096±0.05$^{a}$ | 19.442±0.09$^{b}$ | 19.868±0.06$^{b}$ | 19.469±0.12$^{b}$ | 20.248±0.14$^{c}$ |
| C18:1(n-9)Trans | 2.503±0.10 | 2.477±0.08 | 2.238±0.11 | 2.218±0.18 | 2.474±0.09 | 2.372±0.13 |
| C18:2(n-6)Cis | 6.012±0.10$^{a}$ | 5.184±0.14$^{a}$ | 5.383±0.12$^{a}$ | 5.886±0.15$^{a}$ | 4.862±0.18$^{b}$ | 3.898±0.07$^{c}$ |
| C18:2(n-6)Trans | 0.070±0.06 | 0.076±0.06 | 0.080±0.06 | 0.110±0.07 | 0.074±0.06 | 0.120±0.09 |
| C18:3(n-3) ALA | 0.215±0.07 | 0.255±0.07 | 0.227±0.06 | 0.322±0.08 | 0.253±0.07 | 0.251±0.07 |
| C20:0 | ND | ND | ND | ND | ND | ND |
| C20:1 | ND | ND | ND | ND | ND | ND |
| C20:2 | 1.784±0.10$^{b}$ | 1.858±0.10$^{b}$ | 1.620±0.13$^{c}$ | 1.890±0.12$^{b}$ | 2.111±0.16$^{a}$ | 2.312±0.15$^{a}$ |
| C20:3 n-9 | 0.719±0.20$^{a}$ | 0.173±0.10$^{b}$ | 0.136±0.11$^{b}$ | 0.162±0.10$^{b}$ | 0.200±0.12$^{b}$ | 0.177±0.11$^{b}$ |
| C20:3 n-3 | 1.558±0.08 | 1.510±0.08 | 1.441±0.09 | 1.403±0.09 | 1.539±0.09 | 1.464±0.09 |
| C21:0 | 0.690±0.07 | 0.613±0.07 | 0.654±0.06 | 0.681±0.07 | 0.747±0.06 | 0.556±0.09 |
| C20:4(n-6) ARA | 4.756±0.02$^{a}$ | 3.977±0.04$^{b}$ | 3.534±0.04$^{b}$ | 3.605±0.05$^{b}$ | 3.991±0.03$^{b}$ | 3.692±0.07$^{b}$ |
| C22:0 | 0.272±0.05 | 0.210±0.09 | 0.316±0.09 | 0.308±0.09 | 0.260±0.08 | 0.272±0.08 |
| C22:1 | 0.181±0.07 | 0.192±0.07 | 0.203±0.08 | 0.088±0.07 | 0.248±0.08 | 0.179±0.09 |
| C20:5(n-3) EPA | 2.752±0.07 | 3.271±0.09 | 2.977±0.07 | 2.930±0.07 | 2.726±0.07 | 2.784±0.09 |
| C22:4 n-6 | 1.156±0.07 | 1.062±0.08 | 1.042±0.09 | 1.045±0.07 | 1.132±0.08 | 0.957±0.08 |
| C24:0 | 0.824±0.05 | 0.767±0.06 | 0.751±0.06 | 0.832±0.07 | 0.828±0.07 | 0.929±0.09 |
| C22:4 n-6 | 0.953±0.06 | 1.210±0.09 | 1.035±0.09 | 1.298±0.13 | 1.362±0.09 | 1.053±0.09 |
| C22:5 n-6 | 0.565±0.06 | 0.666±0.07 | 0.616±0.07 | 0.568±0.07 | 0.620±0.07 | 0.641±0.08 |
| C22:6(n-3) DHA | 27.106±0.07 | 28.600±0.07 | 28.428±0.07 | 28.480±0.07 | 28.796±0.07 | 29.126±0.06 |
| SFA | 29.125±0.13 | 28.784±0.13 | 29.253±0.15 | 28.791±0.13 | 28.072±0.15 | 27.835±0.16 |
| MUFA | 23.154±0.10 | 23.262±0.14 | 24.533±0.16 | 24.938±0.14 | 24.139±0.15 | 24.913±0.12 |
| PUFA | 47.646±0.12 | 47.842±0.15 | 46.519±0.11 | 47.699±0.13 | 47.666±0.14 | 47.475±0.12 |
| EPA+DHA | 29.858±0.07$^{a}$ | 31.871±0.08$^{b}$ | 31.405±0.07$^{b}$ | 31.41±0.07$^{b}$ | 31.522±0.07$^{b}$ | 31.91±0.07$^{b}$ |

ALA, alpha-linolenic acid; ARA, arachidonic acid. ND, not detected Data are means of triplicate determinations. Values are expressed as means ± S.D. Values in the same row with different superscript letters are significantly different ($p < 0.05$). Absence of letters indicates no significant difference between treatments ($p > 0.05$).




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## Discussion

The present study was aimed to valorize OP as a partial substitution of wheat flour and corn meal in the diet of rainbow trout and evaluated its effects on some biological parameters, including growth performance, hematological and biochemical indices, as well as sensory evaluation. The results illustrated no significant changes in the growth performance and fed utilization in the experimental fish fed the diets containing OP compared to the control group.Moreover, similar feed conversion ratio and specific growth rate was observed between the control diet and those containing OP. Harmantepe *et al*. (2015) showed that up to 12% olive cake (OC) induced no addvers effects on the growth performance and FCR in juvenile hybrid tilapia *(Oreochromis niloticus* × *Oreochromis aereus)*. Another supportive research by Banavreh *et al*. (2019a) demonstrated that wheat flour could be replaced with OP up to 100 g/kg diet without no conciderable difference in the final weight, FCR, FI, SGR, and CF between the treated fish and the control group.

Blood biochemistry indices reveal the health and nutritional status of fish ( Chen *et al*., 2018; Dadras *et al.,* 2016). Harmantepe *et al*. (2016) stated that olive cake induce no negative effect on hematological factors such as RBC, WBC, Hct and biochemical factors such as glucose, total protein, HDL and LDL. However, high proportion of olive cake significantly increased the cholesterol and triglyceride levels in hybrid tilapia (*Oreochromis niliticus*×*Oreochromis aereus*). In the present study, hematocrit and hemoglobin showed an OP-depenent decrease, with being the lowest levels in the fish recieved the highest OP levels, and these findings could be attributed to higher amount of tannin in the diet containing 100 g/kg OP which, in turn, reduces the iron absorbtion. This findings are in consistent with those reported by Francis *et al*. (2001) who showed that tannin interfere the binding of minerals. This polyphenolic compound functions as an immunostimulant and antioxidant and thereby improving the immune system (Banavreh *et al*., 2019b; Cioffi *et al*., 2010; Dadras *et al*., 2016; Dal Bosco *et al*., 2012). However, WBCs increased following increasing the concentration of OP, and the data is differ from that reported by Harmantepe *et al.* (2016) and Banavreh *et al*. (2019b) who reported no conciderable change in WBC counts following increasing the concentration of diatry OP. The variation in obtained results might be due to the different immune systems between fish species. Nevertheless, Notash *et al* (2013) suggested that polyphenols in green tea induce a direct influence on antioxidant defense system of rainbow trout. Support for this suggestion have come from the observed increase in SOD activity at higher OP concentrations, especially in the $OP_{10}$ group.

Previous studies demonstrated that diets containing plant-derived ingredients reduce the plasma LDL in rainbow trout, because of reducing LDL receptor gene expression in the liver (Richard *et al.,* 2006; Richard *et al.,* 2006).





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In the present study, serum LDL decreased with the increasing dietary OP, but HDL increased and the findings indirectly indicate that the increase of cellular lipid and metabolism as an energy source improved the growth performance through saving protein. However, Harmantepe *et al*. (2015) observed no significant difference between serum LDL and HDL values in juvenile hybrid tilapia.

Lysozyme is an important defensive molecule of the fish innate immune system and prevents biofilm formation as well as adherence and colonization of exogenous gram-positive and gram-negative pathogens (Chen *et al*., 2017; Dadras *et al*., 2016). The current research revealed that the diet containing the highet level of OP enhanced the serum lysozyme activity, suggesting different effect of polyphenols on the immune system of the treated fish.

The lipid composition of fish tissues is mirror the diet composition (Hosseini *et al*., 2010; Lin and Cheng, 2017; Nasopoulou, Stamatakis, Demopoulos, and Zabetakis, 2011). The present study demonstrated that liver DHA content increased in the fish treated with OP. This finding is supported by desaturation and elongation of C18:3n-3 to C18:4n-3, EPA to DHA, and linoleic acid to arachidonic acid, a well-established phenomenon in freshwater fish species (Almaida-Pagán *et al*., 2007; Li *et al*., 2016; Palmegiano *et al*., 2005; Sargent *et al.,* 1992). Our results corroborate the finding of a previous research which was conducted on Siberian sturgeon (Banavreh *et al*., 2019a). Some FAs such as ALA (18:3n-3) and 20:4n-6 increased in the fish received OP, although the diets containing OP exhibited lower levels of ALA compared to the control diet. This difference could be attributed to different responses between organs to dietary lipid composition (Castell *et al.,* 1972).

Sensory evaluation of the fish fed the diets containing OP illustrated an increasing taste and odor acceptability by increasing OP percentage; hence, the observed acceptability could be related to the high presence of phenolic compounds, especially oleuropein which inhibit muscle lipid oxidation and the strong off-smell in trout fillet (Balasundram *et al.,* 2006; Khoshkholgh *et al*., 2013; Pazos *et al.,* 2006; Sicuro *et al*., 2010) and, in turn, increasing the taste and odor of the fish with higher intake of OP. The present study showed that it is possible to partially replace wheat flour and corn meal with OP without compromising growth or nutrient utilization in rainbow trout, and OP significantly enhances non-specific immune parameters in fish. Moreover, this plant-originated by-product increases some of the nutritionally valuable unsaturated fatty acids in flesh fish and thereby could enhance the overall acceptability of its odor and taste.

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## Conflicts of interest

None of the authors has any conflicts of interest to declare.

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