

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

M. Khoshkholgh^{1*}, M. Mosapour Shajani¹, M. Mohammadi¹

¹ Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran

Received: April 2020

Accepted: August 2020

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 different 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 experimental 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 compared to the control treatment.

***Correspondence** M. Khoshkholgh, Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran (e-mail: majidreza@guilan.ac.ir).

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.

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 identification and utilization of easy available and sustainable food resources for aquatics (Kamalam *et al.*, 2017).

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 muscle of the fish received 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 of 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

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

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 $10 \pm 3^\circ\text{C}$ and 10 L/s, respectively. Before 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 experiment, 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/m³). 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, %): $100 \times (\text{final weight (g)} - \text{initial weight (g)})/\text{initial weight (g)}$

Food conversion ratio (FCR): $(\text{dry feed intake (g)})/(\text{wet weight gain (g)})$

Specific growth rate (SGR %/day): $[\text{Ln (final body weight, g)} - \text{Ln (initial body weight, g)}]/63 \times 100$

Survival rate (SR, %): $100 \times (\text{final fish number})/(\text{initial fish number})$

Condition factor (CF): $\text{final weight (g)}/\text{final body length (cm)}$

Body weight index (BWI, %): $100 \times (\text{final body weight (g)} - \text{initial body weight (g)})/\text{initial body weight (g)}$

Hepatosomatic index (HSI, %): $100 \times [\text{liver weight (g)}]/[\text{body weight (g)}]$

Viscera somatic index (VSI, %): $100 \times [\text{viscera weight (g)}]/[\text{body weight (g)}]$

Hematological assays

To assess hematological indices, the feeding was stopped for 24 h and then the blood was collected 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 heparinized and unheparinized syringes and centrifuged (3000g, 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:

$$\text{MCHC (g/L)} = \text{Hb (g/L)} / \text{Hct (\%)}$$

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

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

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). Briefly, 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-point 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

(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- μ m film thickness, BPX70, 60 m \times 0.32 mm ID, SGM, Australia), and a flame ionization detector was adjusted to identify fatty acids. Different FAs were 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 \pm 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 \pm 3.18	186.20 \pm 3.39	187.00 \pm 3.64	182.40 \pm 3.63	185.00 \pm 4.67	186.40 \pm 5.91
Wf (g)	397.95 \pm 8.30	395.05 \pm 9.42	403.62 \pm 9.73	397.62 \pm 8.83	393.87 \pm 7.58	403.00 \pm 8.98
BG (g)	216.75 \pm 5.74	208.85 \pm 6.40	216.62 \pm 6.68	215.22 \pm 6.23	208.87 \pm 6.12	216.60 \pm 7.44
SR (%)	100	100	100	100	100	100
CF	1.23 \pm 0.28	1.30 \pm 0.22	1.28 \pm 0.18	1.26 \pm 0.25	1.31 \pm 0.37	1.27 \pm 0.23
SGR (%)	1.269 \pm 0.11	1.213 \pm 0.15	1.241 \pm 0.13	1.257 \pm 0.19	1.219 \pm 0.17	1.243 \pm 0.16
BWI	119.62 \pm 7.20	112.16 \pm 6.40	115.84 \pm 5.73	117.99 \pm 9.42	112.90 \pm 8.37	116.20 \pm 6.57
FCR	1.476 \pm 0.05	1.532 \pm 0.09	1.477 \pm 0.07	1.487 \pm 0.07	1.532 \pm 0.07	1.492 \pm 0.07
HSI (%)	1.26 \pm 0.03	1.49 \pm 0.04	1.48 \pm 0.08	1.31 \pm 0.04	1.51 \pm 0.28	1.37 \pm 0.16
VSI (%)	11.44 \pm 0.20	10.47 \pm 0.16	11.08 \pm 0.72	11.06 \pm 0.31	11.93 \pm 0.23	12.18 \pm 0.99

Data are means of triplicate determinations. Values are expressed as means \pm 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 higher 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

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 ($\times 10^4$ 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 ($\times 10^2$ 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 ($\times 10^3$ 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 \pm 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 considerable change when compared to the control group.

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

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 ($p > 0.05$).

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 feed 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 adverse 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 considerable 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 niloticus* × *Oreochromis aereus*).

In the present study, hematocrit and hemoglobin showed an OP-dependent decrease, with being the lowest levels in the fish received 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 absorption. These 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 considerable change in WBC counts following increasing the concentration of dietary 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 OP₁₀ 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).

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 highest 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.

Acknowledgements

This research was funded by the Ministry of Science, Research and Technology, Iran (No. 1648-15). The authors would like to thank Guilan University for laboratory services. Our appreciation is extended to "Hamid Salari Joo" for his Academic English Language Editing Service during the preparation of the manuscript.

Conflicts of interest

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

References

Association of Official Analytical Chemists. 1990. Official methods of official analytical chemist international (15th ed). Arlington, VA, USA.

Al-asgah, N., Younis, E. ., Abdel-Warith, A. A., El-Khaldy, A. A. and Amanat, A., 2011. Effect of Feeding Olive Waste on Growth Performance and Muscle Composition of Nile Tilapia (*Oreochromis niloticus*). *International Journal of Agriculture and Biology*, 13(2), 239–244.

Almaida-Pagán, P. F., Hernández, M. D., García García, B., Madrid, J. A., De Costa, J. and Mendiola, P., 2007. Effects of total replacement of fish oil by vegetable oils on n-3 and n-6 polyunsaturated fatty acid desaturation and elongation in sharpsnout seabream (*Diplodus puntazzo*) hepatocytes and enterocytes. *Aquaculture*, 272(1–4), 589–598.

Balasundram, N., Sundram, K., and Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99(1), 191–203. <https://doi.org/10.1016/j.foodchem.2005.07.042>

Banavreh, A., Soltani, M., Kamali, A., Yazdani-Sadati, M. A. and Shamsaie, M., 2019a. Effects of olive pomace on growth performance, digestibility, body composition and fatty acid profile in yearling Siberian sturgeon, *Acipenser baerii* (Brandt 1896). *Aquaculture Nutrition*, 25(2), 333–342. <https://doi.org/10.1111/anu.12858>

Banavreh, A., Soltani, M., Kamali, A., Yazdani-Sadati, M. A. and Shamsaie, M., 2019b. Effects of olive pomace on growth performance, digestibility, body composition and fatty acid profile in yearling Siberian sturgeon, *Acipenser baerii* (Brandt 1896). *Aquaculture Nutrition*, 25(2), 333–342. <https://doi.org/10.1111/anu.12858>

Banavreh, A., Soltani, M., Kamali, A., Yazdani-Sadati, M. A., and Shamsaie, M. 2019c. Immuno-physiological and antioxidant responses of Siberian sturgeon (*Acipenser baerii*) fed with different levels of olive pomace. *Fish Physiology and Biochemistry*, 45(4), 1419–1429. <https://doi.org/10.1007/s10695-019-00649-y>

Blaxhall, P. C., and Daisley, K. W., 1973. Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5(6), 771–781. <https://doi.org/10.1111/j.1095-8649.1973.tb04510.x>

Castell, J. D., Lee, D. J., and Sinnhuber, R. O., 1972. Essential Fatty Acids in the Diet of Rainbow Trout (*Salmo gairdneri*): Lipid Metabolism and Fatty Acid Composition. *The Journal of Nutrition*, 102(1), 93–99. <https://doi.org/10.1093/jn/102.1.93>

Chen, S.J., Guo, Y.C., Espe, M., Yang, F., Fang, W.P., Wan, M.G., Niu, J., Liu, Y.J. and Tian, L.X., 2018. Growth performance, haematological parameters, antioxidant status and salinity stress tolerance of juvenile Pacific white shrimp (*Litopenaeus vannamei*) fed different levels of dietary myo-inositol. *Aquaculture Nutrition*, 24(5), 1527-1539. <https://doi.org/10.1111/anu.12690>

Chen, Y., Li, M., Yuan, L., Xie, Y., Li, B., Xu, W., Meng, F. and Wang, R., 2017. Growth, blood health, antioxidant status and immune response in juvenile yellow catfish *Pelteobagrus fulvidraco* exposed to α -ethinylestradiol (EE2). *Fish and Shellfish Immunology*, 69, 1-5. <https://doi.org/10.1016/j.fsi.2017.08.003>

Cioffi, G., Pesca, M. S., De Caprariis, P., Braca, A., Severino, L., and De Tommasi, N., 2010. Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. *Food Chemistry*, 121(1), 105-111.

Dadras, H., Hayatbakhsh, M. R., Shelton, W. L., and Golpour, A., 2016. Effects of dietary administration of Rose hip and Safflower on growth performance, haematological, biochemical parameters and innate immune response of Beluga, *Huso huso* (Linnaeus, 1758). *Fish and Shellfish Immunology*, 59(November), 109-114.

Dal Bosco, A., Mourvaki, E., Cardinali, R., Servili, M., Sebastiani, B., Ruggeri, S., Mattioli, S., Taticchi, A., Esposto, S. and

Castellini, C., 2012. Effect of dietary supplementation with olive pomaces on the performance and meat quality of growing rabbits. *Meat Science*, 92(4), 783-788. <https://doi.org/10.1016/j.meatsci.2012.07.001>

Folch, J., Lees, M., and Stanley, G. H. S., 1957. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *Journal of Biological Chemistry*, 497-509. Retrieved from <http://ci.nii.ac.jp/naid/10005446045/>

Gatlin D. M, Barrows F. T, Brown P., Dabrowski K., Gaylord T. G., Hardy R.W. and Wurtele E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture research*, 38(6):551-579. <https://doi.org/10.1111/j.13652109.2007.01704.x>

Hosseini, S. V., Kenari, A. A., Regenstein, J. M., Rezaei, M., Nazari, R. M., Moghaddasi, M., Kaboli, S. A. and Grant, A. A., 2010. Effects of Alternative Dietary Lipid Sources on Growth Performance and Fatty Acid Composition of Beluga Sturgeon, *Huso huso*, Juveniles. *Journal of the World Aquaculture Society*, 41(4), 471-489.

Kamalam, B.S., Medale, F. and Panserat, S., 2017. Utilisation of dietary carbohydrates in farmed fishes: New insights on influencing factors, biological limitations and future strategies. *Aquaculture*, 467, 3-27.

Karantonis, H. C., Tsantila, N., Stamatakis, G., Samiotaki, M., Panayotou, G., Antonopoulou,

S., and Demopoulos, C. A., 2007. *Waste Byproducts*, 32(2008), 443–459.

Khoshkholgh, M., Noverian, H., Mosapour Shajani, M., Mohammadi, M., and Azizi, M. S., 2013. The effect of olive oil cake on growth, body composition and sensory evaluation of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fisheries (Iranian Journal of Natural Resources)*, 66(2), 133–144. Retrieved from https://jfisheries.ut.ac.ir/article_35691_en.html (In Persian)

Li, Q., Zhu, H.Y., Wei, J.J., Zhang, F., Li, E.C., Du, Z.Y., Qin, J.G. and Chen, L.Q., 2017. Effects of dietary lipid sources on growth performance, lipid metabolism and antioxidant status of juvenile Russian sturgeon *Acipenser gueldenstaedtii*. *Aquaculture Nutrition*, 23(3), 500-510. <https://doi.org/10.1111/anu.12418>

Lin, Y. H., and Cheng, M. Y., 2017. Effects of dietary organic acid supplementation on the growth, nutrient digestibility and intestinal histology of the giant grouper *Epinephelus lanceolatus* fed a diet with soybeanmeal. *Aquaculture*, 469, 106–111. <https://doi.org/10.1016/j.aquaculture.2016.11.032>

Martinsdóttir, E., 2010. Sensory quality management of fish. In *Sensory Analysis for Food and Beverage Quality Control* (pp. 293–315). Elsevier. <https://doi.org/10.1533/9781845699512.3.293>

Nasopoulou, C., Stamatakis, G., Demopoulos, C. a, and Zabetakis, I., 2011. Effects of olive

pomace and olive pomace oil on growth performance, fatty acid composition and cardio protective properties of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). *Food Chemistry*, 129(3), 1108–1113. <https://doi.org/10.1016/j.foodchem.2011.05.086>

O'Mahony, M., 1986. *Sensory Evaluation of Food; Statistical Methods and Procedures*. *Taylor and Francis Group* (1st Editio). New York, NY: Routledge. <https://doi.org/10.1088/17518113/44/8/085201>

Pahlow, M., Van Oel, P. R., Mekonnen, M. M., and Hoekstra, A. Y., 2015. Increasing pressure on freshwater resources due to terrestrial feed ingredients for aquaculture production. *The Science of the Total Environment*, 536, 847–857. <https://doi.org/10.1016/j.scitotenv.2015.07.124>

Palmegiano, G.B., Agradi, E., Forneris, G., Gai, F., Gasco, L., Rigamonti, E., Sicuro, B. and Zoccarato, I., 2005. Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*). *Aquaculture Research*, 36(2), 188–195.

Pazos, M., Alonso, A., Fernández-Bolaños, J., Torres, J. L., and Medina, I., 2006. Physicochemical properties of natural phenolics from grapes and olive oil byproducts and their antioxidant activity in frozen horse mackerel fillets. *Journal of Agricultural and Food Chemistry*, 54(2), 366–373. <https://doi.org/10.1021/jf0518296>

Richard, N., Kaushik, S., Larroquet, L., Panserat, S., and Corraze, G., 2006. Replacing dietary fish oil by vegetable oils has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss*). *British Journal of Nutrition*, 96(02), 299. <https://doi.org/10.1079/BJN20061821>

Richard, N., Mourente, G., Kaushik, S., and Corraze, G., 2006. Replacement of a large portion of fish oil by vegetable oils does not affect lipogenesis, lipid transport and tissue lipid uptake in European seabass (*Dicentrarchus labrax* L.). *Aquaculture*, 261(3), 1077–1087. <https://doi.org/10.1016/j.aquaculture.2006.07.021>

Sansoucy, R., 1985. *Olive by-products for animal feed. FAO*. Rome: Food and Agriculture Organization of the United Nations. Retrieved from <http://www.fao.org/docrep/003/X6545E/X6545E00.HTM>

Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, R. J., and Tocher, D. R., 1992. The Metabolism of Phospholipids and Polyunsaturated Fatty Acids in Fish. *Aquaculture: Fundamental and Applied Research*, 43, 103–124.

Sicuro, B., Barbera, S., Daprà, F., Gai, F., Gasco, L., Paglialonga, G., Palmegiano, G.B. and Vilella, S., 2010. The olive oil by-product in 'rainbow trout *Oncorhynchus mykiss* (Walbaum)' farming: productive results and quality of the product. *Aquaculture Research*, 41(10), e475-e486 <https://doi.org/10.1111/j.13652109.2010.02514.x>

Zangeneh, S., and Torki, M., 2011. Effects of b-mannanase supplementing of olive pulp-included diet on performance of laying hens, egg quality characteristics, humoral and cellular immune response and blood parameters. *Global Veterinaria*, 7(September 2015), 391–398.