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ORIGINAL RESEARCH

The effect of partial and full fish oil replacement with poultry fat on growth performance, hematological and serum biochemical parameters, antioxidant capacity, and intestine histology of rainbow trout (*Oncorhynchus mykiss*)

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Abstract This study investigated the effect of replacing dietary fish oil (FO) with poultry fat (PF) on some growth and blood parameters of rainbow trout (*Oncorhynchus mykiss*) to find a suitable alternative to fish oil. For this aim, 600 rainbow trout juveniles weighing 50.72±2.13 g were purchased and after the adaptation period, 25 fish in each pond were released in a completely randomized design with 9 treatments and 3 replications. Five diets were considered for two months containing 100%, 75%, 50%, 25%, and 0% FO (D1, D2, D3, D4, and D5, respectively), where FO was replaced with PF. The treatments were CTL (D1), 25PF (D2), 25PFR (D2, then D1), 50PF (D3), 50PFR (D3, then D1), 75PF (D4), 75PFR (D4, then D1), 100PF (D5), and 100PFR (D5, then D1). FO replacement with PF had no significant influence on growth parameters. Hepatosomatic index (HSI) increased significantly in 75PF and 100PF ($P<0.05$), but not in 75PFR and 100PFR ($P>0.05$). Some hematological, e.g. red and white blood cell counts (RBC and WBC, respectively), and serum biological parameters, e.g. glucose, triglyceride, and albumin, changed significantly ($P<0.05$), especially in 75PF and 100PF. The antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were downregulated in 75PF and 100PF, whereas return to D1 significantly increased the activity of these enzymes in 75PFR and 100PFR ($P<0.05$). Furthermore, intestinal lipase activity fell significantly in 50PF, 75PF, and 100PF ($P<0.05$) but not in those fed D1 on the second month ($P>0.05$). Intestine structure was severely damaged in all groups except 50PF, 75PF, and 100PF, which indicates the oxidative stress imposed on fish fed diets with higher FO. In summary, a balance should be maintained between FO and alternative fat in fish diet to retain the best growth efficiency and prevent from adverse effects of either fat source.

Keywords Rainbow trout *(Oncorhynchus mykiss)* . Fish oil . Poultry fat . Growth parameters . Intestine histology

Introduction

Replacement of fish oil with substitute sources in fish diet has recently been a growing trend (Beheshti Foroutani et al. 2018) as it contributes to the goal of achieving sustainability in aquaculture (Gesto et al. 2021). Lipids play pertinent roles in various aspects of fish metabolic processes and growth including cell formation, energy provision, vitamin absorption and transport, among others (Turchini et al. 2009). Fish oil is currently considered the major source of lipid in fish farming practices because of its richness in polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid

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(DHA, 22:6n-3), which are known as vital components for fish growth and development (Yun et al. 2013). However, one cannot afford to consider fish oil a permanent lipid source in aquaculture because of its limited availability especially with regard to growing demand for using it in different areas (Chen et al. 2020) and its susceptibility to oxidation (Ghelichi et al. 2017).

Different resources have been proposed to replace fish oil in fish diet ranging from plant-based (Ofori-Mensah et al. 2020; Qin et al. 2022; Weerasingha et al. 2022)to animal-based (Fawole et al. 2021; Maldonado-Othón et al. 2022) sources or a mix of them (Carvalho et al. 2020). However, the common concern reported in majority of the studies is the significant reduction of PUFA content especially after the full replacement of fish oil with other sources. Some studies suggested the enrichment of diets with PUFA supplements (Gesto et al. 2021), which might raise issues regarding the overall cost of the practice and economic feasibility of the whole process. Nevertheless, considerable cost-effectiveness following the replacement of fish oil with cheaper resources even at partial levels has surged researchers to continue looking for appropriate options to replace fish oil.

Lipids are considered one of the most essential and important elements in the diets formulated for fish culture operations. Dietary lipids are regarded as major energy source and on the other hand, they are known as carriers of essential nutrients that are fat-soluble. The lipid requirements of rainbow trout was first claimed to be 12% (dry matter) and then it was reported that diets more than 20% (dry matter) lipid result in more efficient growth performance in rainbow trout (Meng et al. 2019). Likewise, deficiency of dietary lipid might bring about impaired digestion and immunity leading to growth inefficiency (Zhu et al. 2017). On the other hand, incorporation of excessive lipid in the diet might lead to the reduction of feed intake and elevation of feed conversion ratio (Yi et al. 2014)and would cause stress in fish as a consequence of oil oxidation (Jin et al. 2013). Poultry fat is a candidate to replace fish oil in aquaculture practices, which is considered a substantially cheaper and more accessible source of fat in fish diet. However, such an replacement has always been a place of concern because poultry fat is known to contain higher amount of monounsaturated fatty acids (MUFA) and lower content of PUFA compared with fish oil (Emery et al. 2014), which might reduce DHA and EPA content and undermine the final quality of fish fillet (Campos et al. 2019). Total n-3 PUFA, EPA+DHA, and n3:n6 in poultry fat were found to be 3.0%, 0.3%, and 0.2, respectively, which are far below compared with those in fish oil being 31.7%, 28.7%, and 10.8, respectively (Bowyer et al. 2012).

In this regard, varying and sometimes contradictory findings have been reported by researchers about the effect of partial and full replacement of fish oil by poultry fat. For instance, Bowyer et al. (2012) and Salini et al. (2015) claimed that the fish fed a diet with full replacement of fish oil with poultry fat had not significant effect on fish growth; in accordance with this finding, Campos et al. (2019) also reported that a diet with 100% poultry fat instead of fish oil had no noticeable influence on growth factors such as feed intake, feed conversion ratio, protein efficiency ratio and whole-body composition; however, they witnessed that the fish fed a diet with 100% poultry fat and no fish oil were influenced by complications in internal organs, e.g. lipid accumulation in lipid. Therefore, one cannot afford to suffice the comparable growth performance of fish fed diets with fish oil or poultry oil in order to make practical decisions. The present study was, thus, formulated to evaluate the effect of various feeding strategies by considering 25%, 50%, 75% and 100% poultry fat in diet on hematological and serum biochemical parameters, digestive enzymes in blood, antioxidant capacity and intestine histology of rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Experimental diets

Poultry by-products were provided from an industrial slaughterhouse and rendered to obtain PF (poultry fat). Five diets were prepared by incorporating 100% FO and 0% PF (D1), 75% FO and 25% PF (D2), 50% FO and 50% PF (D3), 25% FO and 75% PF (D4) and 0% FO and 100% PF (D5). EPA, DHA, Σ SFA, Σ MUFA, Σ PUFA, Σ ω-3, Σ ω-6, Σ ω-9, Σ EPA+DHA, and Σ ω-3/ Σ ω-6 contents of FO were 7.24, 19.40, 26.30, 37.20, 32.1, 28.7, 3.08, 29.4, 26.7, and 9.34 g $100g^{-1}$, respectively, and those of PF were 0.21, 0.51, 27.9, 40.5, 22.5, 1.98, 20.0, 37.0, 0.53, and 012 g $100g^{-1}$, respectively. The pellets were prepared using a

meat grinder after mixing the feedstuffs and moisturizing by adding 300 mL water per kg, followed by a drying stage at 60 °C for 10 min. The ingredients and chemical composition of the diets are presented in Table 1 and the fatty acid profile of the diets is shown in Table 2.

Experimental protocol

Rainbow trout juveniles were purchased from a local farm (Golestan province, Iran) and transported to the laboratory. Afterwards, 600 fish were selected and transferred to 500-L tanks (24 tanks for three replicates tanks for each treatment) at a density of 25 fish per tank. The fish were quarantined for a month and fed a commercial diet (Faradaneh, Iran) for acclimatization. After measuring the weight and length of the fish, 24 homogeneous groups, each including 25 fish (mean body weight and length 50.72±2.13 g and 12.5±0.6 cm, respectively) were randomly assigned to each tank. Overall, 9 treatments were considered as follows: CTL (fed D1 diet for two months), 25PF (fed D2 diet for two months), 25PFR (fed D2 diet for one month and then returned to D1 diet for another month), 50PF (fed D3 diet for two months), 50PFR (fed D3 diet for one month and then returned to D1 diet for another month), 75PF (fed D4 diet for two months), 75PFR (fed D4 diet for one month and then returned to D1 diet for another month), 100PF (fed

Table 1 Ingredients (as percentage) and chemical composition of the experimental diets

	D1	D2	D3	D4	D5
Ingredients					
Fish meal[1]	35	35	35	35	35
Meat meal[2]	6	6	6	6	6
Soybean meal[3]	17	17	17	17	17
Wheat meal[4]	25	25	25	25	25
Fish oil[1]	8	6	4	2	0
Poultry fat	0	2	4	6	8
Sodium bentonite[5]	3	3	3	3	3
Vitamin premix[6]	1.5	1.5	1.5	1.5	1.5
Mineral premix[7]	1.5	1.5	1.5	1.5	1.5
Lysine[8]	1	1	1	1	1
Methionine[8]	0.5	0.5	0.5	0.5	0.5
Choline chloride[9]	0.2	0.2	0.2	0.2	0.2
Molasses[10]	0.8	0.8	0.8	0.8	0.8
Salt	0.5	0.5	0.5	0.5	0.5
Proximate composition					
Moisture (%)	10	9.8	9.7	9.8	10.1
Crude ash (%)	8	7.9	8.1	7.9	7.7
Crude protein (%)	42	42.3	42.3	42.2	42.3
Crude lipid (%)	18.3	18	18.1	18.2	18.3
Crude fiber (%)	3	3.1	2.9	3.1	2.9
NFE (%)*	18.7	18.9	18.9	18.8	18.7
Gross energy (kJ g^{-1})**	20.87	20.88	20.88	20.88	20.87

Diet abbreviations, D1: 100% fish oil and 0% poultry fat; D2: 75% fish oil and 25% poultry fat; D3: 50% fish oil and 50% poultry fat; D4: 25% fish oil and 75% poultry fat; D5: 0% fish oil and 100% poultry fat.

[1]Negin-Powder Co., Fisheries Industrial Complex, Amirabad Port, Neka, Iran.

[2]Tonekadasht-e-Shomal Co., Mazandaran, Iran.

[3]Kimiya Tejarat Zar Co., Tehran, Iran.

[4]Eris Trade Group, Tehran, Iran.

[5]Sina Tolid Co., Tehran, Iran.

[6]Sciance Laboratories, Alborz Industrial City, Qazvin, Iran. Provides the following (mg kg^{-1} diet): vitamin E (30), vitamin K (3), niacin (40), thiamine (2), riboflavin (7), pyridoxine (3), folacin (1.5), pantothenic acid (18), biotin (0.7), and cyanocobalamin (0.18).

[7]Sciance Laboratories, Alborz Industrial City, Qazvin, Iran. Provides the following (mg kg^{-1} food): Mg (100), Zn (60), Fe (40), Cu (5), Co (0.1), I (1), and antioxidant (100).

[8]Shimi Gostar Taban Co., Tehran, Iran.

[9]Alborz Gostar Darou, Karaj, Iran.

[10]Iranmalas Co., Leia Industrial Town, Iran.

* NFE: nitrogen free extract = 100 – (moisture + crude ash + crude protein + crude lipid + crude fiber).

**Calculated on the basis of 23.6, 39.5 and 17.2 kJ g^{-1} of protein, lipid and carbohydrate, respectively.

D5 diet for two months), and 100PFR (fed D5 diet for one month and then returned to D1 diet for another month). Feeding was carried out manually based on 3% of the tanks' biomass, at three meals. The water flow rate of each tank was set at 3 l.min^{-1}. Water temperature and pH were measured to be 15.20±0.69 ˚C and 7.08±0.21, respectively, and the dissolved oxygen was near saturation.

Growth performance calculations

Growth parameters including weight gain (WG), special growth rate (SGR), daily growth index (DGI), survival rate (SR), feed conversion ratio (FCR), Hepatosomatic index (HSI), and Viscerosomatic index (VSI) were calculated as follows (Campos et al. 2019):

WG = final weight (g) – initial weight (g)

SGR = 100 × [(ln final weight – ln initial weight) / day]

DGI = 100 × [(Final body weight)$^{1/3}$ − (Initial body weight)$^{1/3}$]/days

SR = 100 × (number of final alive fish / number of initial fish)

FCR = dry feed intake (g) / weight gain (g)

HSI = 100 × liver weight (g)/body weight (g)

VSI = 100 × weight of viscera (g)/body weight (g).

Samplings

Sampling was initiated 24 h after fasting and ensured that all digestive tract contents had been excreted. For this aim, six fish were randomly sampled from each treatment by a dip net and anesthetized with 200 mg L^{-1} clove extract solution. Blood samples were collected by syringes from the caudal vein and poured into plastic tubes. The blood serum was separated after centrifugation (3000 rpm, 5 min) and kept at -20 ˚C until further analyses. The fish samples were incised ventrally and the digestive tract was dissected to collect the intestine (Nayak et al. 2003). All animal care and use procedures were approved by Research Ethics Committee of Islamic Azad University.

Table 2 Fatty acid profile (g 100g^{-1} fat) of the experimental diets. Different letters within a row indicate significant differences among the diets (n = 3; P < 0.05).

Fatty acids	D1	D2	D3	D4	D5
C14:0	2.59 ± 0.08a	2.23 ± 0.11b	1.93 ± 0.05c	1.31 ± 0.09d	1.19 ± 0.04d
C15:0	0.75 ± 0.02a	0.69 ± 0.05a	0.50 ± 0.03b	0.29 ± 0.04c	0.26 ± 0.06c
C16:0	21.0 ± 0.85a	21.48 ± 0.49a	22.7 ± 0.12a	23.63 ± 0.32a	23.8 ± 0.18a
C17:0	0.29 ± 0.02a	0.28 ± 0.05a	0.22 ± 0.01b	0.24 ± 0.09ab	0.25 ± 0.03ab
C18:0	5.55 ± 0.21a	5.67 ± 0.09a	6.25 ± 0.12b	6.73 ± 0.23c	6.87 ± 0.21c
C21:0	0.94 ± 0.04a	0.76 ± 0.09b	0.52 ± 0.04c	0.26 ± 0.05d	0.16 ± 0.03d
C23:0	2.45 ± 0.11a	2.11 ± 0.09b	1.37 ± 0.14c	0.64 ± 0.17d	0.40 ± 0.05d
C14:1	0.25 ± 0.04a	0.18 ± 0.01b	0.09 ± 0.01c	0.09 ± 0.00c	0.10 ± 0.03c
C16:1ω-7	7.21 ± 0.37a	7.19 ± 0.26a	6.38 ± 0.32b	5.48 ± 0.24c	5.24 ± 0.24c
C17:1	0.17 ± 0.02b	0.18 ± 0.00b	0.23 ± 0.04a	0.19 ± 0.01b	0.14 ± 0.03c
C18:1ω-9*trans*	3.07 ± 0.43a	2.89 ± 0.21a	2.61 ± 0.13b	2.13 ± 0.37c	2.11 ± 0.27c
C18:1ω-9*cis*	30.5 ± 0.56c	31.90 ± 0.75c	35.1 ± 0.47b	37.96 ± 0.84a	39.0 ± 0.54a
C24:1ω-9	0.39 ± 0.07a	0.23 ± 0.02b	0	0	0
C18:2ω-6*cis*	10.5 ± 0.31c	11.78 ± 0.39bc	13.6 ± 0.78b	14.44 ± 0.61ab	16.6 ± 0.21a
C18:3ω-3	4.44 ± 0.11a	4.02 ± 0.02a	2.83 ± 0.08b	2.23 ± 0.04bc	1.80 ± 0.05c
C20:3ω-3	1.10 ± 0.08a	0.97 ± 0.05ab	0.71 ± 0.02b	0.59 ± 0.01c	0.37 ± 0.01d
C20:5ω-3 (EPA)	0.72 ± 0.04a	0.60 ± 0.03b	0.36 ± 0.02c	0.07 ± 0.00d	0
C22:6ω-3 (DHA)	3.11 ± 0.06a	2.26 ± 0.09b	1.91 ± 0.04bc	1.02 ± 0.09c	0.75 ± 0.07d
Σ SFA	33.57	33.22	33.49	33.2	32.93
Σ MUFA	41.59	42.57	44.41	45.85	46.59
Σ PUFA	19.87	19.63	19.41	18.35	19.52
Σ ω-3	9.37	7.85	5.81	3.91	2.92
Σ ω-6	10.5	11.78	13.6	14.44	16.6
Σ ω-9	33.96	35.02	37.71	40.09	41.11
Σ EPA+DHA	3.83	2.86	2.27	1.09	0.75
Σ ω-3/ Σ ω-6	0.89	0.66	0.42	0.27	0.17

Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05).

Analyses

The proximate composition of the diets and fish fillets were analyzed according to AOAC (2006). Moisture percentage was determined by drying the samples at 70 ˚C for 48 h. Crude protein and crude lipid of the samples were determined by Kjeldahl and Soxhlet methods. Crude ash percentage was determined by burning the samples in a furnace (550 ˚C) for 8h. Crude fiber content was measured by acidic and alkali digestion (Thiex et al. 2012).

Lipid extraction from the formulated diets was performed via the method proposed by Folch et al. (1957) and fatty acid measurement was done through the method explained by Liland et al. (2013). Briefly, 1 g of the extracted lipid was mixed with 10 ml methanol and 0.5 ml potassium hydroxide methanol N. Afterwards, the mixture was extracted by 15 ml of normal hexane and injected into gas chromatography (Shimadzu 14A; Japan) equipped with the flame ionized detector and capillary column (30 cm).

Hematological parameters including red blood cell (RBC), white blood cell (WBC), hematocrit (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were analyzed according to the methods proposed by Feldman et al. (2000). It should be noted that WBC count included neutrophil, lymphocyte, eosinophil, and monocyte counts. Blood biochemical parameters including albumin, cholesterol, triglyceride, glucose, cortisol, and protein were measured using Autoanalyser (Tajhizat Janjesh, Isfahan, Iran) according to the manufacturer's instructions and using commercial kits (Pars Azmun Co., Tehran, Iran). Cholesterol, triglyceride, glucose, and protein were analyzed through the procedures adopted by Burtis et al. (2001), Burtis et al. (2001), Trinder (1969), and Wootton (1964), respectively. Serum antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were measured according to Góth (1991), Marklund and Marklund (1974), and Paglia and Valentine (1967), respectively. Intestinal lipase activity was measured through the method applied by Iijima et al. (1998). For histological analysis, intestine samples were fixed in Bouin solution and stained through the hematoxylin and eosin methods. The measurement of villi length, villi width, and muscle thickness was carried out by means of Image-Pro Plus 6.0 Software. Furthermore, the goblet cell count was obtained under 400 times the field of view.

Statistical analyses

The normal distribution of the data was confirmed by the Kolmogorov-Smirnov test. The data were subjected to one-way ANOVA and Duncan Multiple Range Test to find significant differences among the treatments ($P<0.05$). All analyses were conducted in SPSS v.20.

Results

Dietary fatty acid composition

The fatty acid profiles of the diets administered in the present study are presented in Table 2. According to the Table, the highest amount of linoleic acid (C18:2ω-6) was observed in the diet with full replacement of fish oil by poultry fat (i.e. D5), which was significantly higher than that in all other diets ($P<0.05$) except D4 ($P>0.05$). Nevertheless, the contents of EPA (C20:5ω-3) and DHA (C22:6ω-3) decreased significantly with higher rates of fish oil replacement with poultry fat ($P<0.05$) reaching from 0.72±0.04 and 3.11±0.06 in D1 to 0 and 0.75±0.07 in D5, respectively. Higher levels of MUFA were observed in diets with higher PF, which is mostly due to the higher content of oleic acid (C18:1ω-9cis) in these diets. As expected, the highest and lowest levels of PUFA and Σω-3/Σω-6 were observed in D1 and D5, respectively, which ~~is~~ accounted for the different contents of fish oil in the diets.

Growth performance and biological parameters

All the fish fed different diets in this study weighed almost over 2.5 times higher than at the beginning of the trial with initial and final weights ranging from circa 50 to 52 and from 136 to 138, respectively. No

significant difference was detected in FCR of different groups (P>0.05), which indicates that all the diets were accepted similarly by the fish of control and experimental groups. There were no significant differences among the control and experimental groups in terms of WG, DGI, SGR, and SR (P>0.05). HSI of the fish fed with D4 and D5 were significantly higher than that of other samples (P<0.05) whereas return to D1 in the second month significantly reduced HSI in these two groups (P<0.05). Furthermore, a significant difference was detected between 75PF and 100 PF (P<0.05) while no significant difference in HSI was witnessed among other groups (P>0.05). Finally, there was no significant difference among the groups in terms of VSI (P>0.05) (Table 3).

Hematological parameters

Table 4 present the hematological parameters of the fish fed various diets in the current study. RBC and hematocrit of the fish decreased significantly when 75% and 100% of the FO in the diet was replaced by PF (P<0.05) whereas the return to 100 FO in the diet on the second months significantly recovered RBC in these two groups (P<0.05). Although the RBC reduction also occurred in 50PF, it was not significantly different from that in other groups (P>0.05). WBC was significantly higher in 75PF and 100PF (P<0.05) while no significant difference was detected among other groups (P>0.05). Furthermore, the percentage of eosinophil increased significantly in 75PF and 100PF (P<0.05), while no significant difference was detected among the groups in terms of the percentages of neutrophil, lymphocyte, and monocyte (P>0.05). Blood hemoglobin started to decrease significantly when 50% of FO in the diet was replaced by PF and continued to decrease even more significantly in 75PF and 100PF (P<0.05), whereas return to D1 in the second month of the trial recovered the hemoglobin content in the fish. No significant difference was observed among the groups in terms of MCV, MCH, and MCHC (P>0.05).

Table 3 Growth performance and biological parameters of rainbow trout after two months feeding with different regimens.

	CTL	25PF	25PFR	50PF	50PFR	75PF	75PFR	100PF	100PFR
Initial weight (g)	52.2±1.69a	51.7±3.57a	52.7±1.98a	51.5±1.21a	51.5±1.21a	51.0±2.74a	51.4±1.49a	50.7±1.60a	50.7±1.60a
Final weight (g)	138.1±6.71a	137.9±7.61a	137.5±5.06a	136.0 ±5.97a	136.8±6.01a	136.9±3.08a	136.7±2.57a	138±7.42a	137±9.10a
WG[1] (g)	86.0±1.38a	86.3±2.24a	85.1±1.83a	85.6±3.52a	85.6±1.95a	86.2±0.97a	85.2±2.08a	87.8±1.85a	87.4±1.62a
DGI[2]	1.42±0.11a	1.39±0.08a	1.37±0.15a	1.40±0.05a	1.42±0.17a	1.43±0.02a	1.39±0.12a	1.44±0.18a	1.43±0.09a
SGR[3] (% d^{-1})	1.62±0.03a	1.59±0.08a	1.58±0.11a	1.58±0.03a	1.59±0.01a	1.60±0.04a	1.58±0.09a	1.65±0.08a	1.63±0.06a
FCR[4]	1.08±0.02a	1.06±0.07a	1.09±0.12a	1.09±0.06a	1.10±0.02a	1.07±0.06a	1.10±0.13a	1.01±0.04a	1.01±0.03a
SR[5]	96.3±3.42a	98.3±2.14a	100.0±0.00a	97.6±2.36a	96.6±2.96a	95.4±2.72a	96.7±1.79a	97.3±4.25a	95.3±3.33a
HSI[6]	1.53±0.20c	1.44±0.09c	1.49±0.18c	1.43±0.12c	1.57±0.06c	1.97±0.11b	1.51±0.21c	2.47±0.06a	1.48±0.26c
VSI[7]	18.2±1.01a	18.7±1.05a	18.9±1.20a	18.8±1.91a	19.1±0.94a	18.4±0.81a	18.8±1.06a	18.7±1.54a	19.3±1.23a

Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05).

[1] WG, Weight Gain

[2] DGI, Daily Growth Index

[3] SGR, Specific Growth Rate

[4] FCR, Feed conversion ratio

[5] SR, Survival Rate

[6] HSI, Hepatosomatic index

[7] VSI, Viscerosomatic index.

Table 4 Hematological and serum biochemical parameters of rainbow trout after two months feeding with different regimens.

	CTL	25PF	25PFR	50PF	50PFR	75PF	75PFR	100PF	100PFR
RBC (10^6 mL^{-1})	2.71±0.05a	2.66±0.07a	2.67±0.12a	2.47±0.15ab	2.65±0.16b	2.39±0.02b	2.57±0.09a	2.37±0.05b	2.55±0.12a
WBC (10^3 mL^{-1})	8.43±0.61b	8.48±0.42b	8.45±0.37b	8.40±0.27b	8.50±0.32b	10.84±0.51a	8.68±0.40b	11.23±0.27a	8.30±0.33b
Neutrophil (%)	47.0±2.64a	47.51±2.96a	47.31±3.07a	47.25±3.13a	47.67±2.52a	47.19±2.49a	46.94±3.12a	46.32±2.88a	46.75±1.75a
Lymphocyte (%)	51.67±2.85a	51.86±2.51a	51.59±2.84a	51.75±3.23a	51.73±1.15a	51.23±2.19a	51.83±3.01a	51.67±2.91a	51.85±1.31a
Eosinophil (%)	0.33±0.11b	0.41±0.08b	0.39±0.05b	0.67±0.03b	0.33±0.02b	1.37±0.29a	0.47±0.07b	1.50±0.33a	0.35±0.03b
Monocyte (%)	1.0±0.56a	0.68±0.07a	0.71±0.10a	0.5±0.18a	0.67±0.33a	0.40±0.14a	0.76±0.07a	0.33±0.13a	1.0±0.21a
PCV (%)	61.00±1.00a	59.57±2.08a	60.43±1.87a	55.5±1.26ab	60.00±1.53a	53.29±1.87b	59.04±2.49a	53.25±2.16b	58.67±2.85a
Hb (g dL^{-1})	11.57±0.82a	11.07±0.49a	11.26±0.38a	9.87±0.17b	11.47±0.43a	9.24±0.41c	10.26±0.26a	9.20±0.23c	10.33±0.56ba
MCV (fl)	225.0±4.58a	224.87±6.08a	223.37±5.49a	224.65±3.94a	222.0±5.29a	223.19±4.76a	222.91±6.48a	223.85±2.99a	234.4±2.62a
MCH (pg)	42.83±1.32a	42.07±2.08a	42.44±1.84a	40.20±2.42a	43.42±1.48a	40.80±1.31a	41.57±1.26a	39.15±1.91a	41.83±1.65a
MCHC (g dL^{-1})	18.74±1.46a	17.94±1.66a	18.57±2.17a	17.75±1.21a	19.52±1.55a	17.30±2.07a	18.88±1.63a	17.26±0.84a	17.78±1.80a
Albumin (g L^{-1})	1.67±0.02ab	1.69±0.07ab	1.68±0.06ab	1.80±0.04a	1.64±0.09ab	1.84±0.05a	1.63±0.04ab	1.84±0.07a	1.55±0.09b
Cholesterol (g dL^{-1})	433.33±8.5a	429±12.78a	431.55±9.48a	416.75±15.3a	447.25±20.2a	420.41±8.47a	432.69±9.07a	442.24±17.4a	432.0±37.72a
Triglyceride (g dL^{-1})	294.66±32.8ab	348.17±32.47a	294.08±24.70b	351.5±18.53a	306.29±11.4b	348.91±16.4a	301.29±26.0b	350.66±29.0a	293.33±13.0b
Glucose (g dL^{-1})	124.41±3.07b	133.11±4.74b	123.29±2.90b	195.83±3.52a	205.31±6.55a	214.65±5.26a	208.84±6.41a	213.69±4.59a	209.86±9.02a
Cortisol (ng mL^{-1})	122.3±7.23b	129.67±6.41b	128.04±8.63b	204.87±6.32a	189.28±4.03a	205.38±7.09a	201.61±8.43a	201.1±8.51a	197.23±5.74a
Protein (g L^{-1})	3.17±0.36a	3.16±0.41a	3.16±0.24a	2.73±0.27a	2.73±0.18a	3.08±0.57a	2.91±0.26a	3.13±0.37a	2.89±0.37a

Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05).

Serum biochemical parameters

Serum biochemical characteristics of the fish studied are given in Table 4. According to the results, the replacement of 25% of FO with PF in the diet had no significant effect on the level of serum glucose in the fish as compared with the control group (P>0.05). Nevertheless, a significant increase in glucose level was observed for 50PF, 75PF, and 100PF compared to that in control and 25PF (P<0.05) and even return to D1 on the second month of feeding did not have any significant effect on the level of serum glucose (P>0.05). Exactly a similar trend was observed for the level of serum cortisol. Replacement of FO by PF in the diets at any percentage tested resulted in a significant increase in serum triglyceride levels (P<0.05); hence, return to the diet with 100% FO and no PF on the second month of feeding led to a significant decrease in serum triglyceride (P<0.05), where no significant difference was detected in serum triglyceride in the fish fed D1 on the second month of trial with that of the control group (P>0.05). No significant difference was found between the groups in terms of other serum biochemical parameters including the levels of albumin, cholesterol, and protein (P>0.05).

Serum antioxidant enzymes

The effect of exposing the fish to various diet regimes is depicted in Fig. 1. Serum SOD in the fish fed by D2 and D3 showed no significant difference from that of the control group (P>0.05). However, serum SOD decreased significantly in 75PF and 100PF reaching from over 160 U mL^{-1} in control to approximately 120 U mL^{-1} in 75PF and 100PF (P<0.05), while, return to D1 in the second month increased their serum SOD significantly (P<0.05). Serum CAT decreased significantly in 50PF compared to control and 25PF (P<0.05), but it rose significantly when the fish were fed by D1 in the second month (P<0.05). The lowest serum CAT levels were detected in 75PF and 100PF, which were significantly lower than those in all other groups (P<0.05). Serum GPx fell significantly in 50PF compared to control and 25PF (P<0.05), but a return to D1 in the second month brought the serum GPx back to control and 25PF levels. The lowest serum GPx were in 75PF and 100PF (P<0.05), while return to D1 in the second month resulted in a significant rise in serum GPx in these two groups (P<0.05).

Intestinal lipase activity

Variations of intestinal lipase activity are shown in Fig. 2. As can be seen, the replacement of 50%, 75%,

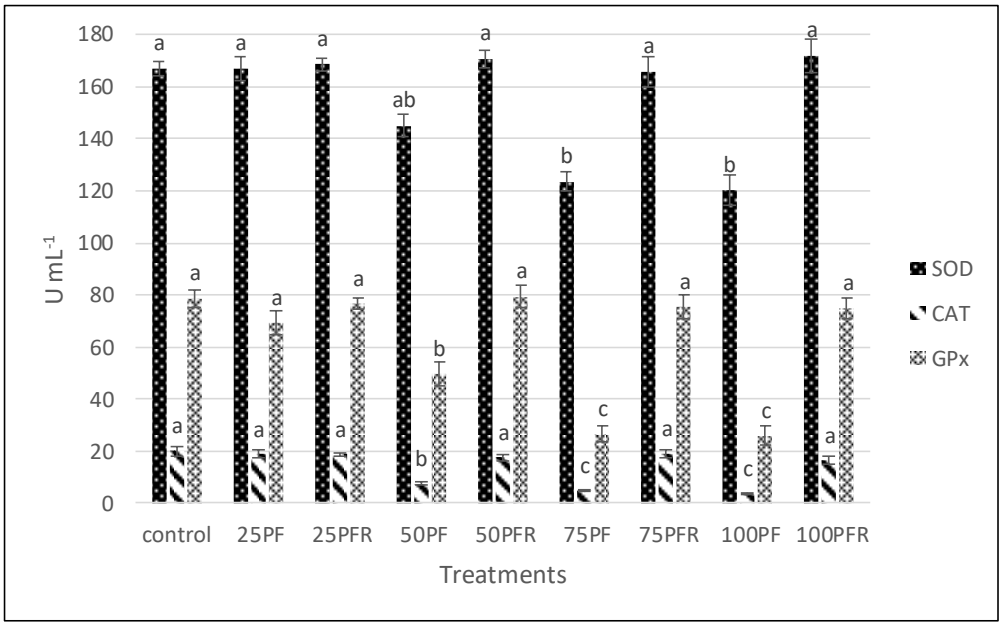

Fig. 1 Serum antioxidant enzymes in rainbow trout after two months feeding with different regimens. Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05).

and 100% of FO by PF resulted in significant reductions in intestinal lipase activity (P<0.05). However, the Figures for the fish fed by D1 on the second month of the trial are not significantly different from the control and 25PF (P>0.05), which indicates that return to the diet with 100% FO led to a significant increase in intestinal lipase activity (P<0.05). No significant difference was observed between the control and 25PF (P>0.05).

Histological analysis of intestine

The histological analysis of intestine sections from fish fed by various feeding regimes is shown in Fig. 3. As can be seen, the intestine in 100PF is completely normal and no necrosis, detachment of intestinal epithelial cell or lamina propria (LP) inflammation is witnessed. Replacement of 50% and 75% of FO by PF in the diet did not have a considerable effect on the histology of intestine, except for negligible necrosis and LP inflammation. However, extensive necrosis was detected in the intestine of 25PF with a minor LP inflammation whereas return to D1 in the second month aggravated the intestine structure and caused ~~the~~ adverse effects. Incorporation of 100% FO in the diet resulted in severe LP inflammation, detachment of intestinal epithelial cells, and extensive necrosis in the intestines of the fish. In addition, villi length and mucus layer thickness increased significantly when 50%, 75% and 100% of FO in the diet were replaced by PO (P<0.05), but return to D1 in the second month in these groups resulted in a significant decrease in villi length and mucus layer thickness (P>0.05) (Table 5).

Discussion

The replacement of FO with PF at any percentage tested resulted in the decrease of EPA and DHA, which is an expected phenomenon because of the high content of these two fatty acids in fish oil (Ghelichi et al. 2021). The present study revealed that replacement of FO with PF at all the ratios experimented, even at 75% and 100%, had no significant influence on growth parameters of the fish. The influencing factor in this regard could be differential lipid deposition areas across different fish species (Campos et al. 2019). Furthermore, the effect of FO replacement with other sources of oil on pellet palatability could undermine the feed intake in fish resulting in difference in growth (Bowyer et al. 2012); however, it seems that the replacement of FO with poultry fat in the present study had no major influence on pellet palatability as evidenced by insignificant differences in growth factors among the treatments studied. A similar observation was previously reported for other species such as European seabass (*Dicentrarchus labrax*) (Campos et al. 2019), largemouth bass (*Micropterus salmoides*) (Yun et al. 2013), barramundi (*Lates calcarifer*) (Wan Ahmad et

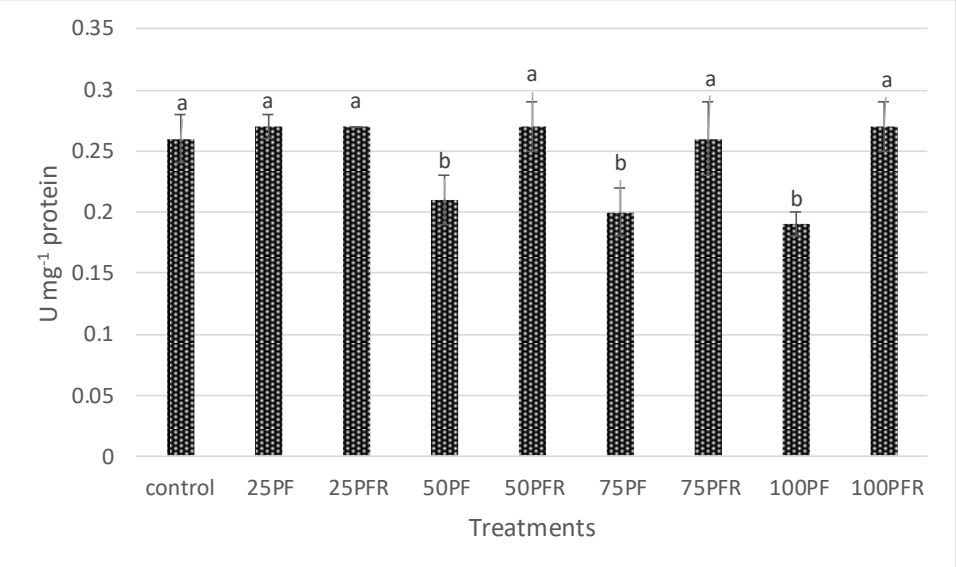

Fig. 2 Intestinal lipase activity in rainbow trout after two months feeding with different regimens. Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05).

al. 2013), and yellowtail kingfish (*Seriola lalandi*) (Bowyer et al. 2012). Chen et al. (2020) reported the replacement of dietary FO with soybean oil even improved growth parameters of largemouth bass (*Micropterus salmoides*). In a recent study by Gesto et al. (2021), it was found that the replacement of FO with rapeseed oil with lower content of EPA and DHA had no significant effect on growth performance of rainbow trout, which is in line with the results of this study. Furthermore, Sáez-Royuela et al. (2022)asserted that the replacement of FO with a blend of vegetable oil at different ratios did not significantly change the growth performance in Juvenile Tench (*Tinca tinca*). Moreover, Higgs et al. (2006)reported that replacement of FO

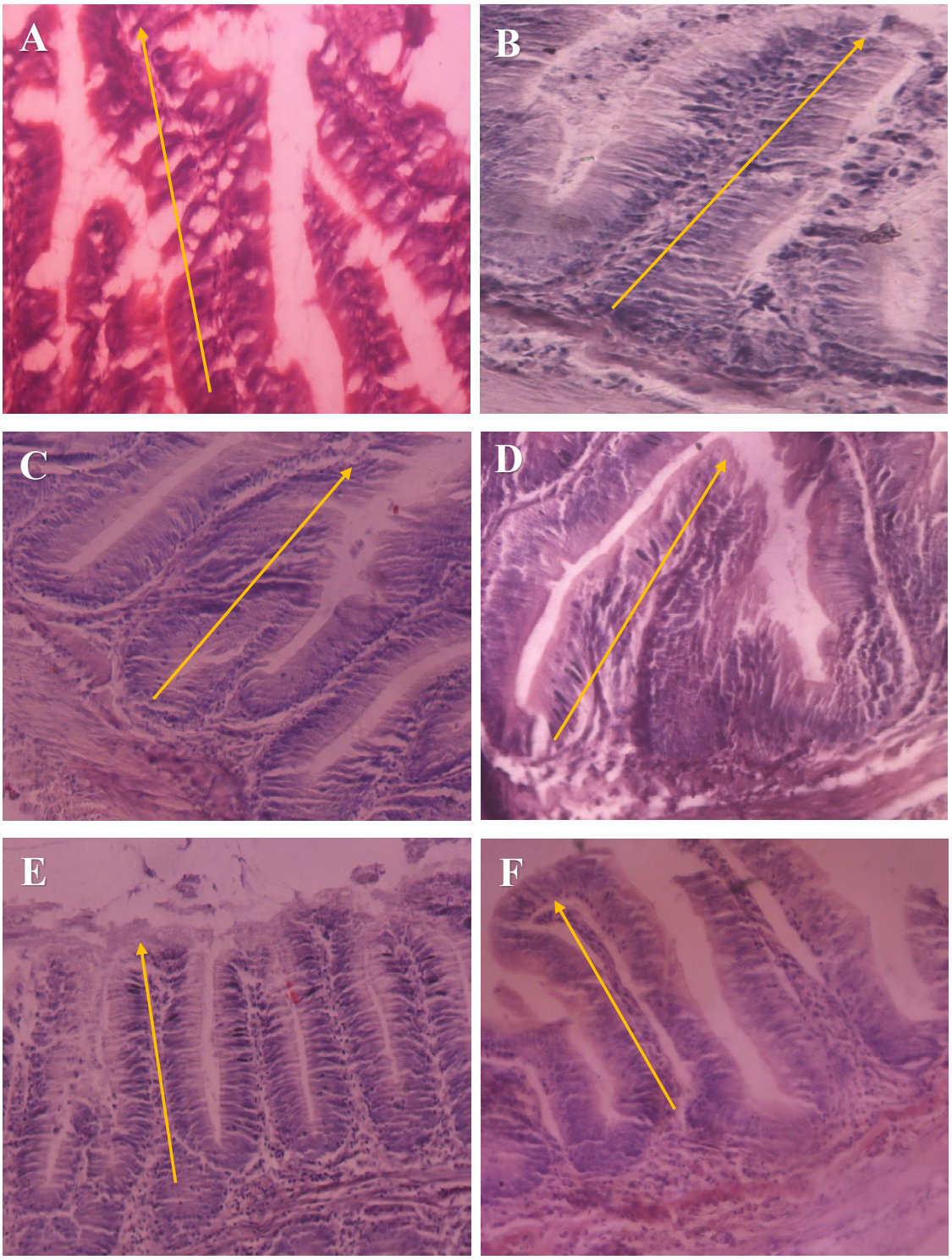

Fig. 3 Histological analysis of intestine sections from rainbow trout after two months feeding with different regimens (H&E, ×20). A-I belong to control, 25PF, 25PFR, 50PF, 50PFR, 75PF, 75PFR, 100PF, and 100PFR, respectively.

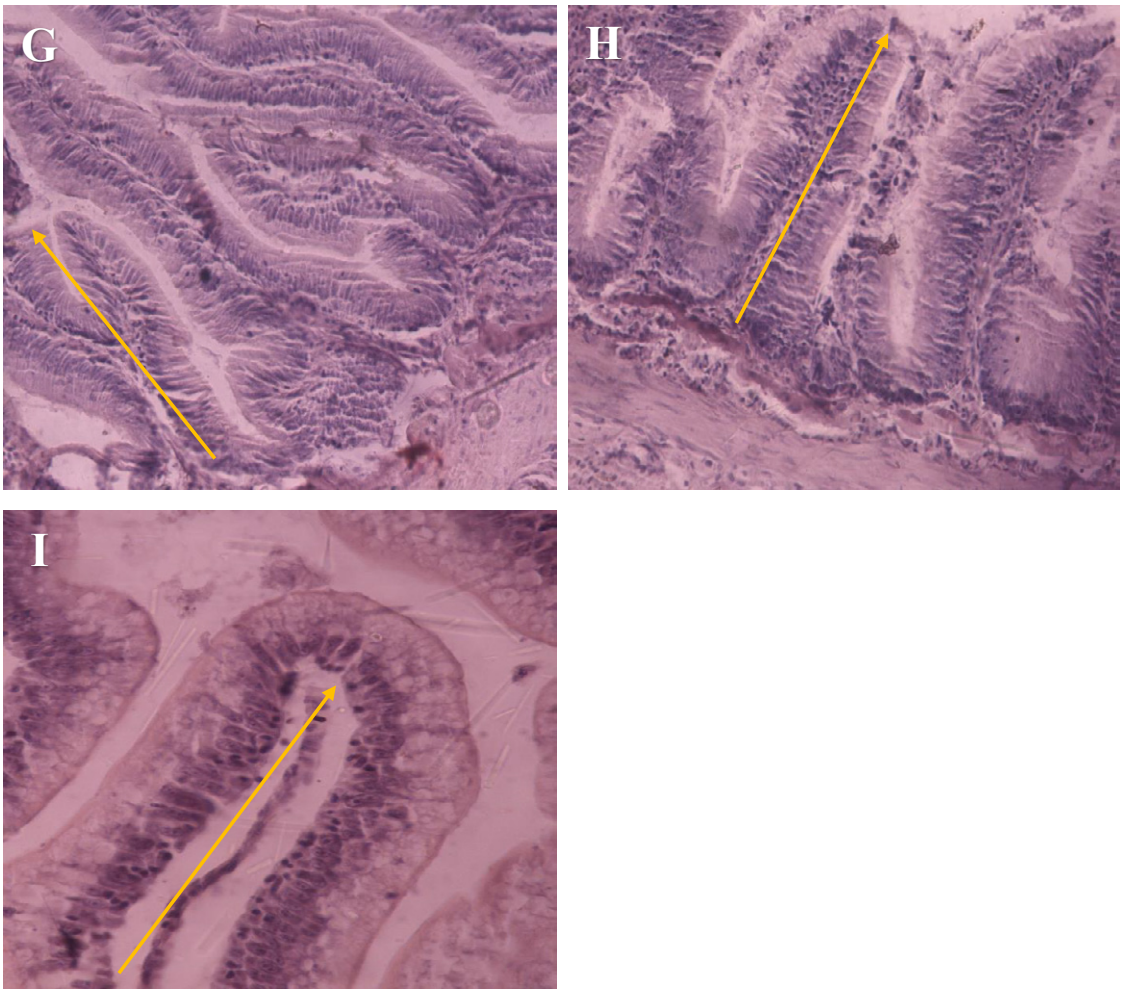

Fig. 3 Continued (Histological analysis of intestine sections from rainbow trout after two months feeding with different regimens (H&E, ×20). A-I belong to control, 25PF, 25PFR, 50PF, 50PFR, 75PF, 75PFR, 100PF, and 100PFR, respectively).

Table 5 The intestine morphological characteristics in rainbow trout after two months feeding with different regimens.

	CTL	25PF	25PFR	50PF	50PFR	75PF	75PFR	100PF	100PFR
Villi length (μm)	428.10±18.4b	424.69±22.3b	427.60±21.4b	467.74±13.4a	429.86±17.94b	473.81±16.3a	431.57±19.2b	476.29±19.0a	429.96±23.4b
Mucus layer thickness (μm)	537.68±24.6b	538.28±21.5b	540.47±18.6b	574.08±25.4a	535.84±27.65b	578.82±20.35a	536.59±19.7b	586.41±27.8a	539.53±19.5b

Different letters within a row indicate significant differences among the treatments (n = 3; P < 0.05).

with an equal blend of canola oil and poultry fat had no significant effect on growth performance of Atlantic salmon (*Salmo salar*). However, a study in European seabass found that total replacement of FO with land animal fats significantly reduced the fish growth performance, while growth performance of the fish was not significantly influenced when 75% of FO was replaced by the alternative fat source (Monteiro et al. 2018). It seems that the controversy in the results of different studies evaluating the effect of replacing FO in fish diet is accounted for the type of the lipid source used to replace FO and the fish species experimented. This is well projected in a review on the effects of various ratios of FO replacement with a variety of vegetable oil sources on growth performance in different fish species where it is evidently shown that fish growth performance as a result of FO replacement in diet is a function of lipid source, percentage of replacement, and species (Nasopoulou and Zabetakis 2012)[A].

Hepatosomatic index in the fish-fed diets with 75% and 100% replacement of FO with PF increased significantly in the present study, which is perceivable because fish received a high level of energy from the diets containing poultry fat and therefore, they stored the additional energy in the liver (Bowyer et al. 2012). This is consistent with the results of previous studies on European seabass (Campos et al. 2019; Monteiro et al. 2018). In contrast, Yun et al. (2013) reported that the replacement of FO with poultry fat had no signifi-

cant influence on the hepatosomatic index of largemouth bass. Such differences were contemplated to be a consequence of variations in dietary lipid contents as well as differential lipid deposition areas in different species (Campos et al. 2019). This is further supported by the results of the current study where the return to the diet containing 100% FO on the second month of the trial in the groups fed diets with a high level of poultry fat resulted in the significant reduction of hepatosomatic index. Moreover, insignificant differences among the treatments in terms of viscerosomatic index in the present study were previously reported in rainbow trout (Trushenski et al. 2011).

The incorporation of high percentage of poultry fat in fish diet instead of FO in the present study resulted in a significant decrease in red blood cell count of the fish. This is in line with the results of (Yildirim-Aksoy et al. 2007) who reported that lower red blood cells in Nile Tilapia (*Oreochromis niloticus*) fed FO as compared with those fed corn oil, beef tallow, and linseed oil. However, they also reported that the fish fed FO also had significantly higher levels of white blood cells and similar levels of hematocrit, which is not consistent with the results of the present study. Furthermore, Medagoda et al. (2022) reported that total replacement of fish oil with tallow in the diet of olive flounder (*Paralichthys olivaceus*) had no significant effect on hematocrit and hemoglobin of the fish, which is in contrast with the results of the present study. Differences in hematological parameters of fish fed diets with varying levels of FO could be attributed to the flexibility and permeability of cell membranes as influenced by highly unsaturated fatty acids in FO (Yildirim-Aksoy et al. 2009). In this regard, three mechanism were explained. Firstly, the fatty acid composition of fish diets plays role in the fatty acid composition of cell membrane phospholipids, which greatly influences the disease resistance since several immune responses are dependent on leucocyte cell membrane interactions. The second mechanism is the modification of signal transductions influenced by protein kinase C. finally, the third mechanism is the production of immunologically active eicosanoids from nonesterified AA, EPA, DHA, and probably another polyunsaturated fatty acid precursor, i.e. 20:3n-6 (Balfry and Higgs 2001).

Replacement of FO with poultry fat in this study did not significantly change circulating level of cholesterol at any percentage tested, which is in line with the results of (Monteiro et al. 2018) on the effect of replacing FO with land animal fats in European seabass. This observation is perceivable because FO and poultry oil both contain high level of cholesterol (Cheng and Hardy 2004). In contrast, the diet devoid of FO but containing poultry oil and canola oil brought about significant decrease in the level of plasma cholesterol in yellowtail kingfish (*Seriola lalandi*) (Bowyer et al. 2012), which can be attributed to lower cholesterol content of plant-based sources (Cheng and Hardy 2004). Therefore, it is concluded that the replacement of FO with poultry fat, whether partially or fully, in rainbow trout would not expose the fish to the risk of hypocholesterolemia. Moreover, the replacement of FO with poultry at any ratio tested, even at 25%, significantly increased plasma triglyceride in the present study, which is contrary to the results of Bowyer et al. (2012) and Monteiro et al. (2018) who witnessed no significant change in the level of plasma triglyceride after replacing fish oil with alternative fats source. Unlike the results of the current study, Pérez et al. (2014) reported that replacement of FO with beef tallow in gilthead sea bream (*Sparus aurata*) diet resulted in the reduction of triglyceride. The hypertriglyceridemia caused by replacing FO with poultry fat in the present study could be considered as an indicator of liver malfunction and compromised nutritional status of the fish (Bowyer et al. 2012). Interestingly, despite the hypertriglyceridemia caused by partial and full replacement of FO with poultry fat in this study, return to the diet containing 100% FO alleviated the condition and plasma triglyceride in all the groups decreased significantly. This indicates that the return to a diet rich in FO could be considered a compensating strategy for hypertriglyceridemia caused by the inclusion of poultry fat in fish diet. The results of this study also showed that replacing FO with poultry at 50% and more resulted in significant increase in plasma glucose even when the fish were fed D1 on the second month, which is in contrast to the results of Monteiro et al. (2018) who witnessed no significant difference in plasma glucose of European seabass after replacing FO with animal fats. Furthermore, replacing FO with poultry fat at over 25% caused irreversible increase in plasma cortisol levels even after return to D1, which indicates that the fish fed the diet with 50% or more of poultry fat instead of FO experienced significant levels of stress (Benítez-Dorta et al. 2013).

Inclusion of 75% and 100% poultry fat instead of FO in rainbow trout diet in the present study significantly decreased the activity of antioxidant enzymes SOD, CAT, and GPx. The increase in antioxidant enzyme activities in fish fed diets with higher levels of FO corresponds to the findings in Russian sturgeon

(*Acipenser gueldenstaedtii*) (Li et al. 2017) and grouper (*Epinephelus malabaricus*) (Lin and Shiau 2007). The activity of antioxidant enzymes is of inevitable importance in fish since the exposure of fish to various pathogens induces the formation of reactive oxygen species (ROS), which should be balanced by the action of antioxidant enzymes (Ishibe et al. 2008). In agreement with the results of the present study, Köprücü et al. (2015) reported that the incorporation of fish oil into rainbow trout diet resulted in the elevation of antioxidant enzyme activities. It is generally perceived that a diet rich in PUFA might cause an increase in the activity of antioxidant enzymes due to the high potential of n-3 fatty acid peroxidation and the formation of free radicals (Kiron et al. 2011). The downregulation of serum antioxidant enzymes in the present study could be an indication of lower oxidative stress, which is accounted for the considerably lower level oxidation-prone fatty acids in poultry fat in the diet. Overall, the upregulation of antioxidant enzymes in the serums of the fish fed diets with high level of PUFA indicates that the enzymes are used for the detoxification of free radicals formed as a result of fish oil oxidation (Köprücü et al. 2015). Intestinal lipase activity significantly decreased in the fish fed D3, D4, and D5 for the whole period of the trial. Lipase activity also decreased in olive flounder after total replacement of fish oil with tallow and emulsifier in diet although the difference, unlike the present study, was not significant ($p>0.05$) (Medagoda et al. 2022), which might be due to the administration of emulsifier with animal fat in the diet in their study. Furthermore, it was reported that replacement of diet fish oil with vegetable oil blend did not have significant effect on lipase activity in European seabass, which indicates the effect of alternative oil sources on the activity of lipase. It is also stated that the differences in intestinal lipase activity in fish could be attributed to differences in species (Nayak et al. 2003). It is noteworthy that returning to the diet with 100% FO again increased the intestinal lipase activity, which denotes the direct relationship between the FO in diet and lipase activity.

The results of the present study revealed that the intestinal structure of the fish experimented was undermined with higher levels of FO in diet, which is likely to be a consequence of oxidative reactions in the diet rich in n-3 fatty acids. Likewise, the extensive destruction of intestinal structure was observed in grouper fed diet containing oxidized fish oil (Long et al. 2022). It was reported that the free radicals caused as a result of unsaturated fatty acids oxidation could bring about extensive damages in the function and structure of fish intestine (Song et al. 2019). Furthermore, villi length and mucus layer thickness were adversely influenced when the fish were fed diets with high level of FO, which is in line with the observations of Long et al. (2022) in groupers fed diets containing oxidized FO. The mucosal barrier of intestinal epithelial cells and cell homeostasis are severely damaged by oxidative stress, which would cause intestine atrophy and cell dysfunction through impairment in the process of restoring intestinal epithelial cells and shedding of intestinal villi epithelial cells (Cuppoletti et al. 2012).

Conclusion

Neither a diet with 100% FO nor the one with 100% poultry fat is recommended to feed rainbow trout juveniles because of adverse effects of caused by n-3 fatty acids in the former and hematological side effects in the latter. Taken together, the replacement of 50% of FO in the diet with poultry fat could be considered a logical strategy both to reduce the cost of using high amount of FO in fish diet and to reduce or preferably prevent from the adverse effects of either oil. It is noteworthy that, according to the results of the present study, return to the diet containing 100% of FO on the second month is not recommended due to the destructive effects of such diet on intestine structure and function.

Competing interests The authors declare that they have no competing interests.

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