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Substituting palm oil for fish oil in feeds for juvenile rohu, *Labeo rohita*: effects on growth performance, fillet fatty acid composition, and antioxidant capacity

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Abstract

The study aims were to test the effects of partial or total replacement of dietary fish oil (FO) by palm oil (PO) on growth, antioxidant capacity, lysozyme activity, muscle fatty acid composition, and fillet quality of rohu (*Labeo rohita*) fingerlings. The rohu fingerlings (3.25 ± 0.13 g) were stocked in 18 circular (water volume 55 L) polyvinyl tanks in triplicate groups (30 fish per tank). Six iso-proteic (400 g/kg) and iso-lipidic (97 g/kg) purified diets were formulated in which FO was replaced by 0, 20, 40, 60, 80, and 100% PO (0 PO, 20 PO, 40 PO, 60 PO, 80 PO, and 100 PO). The diets were fed to the fish for 8 weeks, with meals being given at 8:00, 12:00, and 16:00 h. There were no significant ($P > 0.05$) treatment effects on growth (19.32–22.58 g gain/fish), feed conversion ratio (1.32–1.68), and protein efficiency ratio (1.48–1.89). However, protein retention efficiency was highest (33.25–34.99%) in fish fed the 0 PO and 60 PO diets, and lipid retention efficiency was highest (53.09%) in the fish fed the 100 PO diet. Muscle eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels and n-3/n-6 ratio were highest in fish fed the 100% FO diet and decreased as increasing proportions of FO were replaced by PO. Serum superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and lysozyme activity did not change significantly ($P > 0.05$) up to 60% replacement of FO with PO. Further replacement of FO with PO (80 PO and 100 PO) resulted in decreased serum antioxidant capacity and lysozyme activity. Although muscle atherogenicity and thrombogenicity indices did not change significantly ($P > 0.05$) among treatments, the highest hypocholesterolaemic-to-hypercholesterolaemic (H/H) ratio and fillet lipid quality (FLQ) were found in fish receiving the 0 PO (with 100% FO and no PO). H/H and FLQ values did not differ significantly up to 60% replacement of FO with PO but decreased upon further replacement of FO with PO. FO can be replaced by PO to a level of 60% without hampering the growth and fillet quality of fingerling rohu provided with such diets for a period of a few weeks, but the long-term effects of FO replacement remain to be studied.

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Keywords Feed oils · Nutrient utilization · Proximate chemical composition · Blood chemistry · Fingerling · *Labeo rohita*

Introduction

Lipid is an essential component in the fish diet as it provides metabolic energy and essential nutrients such as fatty acids. Fish oil (FO) is considered an important lipid source in aquaculture feeds due to its high proportions of n-3 LC-PUFA. In comparison to oils of terrestrial origin, FO contains an abundance of EPA (eicosapentaenoic acid; 20:5n-3) and DHA (docosahexaenoic acid; 22:6n-3) (Turchini et al. 2011). These fatty acids prevent neurodegenerative diseases and arteriosclerosis, and cerebrovascular and cardiovascular diseases in humans (Turchini et al. 2012; Golden et al. 2021). Fish must also obtain EPA and DHA through their diet (NRC 2011). Both EPA and DHA play essential roles in accelerating fish growth and neural development, improving the immunity of the organism, and regulating the metabolism. However, with the decline in fish oil production, it cannot meet the escalating demand of the aquaculture industry. Also, the shortage of FO production due to the decrease in capture fishery has led to an increase in the FO price. Although FO is considered the best oil to supply essential fatty acids, it has been reported that PUFAs, especially EPA and DHA present in FO, are highly prone to lipid peroxidation, which get oxidized easily. Feeding fish with oxidized oil results in oxidative stress and liver damage to the organism. Thus, there is a dire need to find economically sustainable alternatives to FO in aquaculture feeds. Reducing the inclusion of FO in aquaculture feeds and ensuring the appropriate proportion of n-3 LC-PUFA in the final product are still a challenge. Vegetable oils (VOs) are the most sustainable alternatives to fish oil due to their wide availability, increasing global production, and lower price. However, a common characteristic of all VOs is an absence of n-3 and n-6 LC-PUFA. They are rich in C_{18} PUFA, such as alpha-linolenic acid (ALA) and linoleic acids (LA), which freshwater fish can convert into C_{20} and C_{22} PUFAs such as arachidonic acid (ARA), EPA, and DHA (Tocher 2003). Biosynthesis includes the desaturation and elongation process of C_{18} PUFAs (ALA and LA). The Elovl5 elongase and Δ6 desaturase are the crucial enzymes involved in this biosynthesis (Tocher et al. 2004). The biosynthesis capacity of these enzymes differs among fish species (Monroig et al. 2011).

Mostly used VOs in aquaculture feeds are linseed, soybean, rapeseed, palm, olive, and sunflower oil. Palm oil (PO) is used in aquaculture due to its wide range of availability and low price. It is abundantly available in several Asian countries and is one of the cheap oils in India. It contains a high proportion of palmitic acid (C16:0) and oleic (C18:1) and linoleic (C18: 2n-6) fatty acids. Compared to other VOs, PO is extremely rich in beta-carotenoids, the precursor of vitamin A which gives it its characteristic reddish-orange color. It is also rich in vitamin E, and antioxidants such as tocopherol and tocotrienol, which protect cell membranes from lipid peroxidation. PO has been used in the replacement of dietary FO in several farmed fish species (Ng et al. 2004; Fonseca-Madrigal et al. 2005; Bahurmiz and Ng 2007; Komilus et al. 2008; Babalola and Apata 2012; Gao et al. 2012; Duan et al. 2014; Huang et al. 2016; Ayisi et al. 2016, 2018; Safiin et al. 2021; Alves et al. 2021).

Freshwater aquaculture in India is mainly dominated by Indian major carp (IMC) species. IMCs, catla, *Catla catla*, rohu, *Labeo rohita*, and mrigal, *Cirrhinus mrigala* are the most important and prime cultivable fish species in India due to high growth rate, taste, and public preference. Rohu is one of the top ten aquaculture species cultured worldwide

(FAO 2022). It consists of almost 35% of the total cultured fish production of India. It can grow up to 800–1000 g in a year. Due to the higher market value of rohu, the farmers in India have moved from three-species to two-species polyculture systems with rohu and catla (FAO 2018). Rohu is an important aquaculture species in India, Bangladesh, Pakistan, and Myanmar (Burma) (Rasal and Sundaray 2020). The high fecundity (2 lakh eggs/per kg), external fertilization, and domestication of this species made it easy for intensive culture (Rasal and Sundaray 2020). With the increasing market demand for rohu, there is a dire need to intensify its culture requiring nutritionally balanced and low-cost aquaculture feeds. Although the information on dietary protein, amino acid, and lipid requirements is available, no information is available on the substitution of FO with PO for rohu fingerling. Therefore, the present experiment was conducted to assess the possibility of replacing fish oil with palm oil and its effects on growth performance, fatty acid profiles, fillet quality, and immune response of rohu fingerling.

Materials and methods

Preparation of experimental diets

Six experimental, purified, iso-proteic (400 g/kg crude protein) and iso-lipidic (97 g/kg crude lipid) diets containing FO and PO were prepared as the main lipid sources. Mechanically extracted unrefined palm oil was obtained from a local factory for the replacement trial. The levels of substituting FO in experimental diets were chosen based on results obtained in feeding trial conducted earlier (Siddiqua and Khan 2022b). In the 0 PO and 100 PO diets, FO and PO were the sole lipid sources, respectively. In 20 PO, 40 PO, 60 PO, and 80 PO diets, the FO was serially replaced by 20%, 40%, 60%, and 80% PO (Table 1). The fatty acid profile of diets is depicted in Table 2. Casein and gelatine (fat free) were used as a protein source and dextrin as a carbohydrate. Protein in the experimental diets was fixed at 400 g/kg, reported optimum for fingerling *L. rohita* (Swamy and Mohanty 1990; Satpathy et al. 2003). The dietary lipid level was fixed at 97 g/kg as per the requirement reported for *L. rohita* fingerling (Siddiqua and Khan 2022a). Mineral and vitamin premixes were prepared and added as per Halver (2002). The experimental diets were prepared as per the method adopted by Abdel-Hameid et al. (2017). Gelatin was dissolved in distilled water by stirring and heating the bowl, followed by the addition of casein at 80 °C. After that, oils were added and mixed (Hobart Corporation, Troy, OH, USA) for about 15 min. When the mixture cooled down to 40 °C, mineral and vitamin premixes were added with continuous mixing. Lastly, carboxymethyl cellulose was added and mixed. The dough thus produced was forced through the 2-mm die of an extruder. The moisture content of the strands was reduced to 100 g/kg by drying at 40 °C in a hot air oven. The strands were then crumbled to the required size (500 µm), packed, and stored at -4 °C. The proximate composition of the test diets and initial and final fish were analyzed using AOAC (2015) methods. Moisture content was determined by putting the samples in a hot air oven maintained at 102 ± 1 °C (Yorko Instruments, New Delhi, India). Crude protein ($N \times 6.25$) was determined using Kjeldahl method in an automatic analyzer (Kjeltec TecatorTM Technology 2300, Hoganas, Sweden). Crude fat was assessed by the solvent extraction method (Socs Plus SCS 4, Pelican Equipments, Chennai, India), and ash by burning the sample in a furnace at 650 °C for 12 h (S.M. Scientific Instrument Pvt. Ltd., Jindal Company, New

Table 1 Composition of experimental diets

	Fish oil replacement level (%)					
	0 PO	20 PO	40 PO	60 PO	80 PO	100 PO
Casein (fat free)[a]	400	400	400	400	400	400
Gelatin[b]	100	100	100	100	100	100
Dextrin	179.8	179.8	179.8	179.8	179.8	179.8
Fish oil	97	77.6	58.2	38.8	19.4	0
Palm oil	0	19.4	38.8	58.2	77.6	97
Mineral mix[c,d]	40	40	40	40	40	40
Vitamin mix[d,e]	30	30	30	30	30	30
α-Cellulose	113.2	113.2	113.2	113.2	113.2	113.2
Carboxymethyl cellulose	40	40	40	40	40	40
Total	1000	1000	1000	1000	1000	1000
Analyzed crude protein	39.9	39.7	39.89	39.87	39.98	40
Analyzed crude lipid	9.71	9.74	9.71	9.72	9.72	9.73
Estimated gross energy (kJ/g)[f]	17.91	17.79	17.9	17.89	17.87	17.86

[a]Crude protein (760 g/kg; Loba chemie, India). [b]Crude protein (960 g/kg; Loba chemie, India). [c]Mineral mixture (g/kg): calcium biphosphate 135.7; calcium lactate 326.9; ferric citrate 29.7; magnesium sulfate 132.0; potassium phosphate (dibasic) 239.8; sodium biphosphate 87.2; sodium chloride 43.5; aluminum chloride. $6H_2O$ 0.154; potassium iodide 0.15; cuprous chloride 0.10; manganous sulfate. H_2O 0.80; cobalt chloride. $6H_2O$ 1.00; zinc sulfate. $7H_2O$ 4.0; Loba chemie, India. [d]Halver (2002). [e]Vitamin mixture (30 g/kg of diet; 10 g vitamin mix + 20 g α-cellulose): choline chloride 5.00; inositol 2.00; ascorbic acid 1.00; niacin 0.75; calcium pantothenate 0.5; riboflavin 0.2; menadione 0.04; pyridoxine hydrochloride 0.05; thiamine hydrochloride 0.05; folic acid 0.015; biotin 0.005; alpha-tocopherol 0.4; vitamin B12 0.0001; Loba chemie, India. [f]Estimated value of the basal diet on Gallenkamp ballistic bomb calorimeter

Delhi, India). Gross energy of the test diets was determined in a Gallenkamp ballistic bomb calorimeter (CBB 330 010L, Gallenkamp, Loughborough, UK).

Fatty acid profile assay

Fatty acid profiles were analyzed following the procedures described by Metcalfe et al. (1966) with some modifications. About 50–100 mg of freeze-dried experimental diet and muscle samples was added into a 20-mL screwed tube with a lid. After that, 1 mL diethyl ether was added and mixed. Then, 1 mL 0.5% methanolic potassium hydroxide (1 N) was added, continuously shaken for 10 min, and placed in a water bath at 75 °C for 20 min. One milliliter hydrogen chloride (1 N) was added after cooling and then heated in a water bath at 75 °C for another 20 min. After that, 2–3 mL petroleum ether was added, shaken continuously for about 1 min, and allowed to get separated into two layers. The upper layer containing fatty acid methyl esters was separated and dried in a water bath for about 20 min. Lastly, 0.5 mL n-haptane was added to the tube. Finally, FAMEs of all the samples (approximately 1 μL) were quantified by a gas chromatograph-mass spectrometer (GC–MS) (Shimadzu QP-2010 Plus coupled with Thermal Desorption System TD 20 and capillary column DB-5MS, and helium as carrier gas). The injection temperature was 250 °C and the total sampling time was 1 min. For the identification of fatty acids detected here, the retention times of the fatty acid

Table 2 Fatty acid composition of experimental diets (% total fatty acids)

Parameters	Fish oil replacement level (%)							
	FO	PO	0 PO	20 PO	40 PO	60 PO	80 PO	100 PO
14:0	4.05	1.98	3.9	3.15	2.26	2.15	2.17	1.81
16:0	14.9	40.7	14.2	16.41	21.19	27.64	34.08	38.56
18:0	2.6	5.68	2.1	2.91	3.39	3.98	4.95	5.51
∑SFA[a]	21.55	48.36	20.2	22.47	26.84	33.77	41.2	45.88
16:1n-7	9.58	0.65	9.41	8.12	6.19	4.71	2.24	0.56
18:1n-9	24.88	35.8	23.48	26.38	28.01	30.78	32.19	34.78
20:1n-9	6.91	0.09	6.89	5.02	4.21	3.21	1.45	0.08
22:1n-9	5.65	0	5.51	4.78	3.98	2.75	1.41	nd
∑MUFA[b]	47.02	36.54	45.29	44.3	42.39	41.45	37.29	35.42
18:2n-6 LA[c]	4.18	11.58	3.89	4.98	5.42	7.45	9.79	10.41
18:3n-6	0.15	0	0.11	0.12	0.02	0.04	0.01	nd
20:4n-6 ARA[d]	2.96	0	2.78	2.64	2.45	2.12	1.08	0.34
∑n-6 PUFA[e]	7.29	11.58	6.78	7.74	7.89	9.61	10.88	10.75
18:3n-3 ALA[f]	0.81	2.18	0.71	0.98	1.01	1.71	1.97	2.05
18:4n-3	1.05	0.25	1.01	0.91	0.78	0.68	0.45	0.12
20:5n-3 EPA[g]	11.81	0	11.67	10.01	8.98	4.98	2.75	nd
22:5n-3	1.45	0	1.31	1.25	1.15	1.04	0.98	nd
22:6n-3 DHA[h]	13.15	0	12.98	11.1	10.67	6.09	3.91	nd
∑n-3 PUFA[i]	28.27	2.43	27.68	24.25	22.59	14.5	10.06	2.17
n-3/n-6 PUFA[j]	3.87	0.20	4.08	3.13	2.86	1.50	0.92	0.20
PIn[k]	195.17	16.85	191.64	167.70	157.57	99.06	66.78	16.75

Some fatty acids, of which the contents are minor, trace amount, or not detected, were denoted as nd in Table 2; [a]*SFA*, saturated fatty acids; [b]*MUFA*, mono-unsaturated fatty acids; [c]*LA*, linoleic acid; [d]*ARA*, arachidonic acid; [e]*n-6 PUFA*, n-6 polyunsaturated fatty acids; [f]*ALA*, alpha-linolenic acid; [g]*EPA*, eicosapentaenoic acid; [h]*DHA*, docosahexaenoic acid; [i]*n-3 PUFA*, n-3 polyunsaturated fatty acids; [j]*n3/n6 PUFA*, n-3 polyunsaturated fatty acids:n-6 polyunsaturated fatty acids; [k]*PIn*, peroxidation index

were compared with the internal standard (methyl heneicosanoate, C21:0; Sigma-Aldrich). The percent of each fatty acid was calculated as the proportion of the area under the peak in question to the total area of all peaks.

Fish maintenance

For this experiment, induced bred *Labeo rohita* fingerling were procured from the hatchery of College of Fisheries, G. B. Pant University of Agriculture and Technology, Pantnagar, and shifted to a feeding trial laboratory. After giving prophylactic treatment to the fish by dipping in $KMnO_4$ (1:3000) solution, they were stocked in cylindrical (water volume 600 L; 1.22 m diameter, 0.91 m height) tanks and acclimatized by feeding H440 dry diet (Halver 2002) for 2 weeks before the start of the feeding trial.

Experimental design and feeding trial

Fifty fish were taken at random from the acclimated stock, and their lengths and weights were recorded (6.41 ± 0.21 cm; 3.25 ± 0.13 g) for calculation of condition factor (CF). The liver and viscera were dissected from 10 fish following anesthetizing the fish in tricaine methane sulfonate solution (MS-222; 200 mg/L; Sigma, St Louis, MO, USA). Livers and viscera were weighed, and the data used for calculating the hepatosomatic index (HSI) and viscero-somatic index (VSI). Thirty fish were taken, weighed, and then stored for the analysis of initial proximate chemical composition. For conducting the feeding trial, 540 fish were randomly distributed (30 fish per tank, triplicate group per treatment) to 18 circular (water volume 55 L) polyvinyl tanks, with the fish being weighed to obtain information about initial weights, and the biomass of fish present in each tank at the start of the trial. The tanks were supplied with water at the rate of 1–1.5 L/min and were run as a flow-through system. The fish were fed their allotted diet to apparent satiation at 8:00, 12:00, and 16:00 h for 8 weeks. A 12-h light and 12-h dark photoperiod was maintained. Following anesthetization in MS-222 (100 mg/L), the fish were weighed at 2-week intervals (Precisa 120A; 0.1 mg sensitivity; Oerlikon AG, Zurich, Switzerland) to monitor weight gain. The fish were not fed on the sampling day to avoid stress.

Water quality parameter analysis

Water quality parameters were measured weekly following methods mentioned in APHA (1992). Water temperature and dissolved oxygen ranged from 25.6 to 28.7 °C and 6.84 to 7.67 mg/L, respectively. Total ammonia nitrogen, alkalinity, free carbon dioxide, and pH ranged between 0.22–0.31 mg/L, 65.4–78.1 mg/L, 6.4–10.5 mg/L, and 7.2–7.5, respectively.

Sample collection and chemical analyses

On the day of the termination of the feeding trial, all fish were anesthetized with MS-222 (100 mg/L) and their mass weight was recorded for calculating their growth metrics. The length and weight of 10 fish from each replicate of the groups were recorded for calculating the CF. After that, their blood samples were rapidly collected from the caudal vein using 2-mL plastic syringes (with 0.6-mm-diameter needles) without anticoagulant in the dried Eppendorf tubes. To collect serum, blood samples were let to settle for about 10 min in a slanted position for clotting at room temperature. The samples were centrifuged ($3000 \times g$, 4 °C, 10 min), and serum was collected and stored at − 20 °C for further biochemical analyses. After that, 5 fish from each replicate were weighed; liver and viscera were dissected to calculate HSI and VSI. After that, muscle from another 5 fish was immediately harvested and stored at − 20 °C for analyzing the fatty acid composition. The final body composition of the remaining fish from each replicate ($n = 3 \times 5$) group was analyzed.

Serum oxidation and antioxidant parameters assay

Five subsamples ($n = 3 \times 5$) from the stored serum samples were subjected to biochemical analyses of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and malondialdehyde (MDA). The method adopted by Buege and Aust (1978) was used to determine the MDA concentration. SOD was assayed by following the method of Misra

and Fridovich (1972), and CAT activity was determined by the method of Takahara et al. (1960) and GPx by Rotruck et al. (1973). Serum SOD, CAT, and GPx activities were expressed in U/mL, whereas MDA concentration was expressed in nmol/mL.

Lysozyme activity analysis

Lysozyme activity of serum was assessed following the turbidimetric method (Hultmark et al. 1980), adopted by Wang et al. (2015). A serum sample (20 μL) with 0.1 M sodium phosphate buffer (pH 6.4) was added to the suspension of 1.2 mL *Micrococcus lysodeikticus.* The absorbance was read in a spectrophotometer after 0.5 and 4.5 min at 530 nm. One unit of lysozyme activity is the sample quantity resulting in a decline in absorbance at 530 nm of 0.001/min compared with the control. The lysozyme activities were expressed in U/mL.

Calculation of growth parameters, biometric indices, and diet peroxidation index

$$\text{Absolute weight gain (g/fish)} = \text{Final body weight (g/fish)} - \text{Initial body weight (g/fish)}$$

$$\text{Specific growth rate (SGR;\%/day)} = \ln \text{final body weight (g)} - \ln \text{initial body weight (g)} / \text{No. of days of the experiment} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \text{Dry feed fed (g)/Wet weight gain (g)}$$

$$\text{Protein efficiency ratio (PER)} = \text{Weight gain (g)/Protein fed (g)}$$

$$\text{Protein retention efficiency (PRE\%)} = \text{Protein gain (g)/Protein intake} \times 100$$

$$\text{Lipid retention efficiency (LRE\%)} = \text{Lipid gain (g)/Lipid intake} \times 100$$

$$\text{Hepatosomatic index (HSI\%)} = \text{Liver weight (g)/Body weight (g)} \times 100$$

$$\text{Viscerosomatic index (VSI\%)} = \text{Viscera weight (g)/Body weight (g)} \times 100$$

$$\text{Condition factor (CF)} = \text{Body weight (g)/Body length (cm)}^3 \times 100$$

$$\text{PIn (Peroxidation index)} = 0.025 \times (\text{\% of monoenoics}) + 1 \times (\text{\% of dienoics}) + 2 \times (\text{\% of trienoics}) + 4 \times (\text{\% of tetraenoics}) + 6 \times (\text{\% of pentaenoics}) + 8 \times (\text{\% of hexaenoics})$$

(Witting and Horwitt 1964; Betancor et al. 2016)

Indices of nutritional quality of fish fillet

The fatty acid composition of muscle was used to determine the nutritional parameters of lipids. The following equations were used to calculate the fillet nutritional quality indices. The n3/n6, n6/n3, PUFA/SFA, and EPA/ARA ratios were also calculated.

$$\text{AI(atherogenicity)} = [12{:}0 + (4 \times 14{:}0) + 16:0]/[\sum \text{MUFA} + \sum \text{n}-6 + \sum \text{n}-3]$$

(Ulbricht and Southgate 1991; Siddik et al. 2019)

$$\text{TI (thrombogenicity)} = (14{:}0 + 16{:}0 + 18{:}0)/[0.5 \times (\sum \text{MUFA} + \sum \text{n-6}) + (3 \times \sum \text{n-3}) + (\sum \text{n-3}/\sum \text{n-6})]$$

(Ulbricht and Southgate 1991; Chen and Liu 2020)

$$\text{H/H index (hypocholesterolaemic / hypercholesterolaemic FA ratio)} = (18{:}1\text{n-9} + 18{:}2\text{n-6} + 18{:}3\text{n-3} + 20{:}4\text{n-6} + 20{:}5\text{n-3} + 22{:}5\text{n-3} + 22{:}6\text{n-3}) / (14{:}0 + 16{:}0)$$

(Santos-Silva et al. 2002)

$$\text{Fillet lipid quality (FLQ)} = (20:5n-3 + 22:6n-3)/\sum \text{total FA}$$

(Abrami et al. 1992)

Statistical analyses

To analyze all the data, a one-way analysis of variance (Sokal and Rohlf 1981) at a level of $P<0.05$ significance was done. The normality of the data was confirmed using the Shapiro–Wilk test before analysis, and the homogeneity of variance was tested using Levene's test. Tukey's honest significant difference test was performed for multiple mean comparisons at a level of significance of $P<0.05$. All the analyses were carried out using SPSS 20.0 (SPSS, USA). Principal component analysis (Wold et al. 1987) was done as an unsupervised pattern for a statistical procedure that converts a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables using orthogonal transformation. The main information in the variables is expressed by a lower number of variables called principal components (PC1, PC2). PCA was carried out on the data matrix of the fatty acid composition of the muscle of fish fed experimental diets using statistical software (Origin version 9.1; Origin Software, San Clemente, CA).

Results

Growth response, conversion efficiency, proximate composition, and biometric indices

The effects of dietary FO replacement with PO on the growth performance of rohu fingerling are summed up in Table 3. Inclusion of PO in the diets did not show any negative impact ($P>0.05$) on growth in *L. rohita* fingerling. Absolute weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) ranged between 19.32–22.58 g/fish, 3.36–3.75%/day, 1.32–1.68, and 1.48–1.89, respectively. However, protein retention efficiency was recorded highest (33.25–34.99%) in fish fed 100% FO diet (0 PO) and 60% replacement of FO in diet (60 PO). Lipid retention efficiency was found highest (53.09%) in fish fed 100% FO replaced diet (100 PO) and lowest (27.89%) in fish fed 20% FO replaced diet (20 PO). No mortality was noted in fish receiving all the diets. Carcass protein was recorded significantly ($P<0.05$) higher (179.13 g/kg, wet basis) in fish receiving 100% FO diet (0 PO), followed by 20% (20 PO), 40% (40 PO), and 60% (60 PO) replacement of FO in diets. The lowest carcass protein (156.51 and 144.64 g/kg) was evident in the fish receiving diets with 80% (80 PO) and 100% (100 PO) replacement of FO. Carcass fat content showed the reverse trend (Table 4). HSI (3.98%) and VSI (6.98%) were recorded highest ($P<0.05$) in fish receiving a 100% FO replaced diet (100 PO) followed by a diet containing 80% replacement of FO (80 PO). The lowest

Table 3 Growth performance of fingerling *Labeo rohita* fed different experimental diets[1,2]

	Fish oil replacement level (%)					
	0 PO	20 PO	40 PO	60 PO	80 PO	100PO
Average initial weight (g)	3.12 ± 0.09^{a}	3.24 ± 0.06^{a}	3.45 ± 0.07^{a}	3.14 ± 0.08^{a}	3.41 ± 0.06^{a}	3.25 ± 0.04^{a}
Average final weight (g)	24.8 ± 0.52^{a}	22.73 ± 0.46^{a}	22.77 ± 0.63^{a}	25.72 ± 0.59^{a}	23.86 ± 0.72^{a}	22.66 ± 0.58^{a}
Absolute weight gain (g/fish)	21.68 ± 1.6^{a}	19.49 ± 1.8^{a}	19.32 ± 1.9^{a}	22.58 ± 1.4^{a}	20.45 ± 1.5^{a}	19.45 ± 1.3^{a}
Specific growth rate (%/day)	3.70 ± 0.04^{a}	3.47 ± 0.03^{a}	3.36 ± 0.05^{a}	3.75 ± 0.04^{a}	3.47 ± 0.05^{a}	3.46 ± 0.03^{a}
Feed conversion ratio	1.41 ± 0.08^{a}	1.68 ± 0.09^{a}	1.59 ± 0.07^{a}	1.32 ± 0.08^{a}	1.46 ± 0.06^{a}	1.51 ± 0.08^{a}
Protein efficiency ratio	1.77 ± 0.04^{a}	1.48 ± 0.03^{a}	1.57 ± 0.05^{a}	1.89 ± 0.03^{a}	1.71 ± 0.04^{a}	1.65 ± 0.04^{a}
Protein retention efficiency (%)	33.25 ± 1.12^{a}	27.70 ± 1.34^{bc}	29.45 ± 1.01^{b}	34.99 ± 1.98^{a}	27.82 ± 1.35^{bc}	24.56 ± 1.56^{c}
Lipid retention efficiency (%)	30.99 ± 1.5^{cd}	27.89 ± 1.25^{d}	33.41 ± 1.45^{c}	34.25 ± 1.56^{c}	46.76 ± 1.34^{b}	53.09 ± 1.25^{a}

[1]Mean values of 3 replicates ± SEM. [2]Mean values sharing the different superscripts in the same row are significantly different ($P < 0.05$)

Table 4 Carcass compositions (g/kg, wet basis), and biological indices of fingerling *Labeo rohita* fed different experimental diets[1,2]

	Fish oil replacement level (%)						
	Initial	0 PO	20 PO	40 PO	60 PO	80 PO	100 PO
Moisture	785.65 ± 4.3	738.92 ± 4.1^{a}	739.41 ± 3.8^{a}	742.51 ± 4.3^{a}	743.81 ± 5.6^{a}	744.41 ± 3.2^{a}	744.46 ± 4.9^{a}
Crude protein	120.41 ± 3.2	179.13 ± 3.5^{a}	176.28 ± 2.7^{a}	177.25 ± 3.5^{a}	176.98 ± 3.9^{a}	156.51 ± 2.6^{b}	144.64 ± 2.8^{c}
Crude fat	40.15 ± 1.8	42.10 ± 1.7^{c}	44.75 ± 1.3^{c}	49.80 ± 1.6^{c}	43.45 ± 1.9^{c}	62.54 ± 1.4^{b}	72.54 ± 1.2^{a}
Ash	30.10 ± 1.2	22.54 ± 0.8^{a}	24.54 ± 0.6^{a}	20.15 ± 0.5^{a}	20.39 ± 0.6^{a}	24.25 ± 0.2^{a}	24.78 ± 0.4^{a}
HSI (%)	1.51 ± 0.06	2.21 ± 0.08^{b}	2.42 ± 0.08^{b}	2.94 ± 0.08^{ab}	2.96 ± 0.08^{ab}	3.39 ± 0.07^{a}	3.98 ± 0.09^{a}
VSI (%)	3.21 ± 0.09	4.86 ± 0.14^{b}	4.94 ± 0.17^{b}	4.48 ± 0.16^{b}	4.91 ± 0.15^{b}	5.54 ± 0.18^{ab}	6.98 ± 0.20^{a}
CF (g/cm^3)	0.87 ± 0.04	1.64 ± 0.03^{a}	1.39 ± 0.02^{a}	1.41 ± 0.04^{a}	1.59 ± 0.02^{a}	1.46 ± 0.02^{a}	1.42 ± 0.03^{a}

[1]Mean values of 3 replicates ± SEM. [2]Mean values sharing the different superscripts in the same row are significantly different ($P < 0.05$). *HSI*, hepatosomatic index; *VSI*, viscero-somatic index; *CF*, condition factor

values for HSI (2.21%) and VSI (4.96%) were recorded in fish fed 100% FO diet (0 PO), followed by 20% (20 PO), 40% (40 PO), and 60% (60 PO) replacement of FO diets. However, the condition factor (1.39–1.64 g/cm^3) remained significantly unchanged ($P > 0.05$) in fish receiving all the diets.

Serum antioxidant and non-specific immune status

Serum antioxidant activities of rohu fingerling are depicted in Table 5. Serum SOD, CAT, and GPx values did not change significantly ($P > 0.05$) up to 60% replacement of FO (60 PO) in diet and then declined with the lowest activity noted in fish fed 100% PO diet (100 PO). Highest ($P < 0.05$) MDA activity (15.76 nmol/mL) was recorded in fish receiving a diet containing 100% FO (0 PO), whereas the activity decreased upon further replacement of FO with PO in diet. The highest ($P < 0.05$) serum lysozyme activity (256.89 U/mL) was recorded in fish receiving diet with 60% replacement of FO (60 PO), followed by 40% (40 PO) and 0% (0 PO) FO replacement diets. The lowest lysozyme activity (240.79 U/mL) was recorded in fish receiving a 100% PO containing diet (100 PO).

Muscle fatty acid composition

The muscle fatty acid profile of rohu fingerling fed test diets is depicted in Table 6. Significantly ($P < 0.05$) highest proportion of SFA in muscle (38.91%) was noted in fish receiving a 100% PO diet (100 PO). The highest proportion of MUFA (30.76%) was noted in fish receiving a 100% FO diet (0 PO) compared to other diets ($P < 0.05$). No significant difference ($P > 0.05$) was recorded in the percentage of ALA (1.09–1.99%) in the muscle of fish fed all the diets. Muscle EPA and DHA did not differ significantly ($P > 0.05$) in fish fed diets up to 60% replacement of FO (60 PO). However, further replacement of FO with PO in the diets (80 PO and 100 PO) led to a decline in muscle EPA and DHA content. Significantly higher ($P < 0.05$) muscle n-3/n-6 ratio (4.12) was recorded in fish receiving 100% FO diet (0 PO) and no significant change ($P > 0.05$) in n-3/n-6 ratio was recorded up to fish

Table 5 Serum antioxidant capacity and lysozyme activity of fingerling *Labeo rohita* fed different experimental diets[1,2]

	Fish oil replacement level (%)					
	0 PO	20 PO	40 PO	60 PO	80 PO	100 PO
MDA (nmol/mL)	15.76 ± 0.07^{a}	14.95 ± 0.45^{ab}	13.08 ± 0.43^{b}	12.14 ± 0.35^{c}	12.59 ± 0.61^{c}	12.94 ± 0.52^{c}
SOD (U/mL)	64.75 ± 0.34^{a}	64.51 ± 0.47^{a}	63.99 ± 0.23^{a}	63.75 ± 0.32^{a}	62.74 ± 0.45^{a}	59.61 ± 0.34^{b}
CAT (U/mL)	38.51 ± 0.17^{a}	38.24 ± 0.25^{a}	37.88 ± 0.27^{a}	37.41 ± 0.31^{a}	36.14 ± 0.06^{a}	34.84 ± 0.05^{b}
GPx (U/mL)	258.94 ± 0.09^{a}	258.41 ± 0.07^{a}	256.45 ± 0.06^{a}	245.71 ± 0.08^{ab}	240.94 ± 0.05^{b}	237.24 ± 0.03^{c}
Lysozyme (U/mL)	255.68 ± 8.79^{a}	252.75 ± 7.51^{a}	254.97 ± 7.01^{a}	256.89 ± 8.92^{a}	245.97 ± 7.91^{b}	240.79 ± 8.19^{b}

[1]Mean values of 3 replicates ± SEM. [2]Mean values sharing the different superscripts in the same row are significantly different ($P < 0.05$). *MDA*, malondialdehyde; *SOD*, superoxide dismutase; *CAT*, catalase; *GPx*, glutathione peroxidase

Table 6 Muscle fatty acid compositions of fingerling *Labeo rohita* fed different experimental diets (% total fatty acids)[1,2]

Parameters	Fish oil replacement level (%)					
	0 PO	20 PO	40 PO	60 PO	80 PO	100 PO
14:0	2.49 ± 0.04^{a}	2.46 ± 0.21^{a}	1.97 ± 0.08^{a}	1.89 ± 0.12^{a}	1.25 ± 0.14^{ab}	1.04 ± 0.06^{ab}
16:0	12.99 ± 0.1^{c}	17.57 ± 0.19^{b}	19.89 ± 0.09^{ab}	20.04 ± 0.21^{ab}	26.09 ± 0.23^{ab}	32.89 ± 0.08^{a}
18:0	6.45 ± 0.12^{a}	5.45 ± 0.14^{a}	5.81 ± 0.11^{a}	5.65 ± 0.14^{a}	4.7 ± 0.21^{ab}	4.98 ± 0.19^{ab}
∑SFA[a]	21.93 ± 0.14^{c}	25.48 ± 0.21^{b}	27.67 ± 0.12^{b}	27.58 ± 0.08^{b}	32.04 ± 0.09^{ab}	38.91 ± 0.21^{a}
16:1n-7	6.59 ± 0.22^{a}	5.12 ± 0.21^{a}	4.34 ± 0.23^{b}	3.87 ± 0.24^{b}	2.84 ± 0.32^{bc}	1.04 ± 0.31^{c}
18:1n-9	14.54 ± 0.22^{b}	16.79 ± 0.23^{ab}	17.65 ± 0.24^{ab}	18.76 ± 0.24^{a}	18.89 ± 0.26^{a}	19.98 ± 0.25^{a}
20:1n-9	3.02 ± 0.34^{a}	2.02 ± 0.31^{a}	2.25 ± 0.33^{a}	1.96 ± 0.24^{ab}	0.98 ± 0.21^{ab}	0.54 ± 0.27^{ab}
22:1n-9	6.61 ± 0.34^{a}	5.78 ± 0.35^{ab}	4.19 ± 0.45^{c}	3.18 ± 0.24^{c}	1.45 ± 0.42^{d}	0.64 ± 0.21^{d}
∑MUFA[b]	30.76 ± 0.64^{a}	29.71 ± 0.78^{a}	28.43 ± 0.89^{a}	27.77 ± 0.56^{ab}	24.16 ± 0.67^{b}	22.2 ± 0.82^{c}
18:2n-6 LA[c]	5.58 ± 0.34^{c}	6.62 ± 0.45^{c}	7.59 ± 0.36^{c}	7.99 ± 0.43^{c}	17.52 ± 0.26^{b}	23.67 ± 0.41^{a}
18:3n-6	0.21 ± 0.42^{a}	1.98 ± 0.45^{a}	1.07 ± 0.43^{a}	1.44 ± 0.45^{a}	2.37 ± 0.41^{a}	3.98 ± 0.54^{a}
20:4n-6 ARA[d]	2.58 ± 0.54^{a}	2.05 ± 0.53^{a}	1.74 ± 0.56^{a}	1.04 ± 0.52^{a}	1.01 ± 0.42^{a}	0.98 ± 0.43^{a}
∑n-6 PUFA[e]	8.37 ± 0.82^{d}	10.65 ± 0.89^{c}	10.4 ± 0.97^{c}	10.47 ± 0.97^{c}	20.9 ± 0.78^{b}	28.63 ± 0.76^{a}
18:3n-3 ALA[f]	1.09 ± 0.02^{a}	1.57 ± 0.09^{a}	1.89 ± 0.08^{a}	1.99 ± 0.06^{a}	1.24 ± 0.07^{a}	1.21 ± 0.06^{a}
18:4n-3	0.14 ± 0.02^{a}	0.32 ± 0.04^{a}	0.06 ± 0.03^{a}	0.67 ± 0.04^{a}	0.98 ± 0.01^{a}	0.41 ± 0.04^{a}
20:5n-3 EPA[g]	14.98 ± 0.45^{a}	13.78 ± 0.65^{a}	13.98 ± 0.55^{a}	13.64 ± 0.43^{a}	9.18 ± 0.46^{b}	5.94 ± 0.49^{c}
22:5n-3	2.01 ± 0.27^{a}	0.98 ± 0.14^{b}	0.49 ± 0.02^{b}	0.02 ± 0.35^{b}	0.58 ± 0.45^{b}	0.45 ± 0.35^{b}
22:6n-3 DHA[h]	16.27 ± 0.39^{a}	15.82 ± 0.35^{a}	15.61 ± 0.25^{a}	15.45 ± 0.35^{a}	10.26 ± 0.35^{b}	6.79 ± 0.45^{c}
∑n-3 PUFA[i]	34.49 ± 1.02^{a}	32.47 ± 1.01^{a}	32.03 ± 0.99^{a}	31.77 ± 0.98^{ab}	22.24 ± 1.01^{b}	14.8 ± 1.04^{c}
n-3/n-6 PUFA	4.12 ± 0.98^{a}	3.04 ± 0.87^{b}	3.07 ± 0.79^{b}	3.03 ± 0.89^{b}	1.06 ± 0.74^{c}	0.51 ± 0.89^{c}
EPA/ARA	5.80 ± 0.34^{c}	6.72 ± 0.25^{c}	8.03 ± 0.45^{b}	13.11 ± 0.24^{a}	9.08 ± 0.25^{b}	6.06 ± 0.35^{c}
AI[j]	0.31 ± 0.21^{a}	0.37 ± 0.24^{a}	0.39 ± 0.25^{a}	0.39 ± 0.35^{a}	0.46 ± 0.45^{a}	0.56 ± 0.35^{a}
TI[k]	0.17 ± 0.31^{a}	0.21 ± 0.41^{a}	0.23 ± 0.15^{a}	0.23 ± 0.24^{a}	0.35 ± 0.25^{a}	0.55 ± 0.45^{a}
H/H[l]	3.55 ± 0.24^{a}	2.82 ± 0.35^{a}	2.67 ± 0.41^{ab}	2.68 ± 0.26^{ab}	2.12 ± 0.45^{b}	1.72 ± 0.24^{b}
FLQ[m]	32.70 ± 0.41^{a}	30.10 ± 0.54^{a}	30.03 ± 0.42^{a}	29.80 ± 0.35^{a}	19.56 ± 0.45^{b}	12.17 ± 0.42^{c}
n-6/n-3 PUFA	0.24 ± 0.02^{a}	0.32 ± 0.03^{b}	0.32 ± 0.04^{b}	0.32 ± 0.04^{b}	0.93 ± 0.03^{ab}	1.93 ± 0.04^{a}
PUFA/SFA	1.95 ± 0.06^{a}	1.69 ± 0.05^{a}	1.53 ± 0.04^{a}	1.53 ± 0.05^{a}	1.34 ± 0.06^{a}	1.11 ± 0.05^{a}

[a]*SFA*, saturated fatty acids; [b]*MUFA*, mono-unsaturated fatty acids; [c]*LA*, linoleic acid; [d]*ARA*, arachidonic acid; [e]*n-6 PUFA*, n-6 polyunsaturated fatty acids; [f]*ALA*, alpha-linolenic acid; [g]*EPA*, eicosapentaenoic acid; [h]*DHA*, docosahexaenoic acid; [i]*n-3 PUFA*, n-3 polyunsaturated fatty acids; [j]*AI*, atherogenicity index; [k]*TI*, thrombogenicity index; [l]*H/H*, hypocholesterolaemic and hypercholesterolaemic fatty acid ratio; [m]*FLQ*, fillet lipid quality

[1]Mean values of 3 replicates ± SEM. [2]Mean values sharing the different superscripts in the same row are significantly different ($P < 0.05$)

fed diet with 60% replacement of FO (60 PO). Further replacement of FO with PO in diets (80 PO and 100 PO) resulted in a significant decline in the n-3/n-6 ratio (1.06 and 0.51, respectively). Data of the fatty acid composition of fish fed different experimental diets

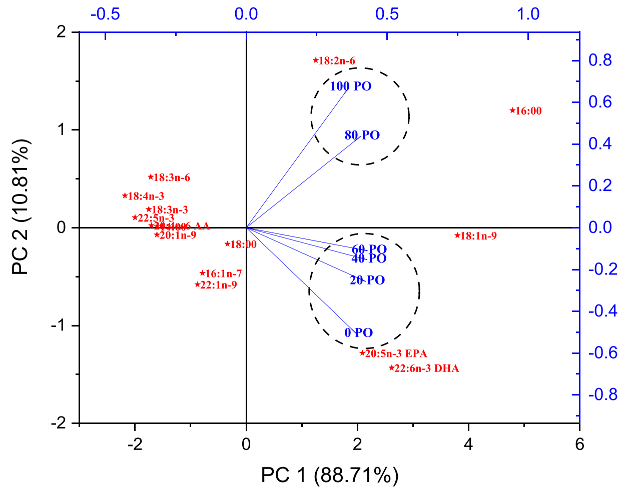

Fig. 1 Principal component analysis of muscle fatty acids of rohu fingerling fed different experimental diets

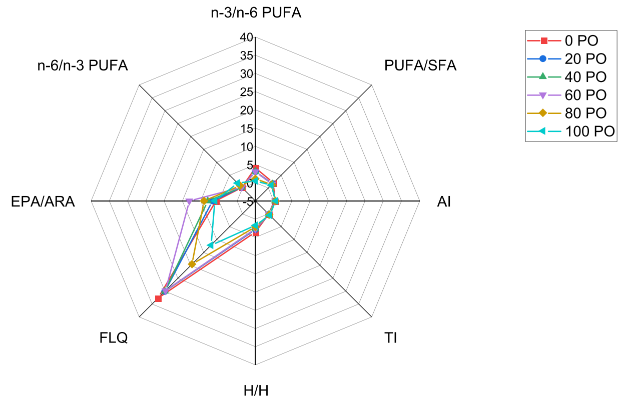

Fig. 2 Radar plot of the indices of nutrition quality of fillet fatty acids of rohu fingerling fed different experimental diets. Values are the mean of triplicate groups of fish

were subjected to principal component analysis (PCA) and the result is shown in Fig. 1. The bi-plot of the first two principal components accounted for 99.52% of total variance with PC1 (main axis 1) of 88.71% and PC2 (main axis 2) of 10.81%, respectively.

Nutritional quality indices of fish fillet

The nutritional quality indices of fatty acids in fish fillet fed test diets are shown in Table 6 and Fig. 2. AI and TI indices in the muscle of rohu fingerling did not differ among treatments ($P > 0.05$). However, highest ($P < 0.05$) H/H (3.55) and FLQ (32.70) values were noted in fish fed 100% FO diet (0 PO). H/H and FLQ values did not differ significantly up to 60% replacement of FO with PO in diet (60 PO). Further replacement FO with PO in diets (80 PO and 100 PO) resulted in decreased H/H ratio and FLQ value. EPA/ARA ratio increased (13.11) up to 60% replacement of FO in diet (60 PO). Further replacement of FO with PO in diets led to a significant decrease ($P < 0.05$) in EPA/ARA ratio. PUFA/SFA and n-6/n-3 ratio did not significantly differ ($P > 0.05$) among all the treatments.

Discussion

Due to the limited availability and escalating cost of fish oil (FO), the search for alternative lipid sources is a priority of the aqua-feed industry. The effectiveness of dietary lipids in promoting growth relies upon the quality and quantity of fatty acids present in the dietary lipid rather than the amount of lipid used in the diet. Herbivorous or omnivorous freshwater fish can convert ALA and LA into ARA, EPA, and DHA (Sargent et al. 2002), but this activity can vary among fish species due to the variation in the ability to desaturate and elongate the fatty acid chains. Growth performances are used to evaluate the effect of the nutrient in the diet (NRC 2011). In this study, replacement of FO with PO in a fish diet did not show any adverse effect on growth performance in *L. rohita* fingerling, indicating that PO can replace FO in diets of *L. rohita* fingerling. However, significant differences in fillet lipid quality of fish fed different diets were recorded. This observation was in accordance with the studies reported on other freshwater fish species (Priya et al. 2005; Karanth et al. 2009; Ren et al. 2012; Babalola and Apata 2012; Kowalska et al. 2010; Jiang et al. 2013; Demir et al. 2014; Zhou et al. 2016; Ayisi et al. 2018; Sankian et al. 2019), where substitution of dietary FO by VOs did not affect fish growth. Once the EFA requirement is satisfied, a considerable quantity of dietary FO may be replaced by other oils without hampering the growth performance, feed intake, and feed efficiency. However, Alves et al. (2021) reported that the inclusion of PO in diet improved growth performances in Nile tilapia, *Oreochromis niloticus*. In the present study, FCR did not differ significantly in all the treatments. Similar observations were also reported by Ayisi et al. (2018) in *O. niloticus*. In the present study, protein retention efficiency (PRE) was found to be highest (33.25–34.99%) in fish fed 100% FO diet (0 PO) and 60% replacement of FO in diet (60 PO). The lowest PRE (24.56%) was recorded in fish fed 100% FO replaced diet (100 PO). Lipid retention efficiency was found highest (53.09%) in fish fed 100% FO replaced diet (100 PO) and lowest (27.89%) in fish fed 20% FO replaced diet (20 PO). This might be due to the imbalance of fatty acids in diets as dietary saturated fatty acid (SFA) increased with increasing dietary PO levels. Increased SFA has been reported to promote lipid deposition (Leamy et al. 2013; Li et al. 2019), resulting in a further increase in carcass lipid content.

The carcass crude lipid and crude protein are important parameters used to evaluate the nutritional quality of fish. In this study, fish fed diets with 80% (80 PO) and 100% (100 PO) replacement of FO exhibited higher carcass lipid than fish fed other diets, whereas carcass crude protein exhibited a reverse pattern. The observations were similar to those of the results of nutrient retention efficiencies. Similarly, the highest HSI and VSI were also recorded in fish fed above-mentioned diets. The higher levels of dietary SFA might have resulted in increased lipid deposition in the hepatic and visceral regions of fish. Similar findings were also reported in rainbow trout, *Oncorhynchus mykiss* (Caballero et al. 2002), gilthead sea bream, *Sparus aurata* (Fountoulaki et al. 2009), African catfish, *Heterobranchus longifilis* (Babalola and Apata 2012), and large yellow croaker, *Larimichthys crocea* (Li et al. 2019).

During normal cellular metabolism, sequential reduction in molecular oxygen generates reactive oxygen species (ROS) in animals causing cell and tissue damage (Nordberg and Arnér 2001; Nayak et al. 2021). A balance between the production and removal of ROS is maintained under normal physiological conditions. However, when an imbalance in ROS production and removal happens, the antioxidant defense mechanism gets stimulated to cope with oxidative stress (Kohen and Nyska 2002; Guillou et al. 2010). This antioxidant defense system includes SOD which accelerates the dismutation rate of superoxide

radicals such as O_2^- into oxygen and hydrogen peroxide (H_2O_2). CAT catalyzes the reduction of H_2O_2 and lipid peroxides into molecular oxygen and water, thus, completing the detoxification process initiated by SOD. GPx, along with glutathione as a hydrogen donor, reduces all the organic lipid peroxides (Jin et al. 2017). Oxidation and breakdown of the fatty acids of membrane lipids that contain more than two methylene-interrupted double bonds result in the production of MDA, an important metabolite used to indicate oxidative damage caused by ROS (Yuan et al. 2019). In the current experiment, the lowest GPx activity was observed in fish fed diet containing 100% palm oil (100 PO), whereas the highest activity was noted in fish receiving 100% fish oil diet (0 PO), followed by a 60% FO replacement diet (60 PO). This signifies that feeding rohu fingerling with palm oil up to 60% replacement can decrease the peroxidative damage by removing excess ROS. In this study, a significant decrease in CAT and SOD activities was noted in the serum of rohu fingerling fed with 80% (80 PO) and 100% (100 PO) fish oil replacement diets. Highest MDA was recorded in fish receiving a 100% FO diet (0 PO). A significant decrease in MDA level with the increase in PO in all the diets was noted, indicating a reduced susceptibility of fish to fatty acid peroxidation. Moreover, in the current study, the PIn (peroxidation index) of the diets was related to the percentage of dietary LC-PUFA and MDA to PIn. High dietary LC-PUFAs present in 100% FO diet (0 PO) caused lipid peroxidation resulting in an increase in MDA level. This signifies that the replacement of FO with PO prevents MDA accumulation by suppressing lipid peroxidation.

Lysozyme plays a vital role in non-specific immunity (Zhang et al. 2017) which in fish is considered to be more important than specific immunity because the latter requires a longer time in specific cellular activations to produce antibodies. It is distributed widely in the mucus, serum, gill, and intestinal tract of the fish body. Lysozyme has antiviral, anti-inflammatory, and antibacterial activities. Fish immunity is regulated by dietary fatty acid composition and serum lysozyme activity (Yu et al. 2020). In this study, lysozyme activity did not change significantly up to 60% replacement of FO with PO (60 PO). However, the activity declined upon further replacement of FO in diet (80 PO and 100 PO). A similar response was recorded where FO substitution with VOs up to a certain level did not affect lysozyme activity as reported in earlier studies, including gilthead sea bream, *Sparus aurata* (Montero and Izquierdo 2010), Eurasian perch, *Perca fluviatilis* (Geay et al. 2015), and Nile tilapia, *O. niloticus* (Ayisi et al. 2018).

The fatty acid profile of cultured fish is directly influenced by the nutrient composition of the diet (Barriviera et al. 2021; Zhu et al. 2022). Muscle fatty acid composition of rohu fingerling was significantly affected by the replacement of FO with PO in the diet. Fish receiving a 100% fish oil diet (0 PO) resulted in the highest EPA and DHA levels in fish muscle, followed by diet containing 20% (20 PO), 40% (40 PO), and 60% (60 PO) replacement of FO. A significantly higher muscle n-3/n-6 ratio was noted in fish receiving 100% fish oil diet (0 PO). Although replacement of FO with PO lowered the muscle n-3/n-6 ratio, it is much higher than their corresponding levels in diets. The content of n-6/n-3 PUFA ratio, PUFA/SFA and H/H ratios, and AI and TI values are used as tools to evaluate the nutritional quality of meat (Chen and Liu 2020). The consumption of a balanced n-6/n-3 ratio is important. High levels of n-6 fatty acid lead to health problems such as coronary artery diseases, obesity, and type 2 diabetes. In contrast, n-3 fatty acids have health benefits in reducing the risk of cardiovascular disease and preventing Alzheimer's disease. N-6/n-3 PUFA ratio lower than 4 and PUFA/SFA ratio higher than 0.45 are recommended for the human diet (Department of Health and Social Security 1984). A PUFA/SFA ratio below 0.45 has been considered undesirable for the human diet as it might increase cholesterol level in blood. In this study, the n-6/n-3 ratios were lower than 4 and PUFA/SFA ratios

were higher than 0.45 in all the muscle samples, indicating that the muscle of rohu fingerling met the requirements for healthy human nutrition. The H/H ratio reflects the influence of fatty acids on the metabolism of cholesterol. Fish flesh with higher H/H values is recommended for human consumption (Santos-Silva et al. 2002; Cortegano et al. 2017; Gonçalves et al. 2021). Also, the larger the fillet lipid quality (FLQ) value, the better it is. In the present study, fish fed 100% FO diet (0 PO) resulted in the highest H/H ratio (3.55 ± 0.24) and FLQ value (32.70 ± 0.41) in fish muscle (Table 5) which did not differ significantly up to 60% replacement of FO with PO in diet (60 PO). However, further replacement FO with PO in diets (80 PO and 100 PO) resulted in a decrease in H/H ratio and FLQ value in the fish muscle.

AI represents arteriosclerosis, a tendency for clot formation in blood vessels (Ulbricht and Southgate 1991). TI characterizes the thrombogenic potential of fatty acids (Ulbricht and Southgate 1991). It is the relation between pro-thrombogenic fatty acids (C12:0, C14:0, and C16:0 acids) and anti-thrombogenic fatty acids (MUFAs, n-3 and n-6 fatty acids). Therefore, lower indices positively affect coronary artery disease prevention (Cortegano et al. 2017). No significant changes ($P > 0.05$) in AI and TI indices were noted among *L. rohita* fingerling fed diets with different experimental diets (Fig. 2) and their values ranged between 0.31–0.56 and 0.17–0.55, respectively. These values are similar to the values obtained by Linhartová et al. (2018) in some freshwater carp species. In the present experiment, all the AI and TI values were below 1 and H/H index was above 1, indicating significant benefit to human health in terms of cardiovascular diseases.

The desaturation and elongation processes of ALA and LA to EPA and ARA, respectively, are mediated by desaturase and elongase enzymes with a preference over the substrate availability (Tocher et al. 2004). Thus, EPA/ARA ratio is a vital fillet nutritional indicator. Moreover, EPA and ARA are the precursors of bioactive mediators such as eicosanoids which are the indicators of inflammatory processes (Gonçalves et al. 2021). In the present experiment, muscle EPA/ARA ratio increased up to 60% replacement of FO in diet (60 PO). However, further replacement of FO with PO in diet (80 PO and 100 PO) resulted in a significant decrease in EPA/ARA ratio, indicating that 60% replacement of FO with PO (60 PO) is optimum for rohu fingerling.

PCA was used to observe the clustering trends of the muscle fatty acid profiles in rohu fingerling fed with different experimental diets. The bi-plot indicated that 16:0, 18:1n-9, 18:2n-6, 20:5n-3, and 22:6 n-3 fatty acids were responsible to cause differences among samples. Diets containing the replacement of FO with PO at 20% (20 PO), 40% (40 PO), and 60% (60 PO) levels are positively correlated and clustered together with diet containing 100% FO (0 PO). However, diets containing the replacement of FO with PO at 80% (80 PO) and 100% (100 PO) levels are negatively correlated with the diet containing 100% FO (0 PO). Also, the bi-plot showed that 16:00 and 18:2n-6 fatty acids in the muscle of fish fed 80% (80 PO) and 100% (100 PO) FO replacement diets resulted in increased lipid deposition. Therefore, it can be concluded that FO can be replaced for up to 60% of the diet without changing the fatty acid composition in fish muscle.

Conclusion

In summary, the current experiment demonstrated that PO can replace FO without hampering growth performance in rohu fingerling. However, health and nutritional benefits were reduced in *L. rohita* fed diets with higher levels of PO (80% and 100% replacement of FO)

due to the decrease in muscle EPA and DHA levels. Antioxidant capacity and lysozyme activity remained unchanged up to 60% replacement of FO with PO in the diet. Although the AI and TI values and PUFA/SFA and n6/n3 ratios did not change among all the muscle samples, the highest H/H and FLQ values were evident in fish receiving a 100% FO (0 PO) diet. H/H and FLQ values did not differ significantly up to 60% replacement of FO with PO in diet (60 PO). Replacing FO with PO at a higher level had negative consequences on the nutritional quality of rohu fingerling. Therefore, FO can be replaced by PO at a moderate level (60%) to avoid the degradation of nutritional quality of the fillet and to formulate cost-effective commercial feeds.

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Data availability The datasets are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval All the experimental procedures, including animal experimentation, were approved by the institutional ethical committee of the Department of Biochemistry, Aligarh Muslim University, Aligarh, India (registration number: 714/02/a/CPCSEA).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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